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The Design, Synthesis and Evaluation of Aryl Guanidinium Derivatives as α_2 Adrenoceptor Antagonists for the Treatment of Depression



**A thesis presented to the University of Dublin
for the degree of Doctor of Philosophy**

by

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September 2012

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Summary

Depression is among the leading causes of illness worldwide and is predicted to be the single largest contributor by 2020. Antidepressants are the most effective treatment but current drugs have slow onset of effects and are only successful for some individuals.

The α_2 -adrenoceptor (α_2 -AR) has emerged as a promising target for the development of improved antidepressants with faster onset of action and reduced side effects. It is found in increased density and hyperactive conformation in the brains of depressed patients and its activation leads to: decreased monoamine levels in the brain, and reduced hippocampal neurogenesis and cell survival, both now seen as fundamental causes of depression. Antagonists have been shown to reverse these effects and, more importantly, lead to antidepressant effects. However, their discovery has been hampered by the lack of a crystal structure of the α_2 -AR and the conflicting pharmacological profile of structurally similar ligands at the receptor.

This project employed a ligand-based drug design strategy to identify the requirements for high binding affinity and antagonistic activity at the α_2 -AR for aryl-guanidinium type molecules. The initial target pyridinyl guanidiniums were first studied computationally, then experimentally after synthesis, and finally were tested for affinity and activity at the α_2 -AR. A full theoretical description of the π -cation and π - π complexes available to the initial ligands was also carried out to model interactions with aromatic amino acid residues thought to be important in the active site, and several structure activity relationships could be drawn.

These and subsequent results guided the choice of four further families of compounds which were prepared and evaluated as α_2 -AR antagonists. Thirty seven compounds in all were synthesised and their purity measured using reverse phase HPLC. Their binding affinity was assessed using competitive radioligand binding assays in prefrontal cortex tissue from depressed suicide victims, and their functional activity was measured using [35 S]GTP γ S assays in the same tissue. The desired antagonistic or inverse agonistic activity was obtained in all cases and several structure-activity relationships were obtained relating to binding affinity. Furthermore, suggested α_2 -AR subtype selectivity has been obtained, lending to the possibility that compounds with high potential as α_2 -AR antagonists for the treatment of depression can be obtained.

Abbreviations

5-HT	5-Hydroxytryptamine, Serotonin
5-HTP	5-Hydroxy-L-Tryptophan
α_2 -AR	α_2 -Adrenoceptor
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACTH	Adrenocorticotrophic Hormone
Ag	Agonist
AIM	Atoms in Molecules
ALD-D	Aldehyde Dehydrogenase
ALD-R	Aldehyde Reductase
AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid
Ant	Antagonist
AR	Adrenoceptor
ATR	Attenuated Total Reflectance
BA	Behavioural Activation
BBB	Blood-Brain Barrier
BCP	Bond Critical Point
BDNF	Brain-Derived Neurotrophic Factor
CAM	Calcium-Dependent Kinases
cAMP	Cyclic Adenosine Monophosphate
CBT	Cognitive Behavioural Therapy
CNG	Cyclic Nucleotide-Gated Ion Channel
CNS	Central Nervous System
CoMFA	Comparative Molecular Field Analysis
COMT	Catechol <i>O</i> -Methyl Transferase
COX	Cyclooxygenase
CREB	cAMP-Responsive-Element-Binding Protein
CRF	Corticotrophin-Releasing Factor
DA	Dopamine
DAT	Dopamine Reuptake Transporter
DFT	Density Functional Theory
ECT	Electroconvulsive Therapy

E _I	Interaction Energy
EPSP	Excitatory Post-Synaptic Potential
E _q	Equivalents
FDA	Food and Drug Administration
FST	Forced Swim Tests
FTIR	Fourier-Transform Infrared Spectroscopy
GABA	γ-Aminobutyric Acid
GDP	Guanosine Di-Phosphate
GIAO	Gauge-Independent Atomic Orbital
GPCR	G-Protein Coupled Receptor
GTP	Guanosine Tri-Phosphate
HB	Hydrogen Bond
HF	Hartree Fock
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal
HPLC	High Performance Liquid Chromatography
IMHB	Intramolecular Hydrogen Bond
IPSP	Inhibitory Post-Synaptic Potential
IRC	Intrinsic Reaction Coordinates
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitor
MDD	Major Depressive Disorder
MHPG	3-Methoxy-4-hydroxyphenylglycol
MST	Magnetic Seizure Therapy
NA	Noradrenaline
NAS	Nucleophilic Aromatic Substitution
NBO	Natural Bond Orbital
NET	Noradrenaline Reuptake Transporter
NICS	Nucleus-Independent Chemical Shift
NMDA	<i>N</i> -Methyl-D-Aspartate
NMJ	Neuromuscular Junction
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NT	Neurotransmitter

PCM.....	Polarisable Continuum Model
PDE.....	Phosphodiesterase
PFC	Prefrontal Cortex
PKA	Protein Kinase A
QSAR.....	Quantitative Structure-Activity Relationship
QTAIM	Quantum Theory of Atoms in Molecules
RT	Room Temperature
SAR.....	Structure-Activity Relationship
SERT.....	Serotonin Reuptake Transporter
SNRI	Serotonin-Noradrenaline Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
TFA.....	Trifluoroacetic Acid
TFAA.....	Trifluoroacetic Anhydride
TLC.....	Thin-Layer Chromatography
TST	Tail Suspension Test
VMA	Vanillylmandelic Acid
VMAT.....	Vesicular Monoamine Transporter
WHO.....	World Health Organisation

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Chapter 1 – Introduction

1.1. The Disease of Depression

Feelings of unhappiness and disappointment are common in the general population, affecting all people at some time in their lives; it is even thought that one third of people experience what can be described as a depressive episode during their lives. For the majority of these people the feelings dissipate and normal mood returns within a reasonable time period. However, when these feelings become exaggerated, pervasive or interfere with a person's ability to function in life, they can be considered as pathological depression, or Major Depressive Disorder (MDD).¹ Depression is a common and costly disorder that affects emotion, cognition, physical well-being and behaviour. The World Health Organization (WHO) characterises the disease by depressed mood, anhedonia, loss of interest or pleasure, feelings of guilt or low self-esteem, disturbances in sleep and/or appetite, low energy and poor concentration.

There is a significant genetic risk factor which is slowly becoming more understood. Offspring of depressed parents are at an increased risk of developing depression during their lifetime. The risk of depression in the first 16 years of life for the offspring of a mother who suffered from post-natal depression was 4.7 times greater than for non-exposed offspring.² While genetic factors are known to play an important role in the development of the disease, to date, no single genetic mutation has been shown to significantly increase the risk of depression and it is likely that a convergence of multiple genetic factors and environmental influences are required for the emergence of the disorder.³

For most people who suffer an episode of depression, the disease becomes chronic in nature. A study carried out by Derek Richards at Trinity College Dublin⁴ found that a person who suffers a first episode of depression has a 25-40% chance of recurrence within two years; this risk increases to 60% after five years and to 85% after 15 years. The study also found that the risk of recurrence increases further by 16% with each subsequent episode of depression.

Depression is recognised by the WHO as one of the leading causes of disability in the world. In a report entitled “The Global Burden of Disease” (commissioned in 2004 and updated in 2008),⁵ health data from across the globe was collected and compared in an effort to describe health problems, identify trends and help decision-makers set priorities. In the report global health is assessed by measuring the burden of disease – the loss of health from all causes of illness and deaths worldwide. It details the leading causes of deaths both globally and in every region, and provides information on more than 130 diseases.

One of the most staggering findings of the report was that mental health problems were the second leading cause of disability, surpassing all cancers combined and even HIV infection, being outranked only by cardiovascular diseases. Considering depression as a single condition, it was found to be the leading cause of disability worldwide.⁶ This outcome has provided the impetus for a series of further WHO studies on the effectiveness of mental health care and, more importantly, it has advocated the need to support these systems as the huge loss of human productivity due to mental illnesses is becoming exposed (*vide infra*).

The overall prevalence of depression is difficult to measure and subject to variation as many cases go undiagnosed due to differing cultural attitudes worldwide and lack of proper infrastructure for diagnosis and treatment. Studies have concentrated on the United States and Europe and there is a shortage of data available for the developing world. One such study from the United States carried out by the Baltimore Epidemiologic Catchment Area reports that in the adult population the overall prevalence of depressive disorders is 5.2% for any given one month period, 5.8% over a six month period, 6.3% over twelve months, and lifetime prevalence is 8.3%.⁷ It is likely that these figures seriously underestimate the reality, as they are taken only from patients who have sought professional help for their depression, and the same study indicates that this represents less than 50% of the total sufferers. Taking this into account, and in general terms, more comprehensive studies indicate that as many as 17% of people will experience episodes of depression at some stage in their lives.⁸

In Ireland, figures collected by the Central Statistics Office state that at any one time 7% of the population is suffering from depression.⁹ Epidemiologically, women are almost twice as likely as men to experience depression – however an outcome of

suicide is more likely in males, accounted for by the fact that men are less likely to seek appropriate medical help.¹⁰ In terms of age the highest risk periods are 15-24 and over 65. Within the younger of these age brackets, suicide is the second highest cause of death, according to the WHO.¹¹ Unfortunately, for the particularly vulnerable over 65 age group depression is often misconceived as a natural part of ageing and so goes under-reported and under-treated, meaning suicide is also common in this age group. As is the case with many other diseases, people with difficulties in their personal lives such as widows, separated people, those with marital problems, those who experienced childhood emotional trauma and those with financial worries are more disposed to developing depression. Comorbidity due to diseases such as cancer, diabetes and HIV are also common.¹²

The high prevalence of depression is of considerable concern, principally as it causes significant distress to the sufferer and the people around them. If any further motivation were needed to work at alleviating this problem, the enormous societal impact and the economic costs that depression causes are substantial enough to provide it. Mental health problems are estimated to account for 35-45% of absenteeism from work.¹¹ In the United States studies have examined the economic costs of this absenteeism and have found that they reach such an extent that it would be far more cost effective for employers to provide treatment to employees with MDD.¹³

A comprehensive study in Europe, commissioned by the WHO across 28 countries and a population of 466 million, determined that at least 21 million of these suffered from depression. Estimates of the annual cost of this in 2004 totalled €118 billion, €42 billion of that incurred through outpatient care, drug treatments and hospitalisation. This has to be considered in the context that less than half of people suffering from MDD seek medical attention; however, it is colourless that the costs of managing the disease are far outweighed by the indirect costs from morbidity and mortality.¹⁴

Morbidity and Mortality

It is not instantly clear where this morbidity and mortality arise from, and where such large economic costs are incurred. One suggestion is non-compliance with treatment regimens; an analysis of published articles dealing with patients suffering from

depression revealed that depressed people were three times more likely than non-depressed people to be noncompliant with medical treatment recommendations. This in turn resulted in reduced health and subsequent increases in the use of health care resources.¹⁵

Patients of depression are also at increased risk for certain co-morbidities such as cardiovascular disease and stroke. The links are poorly understood, but greater platelet activation and aggregation, endothelial dysfunction and impaired autonomic function commonly observed in depression are implicated in the onset of cardiovascular disease.¹⁶ According to a study carried out by the National Institute of Mental Health (U.S.A), over a 14-year period, persons with a history of MDD were four times as likely to suffer a myocardial infarction as non-depressed individuals and the prognosis after the event was worse than for patients who had not been suffering from depression.¹⁷ The increased risk of stroke in depression is seen as independent of other risk factors and the prognosis for stroke victims who also suffer from depression is similarly worse, increased mortality being recorded.¹⁸

Anxiety disorders are the most frequent comorbidities, affecting up to 50% of patients with major depression. These are blamed for leading to more severe depressive symptoms and less favourable responses to treatment.⁴ Depressed people are more likely to have eating disorders and other self-destructive habits such as substance abuse and alcoholism which can lead to serious, life-long problems.¹⁹ Overall, patients of depression are more likely to be hospitalised for some other cause than non-depressed people. A potential explanation of this was proposed in an interesting investigation on the possibility that MDD suppresses immune system responses. The study of older adults monitored their ability to generate white blood cells and found poorer lymphocyte T cell responses for those subjects with depression.²⁰

However, as mentioned earlier, by far the most significant and serious contribution to mortality in individuals with depression is suicide. An estimated 90% of suicides are associated with depression and between 2% and 9% of patients with diagnosed MDD take their own lives.²¹ Suicide is the second leading cause of death among individuals aged 15 to 24 years¹¹ and the fact that the rate of suicide from depression increases with age is an indication of the gravity of the problem. A further significant difficulty is attempted suicide which is reported to be as high as 56% for patients with depression.²²

Clinicians often cite such attempts as a call for help but what is clear is that the probability of death is greatly increased with every attempt.²³

1.2. Synaptic Neurotransmission

To properly explain any theory on the nature of depression, or the strategies for its treatment, the processes of neurotransmission must first be described. It was only in the 1930s that the now well established link between chemistry and the nervous system was accepted. Otto Loewi received the Nobel Prize for physiology or medicine in 1936 for his discovery of the first neurotransmitter (NT). He proved that the transmission of nerve impulses between neurons was by chemical and not (always) electrical means. The chemical in question which had been secreted by the vagal nerve turned out to be acetylcholine (ACh). His work stimulated the search for the chemicals involved in neurotransmission and their role in diseases of the central nervous system (CNS). Along with the idea that external stimuli and exogenous compounds could initiate changes to the pathways of the brain came the need for a better understanding of the processes involved in neurotransmission.

The key functional unit of the CNS is the neuron, a highly specialised cell capable of receiving, processing and transmitting information. Neurons receive information from sensory organs and either send information to motor organs or share it with other neurons. The process of communicating information is similar for each type of connection. By far the largest number of neuronal connections is with other neurons within the CNS.

Neurons consist of a central nucleus from which wire-like structures called dendrites and axons protrude (Fig. 1.1). The role of the dendrite is to receive signals from other cells in the form of action potentials. The cell body (nucleus) is then responsible for processing and transmitting these signals through the axon which branches into a large number of dendritic regions before connecting with many neighbouring neurons simultaneously at junctions called synapses.

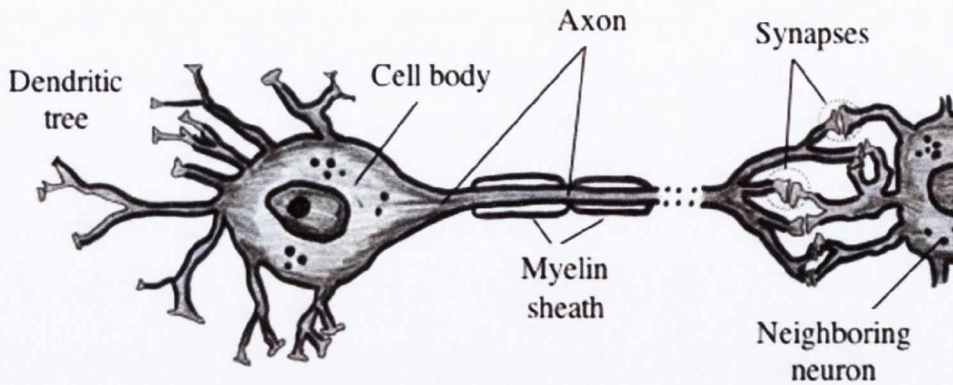


Fig. 1.1. Schematic representation of the features of a neuron-neuron connection – a synapse (adapted).²⁴

When a neuron is at rest it maintains a voltage gradient between its contents and the surrounding environment. This resting membrane potential is approximately -70 mV at the axon hillock, the point where the axon leaves the cell body. When a neuron receives an input it can either depolarise or hyperpolarise depending on the type of input. An action potential is triggered when enough depolarisation occurs to raise the membrane potential above a threshold of approximately -55 mV. When this occurs the voltage gradient rapidly collapses and a wave of depolarisation sweeps along the cell body from dendrite to axon; the neuron can then repolarise in preparation for the next action potential. This process of depolarisation and repolarisation is regulated by voltage-gated sodium and potassium channels and takes less than one millisecond.²⁵

When the action potential reaches the axon terminus, depolarisation gives rise to an influx of Ca^{2+} ions – through voltage-gated ion channels – which bind to calmodulin, inducing vesicles storing NT to fuse with the cell membrane (Fig. 1.2) and release the NT into the synaptic cleft by exocytosis. The released NT then diffuses towards the dendrite of a neighbouring cell through the synapse, the gap between an axon terminus and a neighbouring cell's dendrite. Several different types of receptor are expressed here, depending on the neuron, and the NT can bind to these receptors, propagating the signal.²⁵

Ligand-gated ion channels (ionotropic receptors) transport ions such as K^+ and Cl^- into the post-synaptic neuron on activation. When metabotropic or G-protein coupled

receptors (GPCRs) are activated they act through intracellular secondary messenger systems such as by switching on or off cyclic adenosine monophosphate (cAMP) production, later having effects on the entry of ions into the cell. Through these signals NTs transmit the signal to neurons whether to produce an action potential (excitatory post-synaptic potential, EPSP) or suppress an action potential (inhibitory post-synaptic potential, IPSP).²⁵

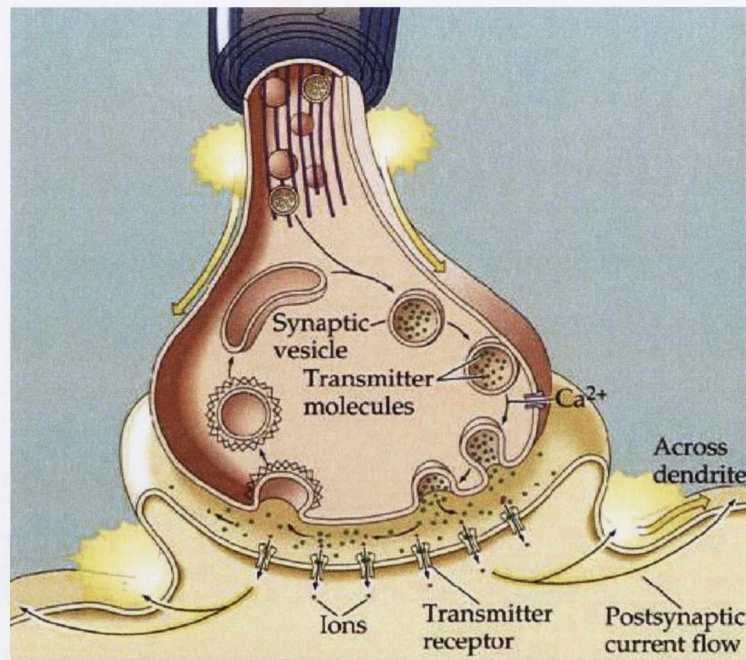


Fig. 1.2. A schematic depiction of synaptic neurotransmission.²⁶

After release into the synaptic cleft, interaction with post-synaptic receptors is not the only option available to NTs. What is more, NTs must be deactivated to allow the membrane potential of neurons to return to rest. Several regulatory mechanisms are in place to achieve this, one of which is degradatory enzymes – such as catechol *O*-methyl transferase (COMT), monoamine oxidase (MAO) and acetylcholinesterase (AChE) – which metabolise NTs to regulate their levels.²⁷

There are also several receptors on the pre-synaptic neuron which NTs can interact with. An example of these is the autoreceptors, a further regulatory mechanism for ensuring too much NT is not released into the synapse. When NT interacts with an autoreceptor a negative feedback loop is activated, through intracellular second messengers, which stops the further release of NT by the cell during subsequent action

potentials. The closely related heteroceptors have the same effect of switching off release but are activated by a different NT than the one being released by the cell.

A further class of proteins located on the pre-synaptic neuron is that of the reuptake transporters. These are generally symporters, meaning they combine facilitated diffusion with active transport of a molecule in the same direction across a membrane. Generally, an ion moves down its concentration gradient to facilitate the disfavoured movement of the NT back into the presynaptic neuron. Once the NT has been taken back into the cell it is either re-stored in vesicles or degraded by metabolic enzymes such as COMT, MAO and AChE, depending on the type of neuron.²⁷

As well as this chemical neurotransmission, action potentials can be transmitted electrically if the axon terminus and the dendrite of a post-synaptic neuron are sufficiently close.²⁸ For this to occur, it has been estimated that the gap should be less than 3.5 nm, whereas the gap at chemical synapses can be 20–40 nm. This transfer is facilitated by a series of hydrophilic channels which cross both the pre and post-synaptic membranes, allowing for the direct transport of ions between the cells at these “gap junctions”. Transmission at these synapses is faster than at chemical synapses but can only result in EPSPs and these can only be equal to or less than the input signal. They are mostly found in neural systems involving functions requiring fast responses such as defensive reflexes.

1.2.1. Neurotransmitters of the Central Nervous System

There are certain criteria to be met for a molecule to be classed as a NT. The molecule must be synthesised in the neuron and be present at the axon termini of pre-synaptic neurons. Its release into the synaptic cleft must induce changes to the post-synaptic membrane potential; either excitatory or inhibitory. The effects of its endogenous release must be replicable by exogenous administration of the same compound. While in the synaptic cleft it must be acted on by specific removal mechanisms, such as degradation or reuptake into the pre-synaptic neuron.²⁹

There are two major classes of NT, defined by their storage and release mechanisms. The first class are small molecules such as ACh, noradrenaline (NA) and serotonin (5-HT), and are stored in small vesicles before release into the synaptic cleft by exocytosis

at active zones on the axon termini. Their release is associated with the opening of Ca^{2+} channels during an action potential. The second class are protein molecules which are packaged in large vesicles and can be released anywhere from the presynaptic neuron. In general, neurons contain both types of NT, but in different relative concentrations.³⁰

The full list of chemicals which could be called NTs is expansive; more than 100 such molecules are known to exist, and new NTs are still being discovered. However, the most important NTs comprise a short list of molecules, many of which have been thoroughly studied with regard to their biochemistry, pharmacology and metabolism, as well as their relevance to human behaviour and cognition. The major classes of NTs associated with depression are discussed in the following sections.

1.2.1.1. The Amino Acids

Amino acid transmitters provide the majority of excitatory and inhibitory neurotransmission in the nervous system. It has proven difficult to determine the localisation of amino acid NTs in the brain, particularly those which are proteinogenic, as they are present in protein structures as well as for neurotransmission.³¹

Glutamate (Fig. 1.3) is the principal excitatory NT in the CNS. It is produced in the mitochondria of neurons as part of the Krebs cycle (the generation of energy through oxidation of acetate derived from carbohydrates, fats and proteins into carbon-dioxide). When glutamate is released it can interact with both ionotropic and metabotropic receptors. There are three families of ionotropic receptors with intrinsic cation-permeable channels; *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate, each of which is linked with opening Na^+ channels on post-synaptic membranes. There are also three groups of metabotropic, G-protein coupled glutamate receptors (mGluR) which modify neuronal excitability through G-protein subunits acting on membrane ion channels and second messengers such as diacylglycerol and cAMP.³²

NMDA receptors are closely associated with neuroplasticity and learning, and require both glutamate and glycine as co-agonist to be present for activation, although aspartate (Fig. 1.3) has also been shown to activate the NMDA receptors.³³ The kainate receptors

are less well understood but are under investigation as a target for anti-epileptic drugs due to their role in seizure induction.³⁴

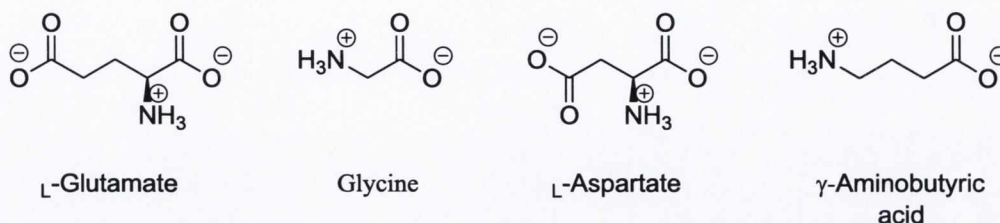


Fig. 1.3. The principal amino acid NTs in the CNS, in their zwitterionic form.

As well as being a required co-agonist for the excitatory effects of glutamate at NMDA receptors, glycine is an inhibitory NT in areas of the CNS such as the spinal cord, brainstem, and retina.³⁵ When glycine ionotropic receptors are activated, chloride enters the neuron, causing an IPSP. Strychnine is a strong antagonist at ionotropic glycine receptors, exerting its toxicity at the motor nerves in the spinal cord that control muscle contraction.³⁶

The second major inhibitory amino acid NT is γ-aminobutyric acid (GABA, Fig. 1.3), which mediates the majority of inhibitory synaptic actions in the CNS. It is synthesised from glutamate on decarboxylation by the enzyme glutamic acid decarboxylase. There are two families of receptors at which GABA acts; the ionotropic GABA_A receptors are mostly post-synaptic and the metabotropic GABA_B receptors are largely pre-synaptic. Benzodiazepines and barbiturates bind to the GABA_A receptor, enhancing the ability of GABA to bind and induce the entry of Cl⁻ into the neuron, resulting in hyperpolarisation.³⁷ The sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties of these agents has made them useful in the treatment of alcohol dependence, seizures, anxiety, panic and insomnia.³⁸ GABA_B agents are less exploited therapeutically but agonists are under investigation for similar indications, particularly the treatment of alcoholism.³⁹

1.2.1.2. Acetylcholine

Acetylcholine (Fig. 1.4) is the only small molecule NT that is not either an amino acid or a derivative of an amino acid. It is synthesised from acetyl-CoA and choline, which cannot be synthesised by the body and is obtained from the diet (through foods such as eggs and fatty meats). Because of this choline is also recycled in the body after use and breakdown in a tightly regulated process. Acetylcholine is less prominent in the CNS than the amino acid NTs, being more prevalent at neuromuscular junctions (NMJ) where it initiates muscular contraction.

At the NMJ, ACh receptors are divided into the post-synaptically located nicotinic (ionotropic) and pre-synaptically located muscarinic (metabotropic) receptor classes. Direct agonists of the ACh receptors are used to treat myasthenia gravis, an autoimmune neuromuscular disorder leading to muscle weakness, as antibodies present in this condition are known to block the nicotinic ACh receptors.⁴⁰ Inhibitors of AChE which indirectly increase ACh activity by preventing its breakdown have been employed as nerve agents (*e.g.* Sarin, Fig. 1.4) and pesticides.

In the CNS ACh acts as an excitatory NT and is associated with alertness and arousal.⁴¹ Reversible inhibitors of AChE such as Rivastigmine (Fig. 1.4) are employed to treat the symptoms of Alzheimer's disease, due to the fact that the reduced function of presynaptic ACh receptors and the degeneration of neurons that produce ACh have been linked to Alzheimer's disease.⁴²

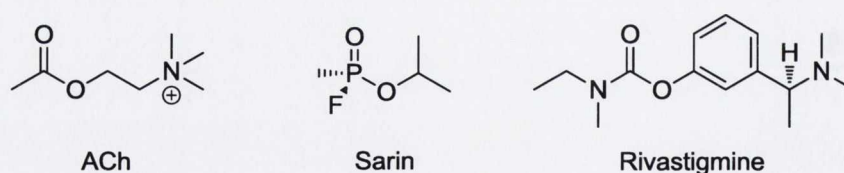


Fig. 1.4. The structures of ACh, Sarin and Rivastigmine.

1.2.1.3. Neuropeptides

Neuropeptides are derived from secretory proteins formed in the cell body of neurons. They are first processed in the endoplasmic reticulum and then moved to the Golgi apparatus before being secreted in large vesicles and transported down the axon in

preparation for release by exocytosis. More than 50 peptides that act as NTs have been isolated from nerve cells including Substance P (Fig. 1.5) and the endorphins, which produce feelings of analgesia and well-being on release by acting at opioid receptors. They can have either excitatory or inhibitory effects on post-synaptic potentials and can also act as neuromodulators, affecting the amount of NT released during action potentials.⁴³

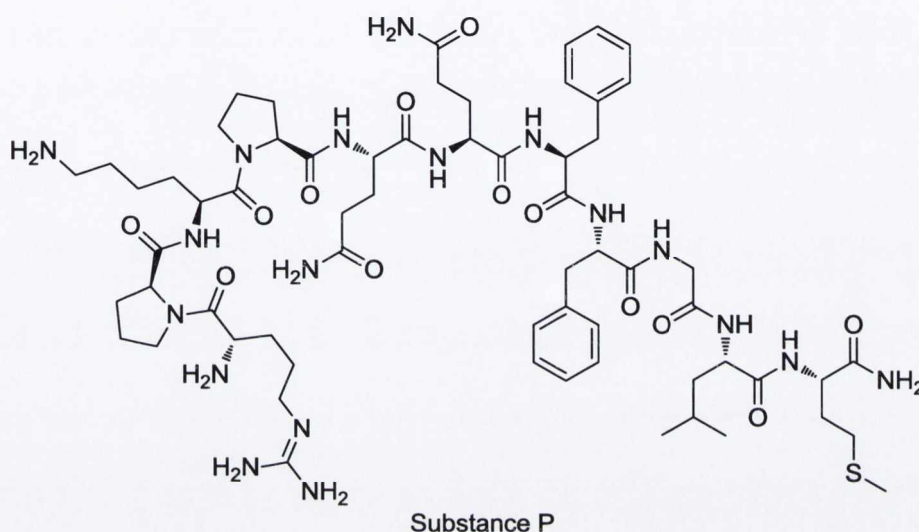


Fig. 1.5. The undecapeptide, Substance P, which acts as a NT and neuromodulator.

Many neuropeptides also act as neurohormones, and the physiological and behavioural response to their release may be long-lasting, as is the case in opioid-withdrawal induced hypersensitivity to pain, which has been associated with the action of the cholecystokinin-8 neurohormone.⁴⁴ Substance P is also associated with inflammatory processes and pain.⁴³ Its sensory function is thought to be related to the transmission of pain information into the CNS through activation of its endogenous receptor, the metabotropic neurokinin 1 receptor.⁴⁵ Substance P is now seen as a potential treatment for type 1 diabetes after its administration to the pancreas of mice cured the disease for up to 4 months.⁴⁶

1.2.1.4. Monoamines

The classical monoamine-NTs include the catecholamines dopamine (DA) and NA, and the tryptamines 5-HT and melatonin (Fig. 1.6). The biosynthetic and metabolic pathways of the monoamine NTs are closely linked. Both DA and NA are synthesised from the amino acid tyrosine, while 5-HT and melatonin are derived from tryptophan. All four of these monoamines are acted on by the degradatory enzyme MAO. While they share many common features, each of these NTs has distinct functions and interacts with its own family of receptors.²⁷

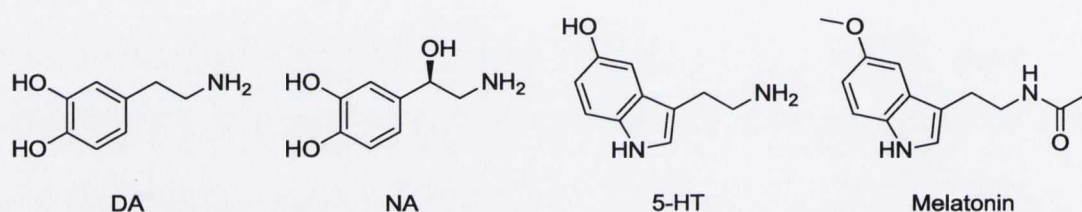


Fig. 1.6. The monoamine NTs; DA and NA are catecholamines, 5-HT and melatonin are tryptamines.

Dopamine, discovered to be a NT in the 1950s by Arvid Carlsson,⁴⁷ is strongly associated with reward mechanisms in the brain. Drugs of abuse like cocaine, opium, heroin and alcohol increase the levels of DA, as does nicotine. Dopaminergic neurons of the midbrain are the main source of DA in the CNS and it is especially prevalent in the substantia-nigra, a region of the brain associated with motor control and reward.⁴⁸ Dopaminergic neurons play an important role in the control of multiple brain functions including voluntary movement and a broad array of behavioural processes such as mood, reward, addiction, and stress.⁴⁸

Dopamine receptors are metabotropic GPCRs and are classified into the D1- and D2-like families. Activation of D1-like DA receptors results in increased concentration of cAMP and, thus, has an excitatory effect at post-synaptic neurons as cAMP is involved in the opening of Ca^{2+} channels. Conversely, activation of the D2-like DA receptors has an inhibitory effect at post-synaptic neurons, through decreased cAMP production.⁴⁹ A decrease in DA activity due to cell death in the substantia-nigra is the reason for the

primary symptoms of Parkinson's disease.⁵⁰ In fact it was also Arvid Carlsson who pioneered the use of L-DOPA, an intermediate in the biosynthesis of DA (Fig. 1.7), to alleviate the symptoms of Parkinson's disease.

Dopamine is synthesised from the amino acid L-tyrosine in the cytosol of neurons. It is first converted to L-dihydroxyphenylalanine (L-DOPA) on addition of a hydroxyl group by tyrosine hydroxylase (this is the rate-limiting step in the synthesis of the catecholamines) that completes the catechol ring (Fig. 1.7).⁵¹ This intermediate is administered to patients of Parkinson's disease to increase DA concentrations and combat the symptoms incurred from DA deficiency.⁵² It is also the substrate for aromatic amino acid decarboxylase (DOPA decarboxylase), which produces DA on decarboxylation of the amino acid.⁵¹ Dopamine is degraded by each of MAO and COMT. A methyl group is added to the *meta* position by COMT and the amine is oxidised to a carboxylic acid by MAO (Fig. 1.7). Regardless of which enzyme acts first the resulting metabolite is homovanillic acid, as both intermediates as well as DA itself are substrates for the metabolic enzymes.⁵¹

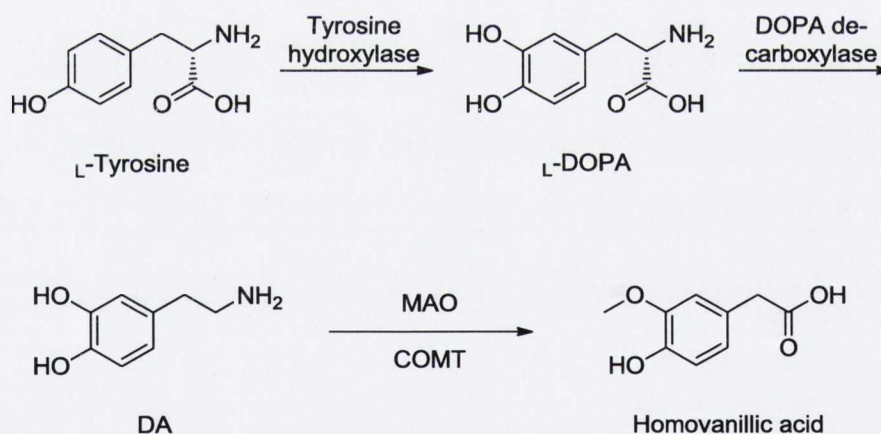


Fig. 1.7. Biosynthesis and catabolism of DA. MAO and COMT act successively to produce homovanillic acid which is excreted.

In dopaminergic neurons the synthesised DA is transported into vesicles by vesicular monoamine transporter 2 (VMAT2) until the cell receives an action potential. Once released into the synaptic cleft DA can have its effects at the D₁- and D₂-like receptors. It can also be re-uptaken into the pre-synaptic neuron by the DA reuptake transporter (DAT). When this transporter is not present DA can also be taken into noradrenergic

neurons by the NA reuptake transporter (NET) in a good example of the recycling and conservation of material that occurs for all NTs in the CNS.⁵³

Dopamine is useful to noradrenergic neurons as it is the precursor to NA. Along with the DA that has been synthesised in the cell cytosol, the reuptaken DA is transported into vesicles by VMAT2 in the same way as in dopaminergic neurons. However, dopamine β -hydroxylase is present in the vesicles of noradrenergic neurons to stereospecifically add a hydroxyl group to the β -position of DA, producing NA (Fig. 1.8).⁵⁴

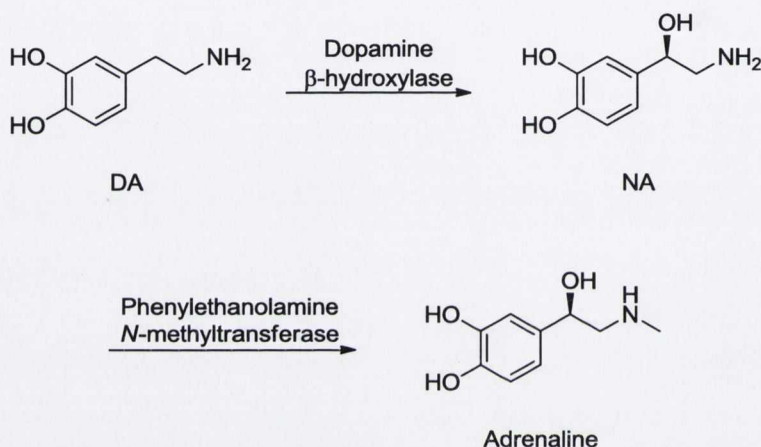


Fig. 1.8. Biosynthesis of NA and adrenaline from DA occurs inside neuron vesicles.

The central noradrenergic system serves a global function of neural modulation, controlling vigilance, attention, and the sleep-wake cycle and contributing to learning and memory processes. It is robustly activated by stress and thus activation of adrenergic receptors by NA in the periphery generally results in effects such as increased heart rate, pupil dilation, blood-flow to skeletal muscle and utilisation of glucose in a response best known as the fight-or-flight response.⁵⁵ Noradrenergic neurons are widespread throughout the CNS and their distribution is diffuse, making it difficult to define a singular role for NA. As well as its roles in alertness it is widely accepted that NA is heavily involved in mood and the stress response, the ability of a person to deal with stressful stimuli.⁵⁶

There are two major classes of noradrenergic receptors, α and β adrenoceptors (ARs), both of which are GPCRs. The β -ARs are principally located in the heart, kidney and

stomach (β_1 subtype), and in the smooth muscles and lungs (β_2 subtype). Adrenaline, synthesised in both the adrenal medulla and adrenergic neurons can be considered as a hormone and a NT.⁵⁷ It is instrumental in this response to stress through its action at β -ARs, at which it has a higher affinity than NA (the opposite is the case for α -ARs). Medications acting on the β -ARs (Fig. 1.9) have been useful in the treatment of cardiac illness (propranolol, non-selective β -AR antagonist) and asthma (salbutamol, β_2 -AR agonist). Both the β_1 and β_2 -AR are coupled with the G_s protein which activates adenylyl cyclase and leads to increased levels of cAMP.

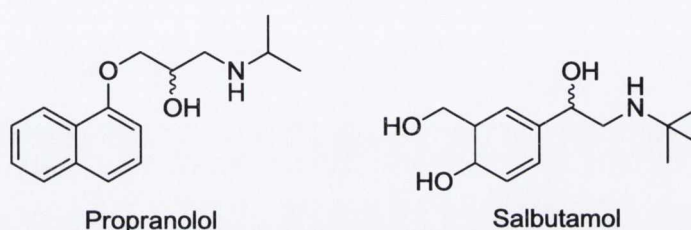


Fig. 1.9. Propranolol (*left*) and salbutamol (*right*) are examples of drugs acting on the β -ARs.

The α -ARs are also subdivided into α_1 - and α_2 -ARs. Specific peripheral actions of the α_1 -AR mainly involve smooth muscle contraction. Its activation causes vasoconstriction in blood vessels, including those of the skin, gastrointestinal system, kidney and brain.^{58,59} Specific peripheral actions of the α_2 -AR include inhibition of insulin release from the pancreas, as well as induction of glucagon release and contraction of the sphincter of the gastrointestinal tract.⁶⁰ For these reasons several drugs exist which exert their action through effects at the α -ARs (Fig. 1.10); antagonists of the α_1 -AR (*e.g.* doxazosin) are used to treat benign prostatic hyperplasia⁶¹ and α_2 -AR agonists (*e.g.* clonidine) have been used as antihypertensives and sedatives.⁶²

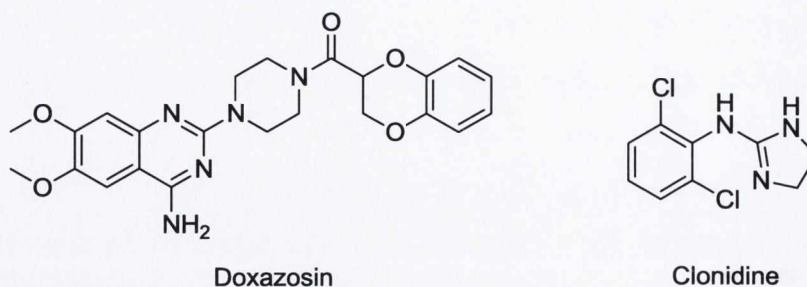


Fig. 1.10. Doxazosin (*left*) and Clonidine (*right*) are examples of drugs acting on the α -adrenoceptors.

The α_1 -AR is coupled with the G_q protein which activates phospholipase C causing an increase in levels of inositol triphosphate and Ca^{2+} that leads to activation of Protein Kinase C.⁶³ The α_2 -AR is associated with the G_i protein which negatively couples to adenylyl cyclase, resulting in decreased levels of cAMP.⁶⁴ Because of its implication in the pathophysiology of CNS disorders the α_2 -AR is further discussed in Section 1.5.2.

Once in the synaptic cleft, NA can also be transported back to the pre-synaptic neuron by NET, inhibition of which has been exploited in recreational stimulants (which also inhibit DA reuptake) and antidepressants. The degradation of NA is complex but is affected in part by the same enzymes that degrade DA; MAO and COMT (Fig.1.11). As with DA degradation either COMT or MAO can initiate the process. Further catabolism by the enzymes aldehyde dehydrogenase (ALD-D) and aldehyde reductase (ALD-R) leads to the excretable products; vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG).⁶⁵

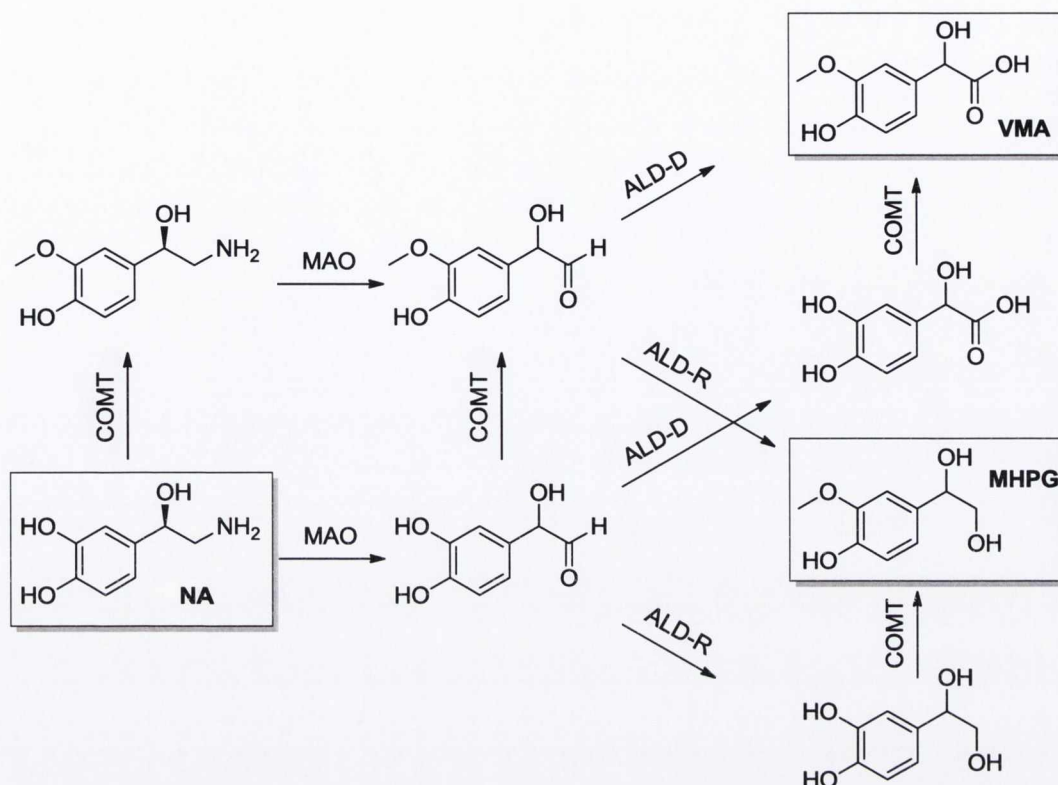


Fig. 1.11. Degradative pathways of NA. Framed compounds are NA and the excreted metabolites VMA and MHPG.

Like the catecholamines the tryptamines have highly related biosynthetic and degradative pathways. As much as 90% of the body's 5-HT is located in the gut, where it is involved in regulating intestinal movements.⁶⁶ The remainder is synthesised in serotonergic neurons in the CNS, where the precursor is the essential amino acid *L*-tryptophan (Fig. 1.12). Hydroxylation at the 5-position of the indole ring of *L*-tryptophan by tryptophan hydroxylase leads to 5-hydroxy *L*-tryptophan (5-HTP), which is then decarboxylated by 5-HTP decarboxylase to give 5-HT. Occurring mainly in the pineal gland, the action of 5-HT *N*-acyl transferase and hydroxyindole *O*-methyltransferase complete the formation of melatonin.⁶⁷

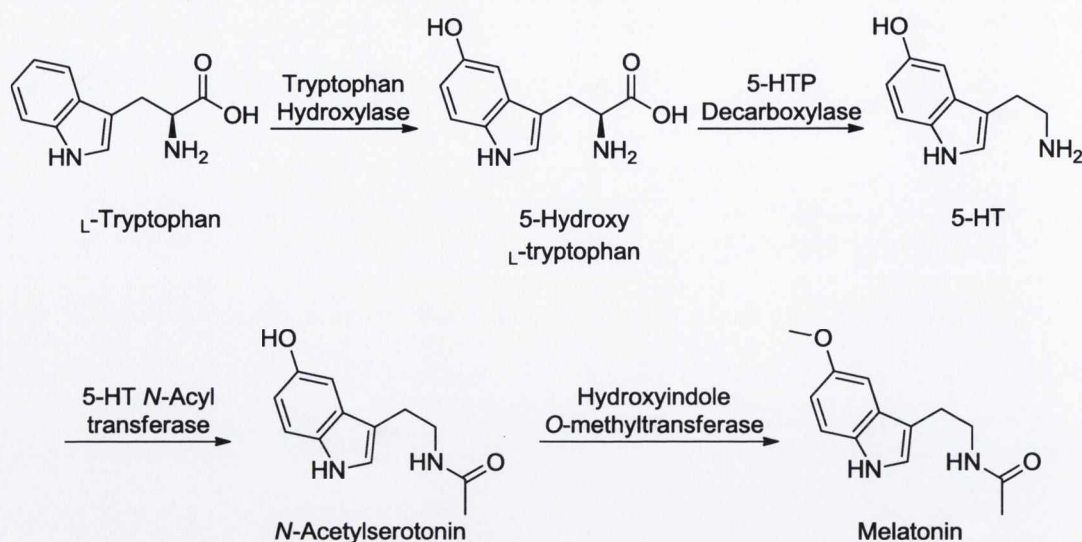


Fig. 1.12. Biosynthetic pathways of 5-HT and melatonin.

Melatonin is subsequently released from the pineal gland during darkness. Its most important role is to provide a hormonal signal of night-time darkness and to regulate circadian rhythms.⁶⁸ It acts at GPCR melatonin receptors to inhibit adenylyl cyclase, reducing cAMP production. Two such human melatonin receptors have been identified; Mel_{1A} distributed in the pituitary gland and hypothalamus, and Mel_{1B} located in the retina where it is thought to interpret the stimulus of light.⁶⁹ Research has suggested that melatonin is involved in the ageing process as circulating levels are seen to reduce with age. This is also cited as the reason for poorer quality and shorter sleep cycles in the elderly.⁷⁰ In relation to its role in mood and depression, some studies have shown reduced efficacy of the antidepressants desipramine and fluoxetine when melatonin supplements are taken simultaneously and it is likely that it plays a role in depression due to its effects on sleep cycles which are often disrupted in the disease.⁷¹

Serotonin itself is a NT with a complex range of functions, famed for its role in mood disorders and the fact that increasing its levels is the target of the antidepressant Prozac (discussed later). It is associated with a wide spectrum of physiological and behavioural responses including mood, anxiety, appetite, sexual arousal, sleep and nociception.⁷² There are seven classes of 5-HT receptor, six of which are GPCRs. Only the 5-HT₃ receptor, which is a ligand-gated ion channel receptor, does not act by a GPCR mechanism. These receptors mediate both excitatory and inhibitory neurotransmission

with different subtypes associated to each of the G_i , G_o , G_q and G_s proteins. Several 5-HT receptors have specific subtypes, 13 distinct receptors have been characterised in all. This makes providing discussion of the entire family impossible.⁷³

What is undoubted, however, is the therapeutic potential of agents which act at these receptors. By tuning the selectivity for various receptors, 5-HT ligands have found many clinical applications; agonists have been used as anxiolytics, antiemetics and appetite suppressants, while antagonists have been used as antipsychotics and antidepressants.⁷⁴ However, possibly of more importance therapeutically has been the serotonin transporter (SERT). The SERT is responsible for reabsorbing 5-HT into the pre-synaptic neuron after release into the synaptic cleft. Inhibitors of SERT, better known as selective serotonin reuptake inhibitors (SSRIs), have become the most prescribed class of antidepressants and are discussed further in Section 1.4. Degradation of 5-HT is affected by MAO and ALD-D in a similar way to NA (Fig. 1.13).²⁵

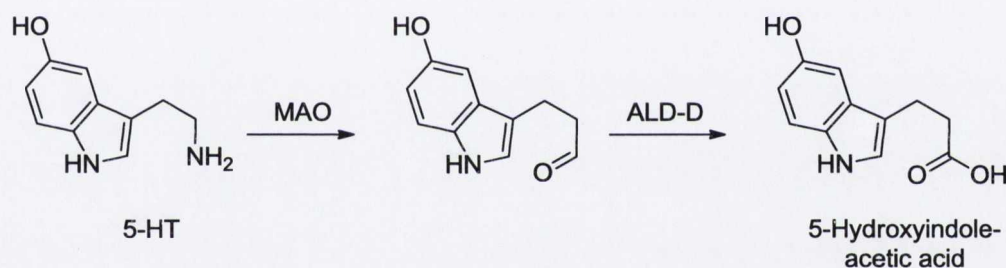


Fig. 1.13. Catabolic pathway of 5-HT.

Interestingly, melatonin exhibits a departure from this familiar pathway for metabolism (Fig. 1.14). The main path for its degradation is through hydroxylation at the 6-position of the indole ring, catalysed by several isoforms of the cytochrome P450 enzymes in the liver, followed by sulfation and excretion. An alternative pathway, also affected by the P450 enzymes, involves *O*-demethylation at the 5-position followed by either glucuronidation or sulfation to give excretable conjugates.⁷⁵ This pathway is less prevalent than the previously described route. The likely reason for this unique catabolic pathway is that melatonin is released from the pineal gland which is outside the blood brain barrier (BBB) where metabolism is regulated in a different manner.

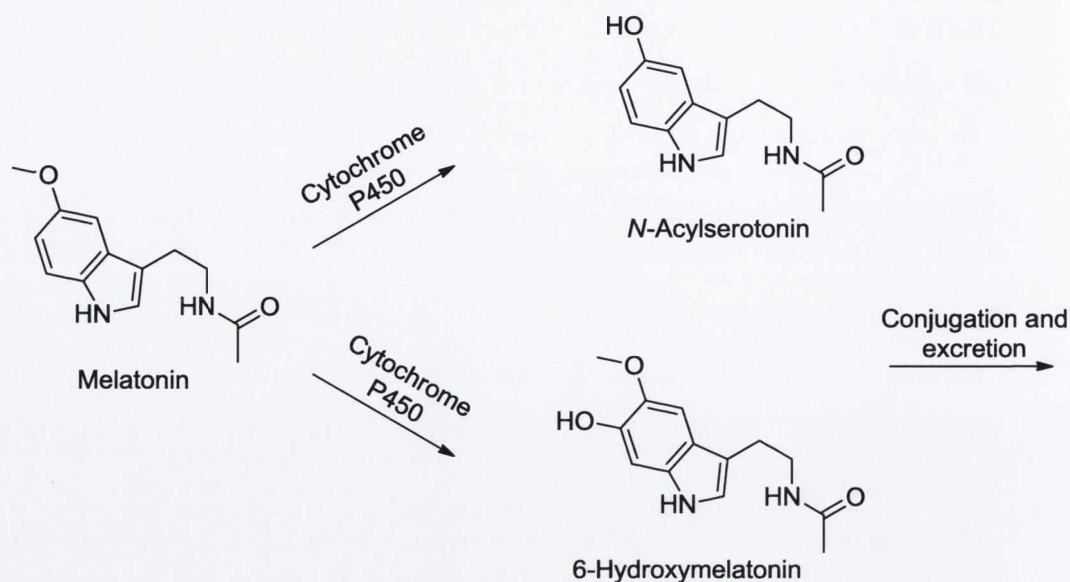


Fig. 1.14. Degradation pathway of melatonin.

1.3. Pathophysiology of Depression

In recent years the psychopathology of depression has become better understood. This is in most part due to discoveries on the role of the genetic, cellular and neuroanatomical changes that have been observed in patients of depression. Thankfully, it is now widely accepted that mood disorders are diseases of the brain and not creations of the mind as was once thought. Yet despite the advances that have been made, many problems remain regarding the effective treatment of a substantial group of depressed patients, albeit a minority.

The disease of depression is highly heritable, with roughly 40-50% of the risk factor being genetic, however the specific genes that underlie this have not yet been identified.⁷⁶ The remainder of the non-genetic risk factor also remains poorly defined, with early childhood trauma, emotional stress, physical illness, and even viral infections all being implicated. The brain regions involved are not well defined and, due to the

heterogeneity of the disease and its varied presentation, it is likely that different regions may be involved in different individuals.

Knowledge of these regions is growing, however, and theories have emerged based on functional imaging⁷⁷ and autopsy⁷⁸ studies which link the symptoms observed in depression to the areas of the brain which control such functions. In particular, abnormalities have been found in several brain regions in patients with depression compared with controls, specifically in terms of blood flow and cell densities. Thus, the hippocampus⁷⁹ and frontal cortex⁸⁰ areas are the subject of most intensive research due to their involvement in cognitive functioning. The amygdala has naturally been investigated as it is responsible for associating rewarding stimuli with the environmental events that activate them.⁸¹ Dopaminergic neurons in the nucleus accumbens trigger reward pathways and due to the prevalence of anhedonia in depression they are also a rational subject of research.⁸² Further, abnormalities in circadian rhythms, as well as insomnia, implicate the hypothalamus.⁸³

From a study of each of these distinct brain regions have come numerous theories for the pathophysiology of depression which are presented below. Each presents a convincing argument for the aetiology and potential treatment of depression; however, it must be kept in mind that the only one which has led to therapeutic application is the monoamine theory, with all current medications for the treatment of depression finding their basis in this theory.

1.3.1. The Monoamine Theory

The first theory aiming to characterise the pathophysiology of depression was formulated during the 1960s after the work of Bunney and Davis⁸⁴ and Schildkraut⁸⁵ eventually led to the catecholamine hypothesis of affective disorders. It is a theory based on clinical observations which were later rationalised. Evidence put forward in Schildkraut's seminal paper came from several observations, including the unlikely source of reserpine, an indole alkaloid that was being used for the treatment of high blood pressure. It was observed that this alkaloid depleted the levels of NA and DA in the brain, while at the same time induced depressed mood in patients. Both of these effects were reversed when treatment with the drug was stopped. It has since been

proven that reserpine acts by blocking the VMAT2 receptor which actively transports DA into storage vesicles in neurons. This explains its diminishing effects on levels of DA and NA, which is subsequently synthesised from DA in these vesicles.⁸⁶

It was also shown around the same time that treatment with the tricyclic antidepressant (TCA) imipramine and the monoamine oxidase inhibitor (MAOI) Iproniazid – both of which were being used clinically as antidepressants at the time despite no knowledge of their mode of action – increased the levels of NA in the brain. Combined with the newly elucidated biochemical pathways of NA biosynthesis and degradation, this new information on the mechanisms of action of imipramine and iproniazid led to the general belief that all effective antidepressant medications were acting by increasing the activity of either the brain's noradrenergic or dopaminergic systems. Following from this the deduction was made that depression must be caused by a depletion of these catecholamine NTs and their activity in the brain.

It was not long before the theory was challenged, with work by Carlsson suggesting a role for 5-HT in the action of TCAs. He suggested that induced improvements to mood could be attributed to blockade of 5-HT reuptake and that increased drive was the result of NA reuptake inhibition.⁸⁸ Incorporation of 5-HT into the hypothesis meant the catecholamine theory evolved to the monoamine theory of depression which remains more or less unchanged to this day. It states that neither the tryptamines nor the catecholamines are exclusively responsible for depression, and that depletion in either might bring about symptoms of the illness. In an effort to describe the varied observations in patients, some researchers have suggested (like Carlsson had) that each system is responsible for different symptomatic presentations; 5-HT for mood, NA for drive, energy and alertness, and DA for anhedonia.⁸⁹

The validity of the theory has long been debated due to several inconsistencies which have been observed. A discrepancy exists between the short time taken to alter the concentration and activity of these NTs at their receptors and the long delay involved before the onset of clinical antidepressant effects. It has also been noted that several psychotropic substances which increase the availability of NA and 5-HT, such as cocaine and amphetamine, have no antidepressant effects.⁹⁰ As such, one modern alternative version of the monoamine theory postulates that dysfunctional monoaminergic systems are not the underlying cause of depression and incorporates the

belief that antidepressants are effective because of adaptive changes to the receptors affected by the increased levels of monoamines, thus accounting for the observed delay in onset of antidepressant effects.

While still not a comprehensive description of the fundamental mechanics of depression the monoamine theory has been enormously valuable in the development of antidepressants and continues to be instrumental in the development of safer and more effective therapeutics for depression. In terms of the future direction of the monoamine theory, it is likely to concentrate on establishing the routes by which antidepressant drugs restore function to brain areas modulated by monoamine neurons, and understanding the adaptive changes that follow enhancement of synaptic levels of monoamines in neuronal circuits of regions such as the frontal cortex, amygdala, and hippocampus.⁹¹

1.3.2. The Hypothalamic-Pituitary-Adrenal (HPA) Axis, Stress and Cortisol in Depression

It is well established that stressful events can initiate a depressive episode in people who are prone to depression. The stress response is primarily coordinated *via* the highly linked hypothalamus, pituitary gland, and adrenal glands. During this response, the action of corticotrophin-releasing factor (CRF) secreted by the hypothalamus activates CRF1 and 2 receptors in limbic regions of the brain, in addition to the hypothalamus and pituitary gland. This leads to release of adrenocorticotrophic hormone (ACTH) which in turn induces the synthesis of glucocorticoids. The hypersecretion of cortisol – such a glucocorticoid (Fig. 1.15) – is a common feature of major depression.⁹²

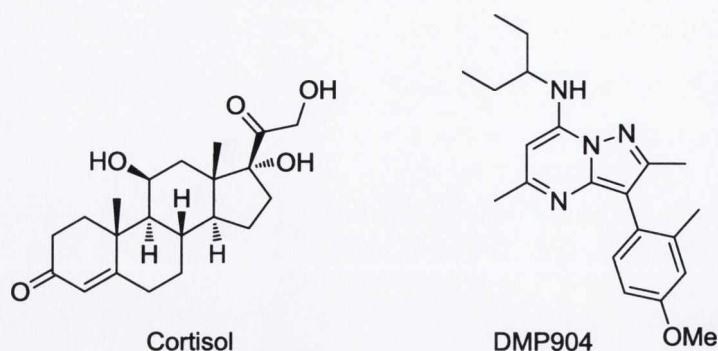


Fig. 1.15. Chemical structures of cortisol and a CRF1 receptor antagonist, which showed inconsistent results across different animal models of depression.

The implications of hypercortisolaemia have been investigated and toxicity to hippocampal neurons in such cases has been shown. These changes have been reversible by antidepressant treatments in animal models and so it has been suggested that hypercortisolaemia is the cause of reduced hippocampal volume often seen in depression.⁹³ Thus CRF antagonists, such as DMP904 (Fig. 1.15), are under investigation as new antidepressants. The CRF1 and 2 receptors are associated with the G_s protein which stimulates adenylyl cyclase to increase cAMP synthesis. However, these compounds have not shown consistent activity in standard antidepressant tests, being active in some models and not in others. They have also suffered from hepatotoxicity issues in rodent testing.⁹⁴ Selective antagonists of the CRF2 receptor continue to be researched due to their more restricted expression in the brain, which should make them less likely to have side effects mediated by the HPA axis.

1.3.3. The Neurotrophic Hypothesis of Depression

Neurotrophins are peptides which stimulate and control neurogenesis, the birth of new neurons from neural stem-cells. Brain-derived neurotrophic factor (BDNF) is a growth factor which supports the survival of existing cells and promotes the growth and differentiation of new neurons and synapses.⁹⁵ It is mainly active in the hippocampus and frontal cortex regions of the brain and is heavily involved in the reduced volume of these areas seen in depression. The neurotrophic hypothesis of depression is based on findings in rodents that acute or chronic stress decreases expression of BDNF in the

hippocampus and that antidepressant treatment produces the opposite effects and prevents the actions of stress.⁹⁶ Further, autopsy of patients with depression showed decreased expression of BDNF in the hippocampus, while deceased patients who had been undergoing antidepressant treatment at the time of death show reversal of these effects.⁹⁷

The effects of BDNF are mediated through the TrkB tyrosine kinase receptor and while agonists are an attractive target for new antidepressants, they are not easily attained as BDNF is a 14 kDa protein which binds the receptor as a dimer. Thus, a strategy that offers great hope is indirect modification of its expression rather than mimicking of its effects. Recently, ketamine has been shown to have rapid antidepressant effects. Ketamine is a NMDA receptor antagonist but these effects are attributed to regulation of synaptogenesis and neuronal plasticity mediated by BDNF. Ketamine is a drug of abuse with severe neurotoxicity, particularly on GABAergic neurons; however, selective NMDA antagonists are being investigated as potential antidepressants.^{98,99}

1.3.4. Phosphodiesterase Inhibitors – Neurokinin Theory of Depression

The principle function of phosphodiesterases (PDE) is to catalyse the breakdown of cAMP and cGMP. For this reason, antagonists of the PDEs have been investigated for decades, as a way to increase the release of NTs into the synaptic cleft (through increased cAMP in neurons), following the monoamine theory.¹⁰⁰ What makes them attractive targets in the search for new antidepressants is that inhibitors of PDE also induce BDNF expression in the hippocampus. The pathway involves cAMP activating the transcription factor cAMP-responsive-element-binding protein (CREB) which goes on to induce transcription of the *bdnf* gene by binding its promoter.¹⁰¹ Thus, they have the effect of increasing BDNF activity in the hippocampus, but are themselves small molecules. Obstacles exist in that inhibition of PDE in the brainstem causes vomiting but, due to their high potential, intensive research is on-going to obtain PDE subtype selectivity that might overcome this.

1.3.5. Neuroplasticity Hypothesis of Depression

Many of the above theories can be tied together by their common effects on the neuroplasticity of the brain, and in particular of the hippocampus. In an effort to find more fundamental mechanisms underlying the pathophysiology of depression, attention has moved away from the monoamine concept towards a description of the effects of stress (as an inducer of depression) and antidepressant treatment (which reverses these effects) on the brain. Histological changes to regions such as the hippocampus, prefrontal cortex (PFC) and amygdala in particular are seen to coincide with onset of depression, and also to be reversed with chronic antidepressant treatment. Observations on these changes have culminated in the neuroplasticity theory of depression.

The main reason for adopting this theory is the fact that antidepressant drugs influence monoamine concentrations as soon as they are absorbed into the body and transported across the BBB; however, no improvements to mood are observed for several weeks, which is when these histological changes begin to take place. Thus, the theory centres on stress as the inducer of depression and attributes the benefits of antidepressants to downstream effects on the systems involved in mediating the response to this stress and the histological changes to brain regions.

The structural changes observed in the depressed brain are mainly in the hippocampus, the PFC and the amygdala. Kempermann was among the first to propose that a failure of adult hippocampal neurogenesis and resultant loss in synaptic connections was the biological and cellular basis of major depression.¹⁰² This explained the observed reduction in volume and was also corroborated by the fact that antidepressant treatments induced resumption of neurogenesis in the hippocampus. As the hippocampus has projections to the PFC and the amygdala, the changes in their histology can also be rationalised.

Atrophy is also observed in the PFC, which coordinates with the hippocampus in the regulation of explicit memory (*i.e.* consciously acquired memory) supporting the fact that stressful life experiences can make individuals more prone to depression. It is also involved in cognitive functions such as attention and memory, seen to be dysfunctional in depression. One striking contradiction is that the amygdala, involved with social and emotional learning, particularly anxiety and fear, is increased in volume in many cases of depression. Furthermore, new synapses are observed in the amygdala.¹⁰³ Tentative

justifications claim that this explains the tendency of depressed people to overreact to stressful situations, though this is mainly speculation and the pathways that lead to increased volume of the amygdala in depression are not well understood.

An important point noted by Kempermann in his paper is that the function of new born neurons in the hippocampus is still not known and that this will need to be elucidated for the advancement of the theory. An interesting animal study demonstrated that the beneficial effects of antidepressant administration, electroconvulsive therapy, and exercise were negated by the absence of TrkB, the receptor for BDNF. Thus, it seems that increased levels of hippocampal neurogenesis are fundamental in both the aetiology and treatment of depression and that modulation of the pathways involved could lead to improved therapeutics.¹⁰⁴

A fascinating enigma that further lends credence to the neuroplasticity theory of depression is tianeptine (Fig. 1.16), indicated for the treatment of major depressive disorder. Tianeptine has structural similarities to the TCAs but has distinct pharmacological actions. It is classed as a selective serotonin reuptake enhancer (SSRE), completely opposite to the SSRI class of antidepressants (in fact co-administration has demonstrated cancellative effects).¹⁰⁵ Its main effects are on glutamatergic systems, an essential participant in many forms of adaptive plasticity, including learning and memory. The actions of tianeptine on the glutamatergic system have yet to be elucidated though it is sure that they could offer new insights into how this compound may be useful in the treatment of depressive disorders and serve to advance the belief that antidepressants act by modulating plasticity as it has depletive effects on serotonin levels.¹⁰⁶

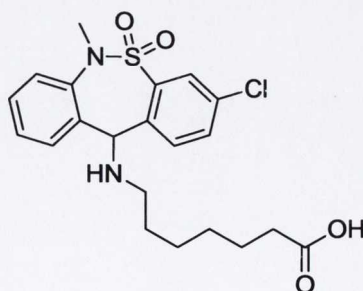


Fig. 1.16. Structure of tianeptine, a clinical antidepressant that lowers 5-HT levels.

Is there a common factor in the effects of conventional antidepressant treatment, tianeptine and the several theories of depression discussed above? It has been attempted to link these pathways and CREB-P (phosphorylated CREB) is the protein that appears to be common point (Fig. 1.17). Antidepressants are known to increase the activity of NA, 5-HT and glutamate at their respective receptors; activation of GPCRs of NA and 5-HT leads to increased cAMP, which activates protein kinase A (PKA), eventually phosphorylating CREB. Increases in the levels of glutamate are observed after antidepressant treatment as well; glutamate acts at ionotropic NMDA receptors to increase Ca^{2+} levels and activate calcium-dependent kinases (CAM), which can also activate CREB by phosphorylation at Ser¹³³. Similarly, increased expression of BDNF on antidepressant treatment culminates in activation of CREB to CREB-P; BDNF action at TrkB receptors activates the Ras and MAPK pathways which initiate CREB kinase to phosphorylate CREB.

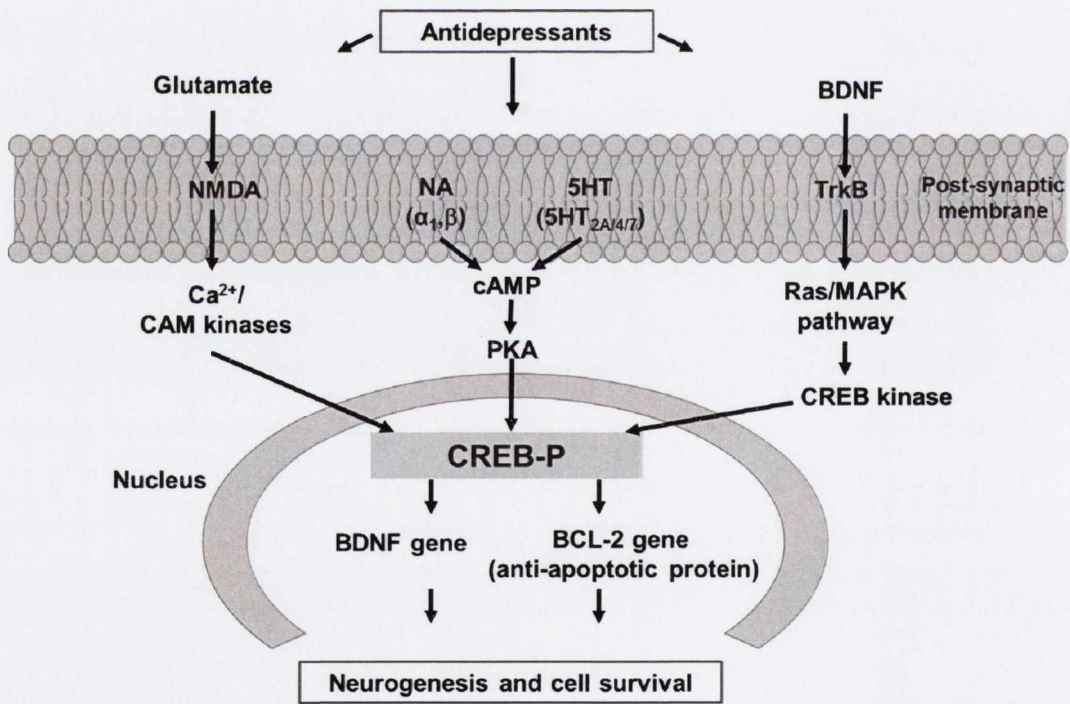


Fig. 1.17. Pathways linking antidepressant treatment with neuroplasticity (adapted).¹⁰⁷

Phosphorylated CREB then binds to DNA to upregulate the expression of the BDNF gene and the gene that encodes for the anti-apoptotic protein B-cell lymphoma 2 (BCL-2). The resulting BDNF-promoted neuroplasticity, coupled with reduced apoptosis from

BCL-2, give rise to increased neurogenesis.¹⁰⁷ It seems that antidepressant drugs indirectly target these pathways and that more directed agents could lead to more efficacious antidepressants.

1.3.6. The Cytokine Theory of Depression

The neuroplasticity theory of depression has been a significant advancement in characterising the disease; however, it is based on observations of the changes that occur on development of the disease and does not offer any explanation on a cause for these changes occurring that can be linked to either genetic or external stimuli. One aspect that is lacking is a link between depression and the comorbidities and subsequent premature mortalities that are associated with depression.

Cytokines (*e.g.* interleukons and interferons) are immunomodulatory signalling molecules that have been known to affect psychological function since 1927 when Julius Wagner-Jauregg discovered that activation of the immune response by an infection affects psychiatric functioning. Subsequently, pro-inflammatory cytokine concentrations were shown to be increased in patients with depression, amounts being approximately proportional to the severity of depression.¹⁰⁸ The similarities between sickness-behaviour and depression are thought to result from an over-production of endogenous proinflammatory cytokines and a dysregulation of the HPA axis.

Both stress and depression are associated with a reduction in immune system function. Thus, it is proposed that hypersecretion of cytokines is responsible for the onset of depression.¹⁰⁹ Depression is widely observed to occur in diseases which either reduce immune function or have treatments that do so; patients of hepatitis C, inflammatory conditions and autoimmune disorders (*e.g.* lupus and multiple sclerosis), as well as those taking cytokine-based therapies (*e.g.* interferon α for hepatitis C) all have high incidences of depression.¹¹⁰

Cytokines in the CNS have functions such as neuroprotection and neurodegeneration and can be regulated by NTs and hormones. Cytokines from the periphery have more access to the brain in times of stress when the BBB loses integrity. The resulting increased presence of cytokines in the CNS is thought to induce depressive state through signalling cascade modification, and has even been implicated in deterring

neuroplasticity.¹¹¹ The chronic effects of increased cytokine production are decreased neuroexcitation and NT activity, leading to a long-term decrease in monoaminergic neurotransmission, decreased neurogenesis and an increase in neural apoptosis.¹¹²

The use of anti-inflammatory approaches for the treatment of depression is being examined at both pre-clinical and clinical levels. Many current antidepressant medications have specific anti-inflammatory effects and significant immunoregulatory activities, such as reducing the number of T cells secreting interferon- γ (IFN- γ).¹¹³ For example co-treatment of fluoxetine with celecoxib, an anti-inflammatory cyclooxygenase (COX) inhibitor showed improved antidepressant activity compared with fluoxetine alone, during animal studies. However, the opposite effects of anti-inflammatories decreasing antidepressant effects have also been observed.¹¹⁰ This indicates that, despite the undoubted associations between depression and cytokines and the possibility that immune responses are the initiator of depression, the processes involved are not yet sufficiently understood to lead to antidepressant therapeutics acting on these pathways.

1.3.7. Thoughts on the Theories of Depression

It is difficult to marry the opposing schools of thought on depression; however, some assertions can be made. It is clear that conventional antidepressants confer improvements to patients' mood. It is also clear that they act at receptors to increase the availability of the monoamine NTs; however, the complexity of the circuits in the brain is staggering and this cannot be attributed as the sole source of antidepressant effects. It is known that there are about 100 billion neurons in the human brain. Each neuron synapses with an average of about 1000 other neurons, making about 100 trillion synapses in the brain.¹¹⁴ Thus, it is no surprise that modulations to one region or system of the brain can lead to adaptive changes in another.

Given that depression is a heterogeneous condition with symptoms varying widely from one patient to another, it is likely that its aetiology is similarly variable and that the underlying biology might differ from patient to patient. Ideally, a diagnosis of depression would be supported not only by psychiatric rating scales, but also by biological factors that would allow for its more effective treatment, as is the case with

other medical conditions such as diabetes and hypertension. The variable presentation of the disease is the likely reason that a large group of patients show no improvements on treatment with current antidepressants. It has been suggested that patients should be treated differently depending on the principal symptoms; for example, patients presenting with anxiety should be prescribed 5-HT augmenting medication, a patient with lack of drive should be treated with drugs acting on the NA system and patients with anhedonia should be given treatment attenuating DA activity.¹¹⁵

Something which must be called into question is the relevance of animal models for depression. For example, sickness behaviour can be modelled in animals, but it is next to impossible to model the higher cognitive functions that are involved in depression. Due to the raft of chemicals which have shown promise in pre-clinical studies only to fail on introduction to humans, either for lack of activity or adverse side-effects, the translational approach of bridging the gaps between the two systems has aimed at addressing the problem. Under this approach increased investigation is carried out on what has gone wrong in failed attempts at attaining the same activity in humans as has been obtained in animals, so that models can be improved in the future.¹¹⁶ A general framework for assessing the validity of animal models of psychiatric disorders is also proposed.

1.4. Managing Depression

There are three broad categories of methods to manage MDD. Psychotherapy and psychiatric medication are by far the most common and can both be administered to outpatients. Only in severe cases in which patients are considered to be a serious risk to themselves, are they admitted to hospital where the third option, electroconvulsive therapy, can be considered. Psychiatric medication is the primary therapy for MDD, but should always be considered alongside psychotherapy and, thus, adjunctive therapy involving both types of treatment is considered to be the optimum course of action, with faster remission being observed compared with each therapy alone.¹¹⁷

Behavioural Approaches to Treatment

The Cognitive Model of Depression is an attractive theory for understanding the development of the disease. Its view that dysfunctional thought processes are created by early life experiences and critical events correlates well with the troubled history associated with many people who develop depression and there is no doubt that therapies based on cognition are valuable in the treatment of the disease, whether alone or in combination with drug therapy.

Behavioural activation (BA) and Cognitive Behavioural Therapy (CBT) are psychological strategies for the treatment of depression based on altering the cognition of patients. Cognition encompasses the group of mental processes including attention, memory and decision making, and this approach posits that depression has its roots in negative cognitions. Aaron Beck, the renowned American psychiatrist and developer of many indices for the diagnosis of depression as well as the pioneer of cognitive therapy, attributed depression to three cognitive states which he termed as the negative cognitive triad; a negative self-view, a negative view of the world and a negative view of the future.¹¹⁸ These core outlooks cause situations to be interpreted in a skewed manner; meaning problems are often seen as one's own fault and as permanent. Such thought processes also serve to exacerbate depression through avoidant behaviour and inability to express feelings or seek help.

The goal of BA is for patients to increase positive environmental reinforcement and reduce punishment. The treatment systematically increases exposure to positive activities by creating a bank of positive statements of varying degrees of significance, thereby helping to alleviate depressive effects.¹¹⁹ A recent study suggests that it can be as effective as drug therapy in the acute treatment of adults with depression¹²⁰ and, while this remains to be seen, it underlines the potential benefits of this type of treatment, particularly in the early stages of the disease.

A similar approach is undertaken in CBT which addresses negative cognition and the way in which people react to situations. Beck believed that the cognitive symptoms of depression actually precede the affective and mood symptoms of depression, rather than the opposite. Thus, the premise of the treatment is that changing negative thinking leads to improvements in behaviour and mood. Therapists help patients to reassess issues

such as magnifying negatives, minimising positives and over-generalising, and replace them with more realistic thoughts and better coping mechanisms.¹¹⁸

These psychotherapeutic approaches to the treatment of depression are preferred where psychiatric medication is not appropriate, such as for children under the age of 18 and individuals sensitive to medication.

Electroconvulsive Therapy

Electroconvulsive therapy (ECT) is a treatment in which either anaesthetised or sedated patients are administered electrical current to induce seizures which result in therapeutic effects. It is generally only considered for patients of MDD who have shown no response to either psychotherapy or psychiatric medication. Treatment with antidepressant medications is generally continued during and after the treatment – this is the practise in the United Kingdom and Ireland according to the Royal College of Psychiatrists.¹²¹ While the clinical benefits of ECT cannot be denied, the severities of the treatment, as well as the risk of cognitive (memory-loss and confusion) and physiological (heart-attack and stroke) side effects induced by seizure, make it a therapy that is generally only used when both antidepressant or psychiatric treatments have failed.

The treatment has a negative image going back to its overuse in the 1940s when it was one of the only known treatments for psychiatric disorders. There is no doubt however that, when used correctly and with proper follow-up, ECT offers the potential for improved quality of life to patients of mania and depression. The exact mechanism of the therapeutic effects remains unknown. A recent study has reviewed the accuracy of three proposed mechanisms; the normalisation of neuroendocrine dysfunction, increased hippocampal neurogenesis and benefit from generalised seizures.¹²² It also attributes cognitive side effects to sub-convulsive seizures in the brain.

The development of magnetic seizure therapy (MST) over the past decade has attempted to devise a therapy with comparable efficacy to ECT, but without the associated cognitive side effects. The strategy for this is that MST uses magnetic fields to induce seizures only in the cortex, without electrical stimulation of brain structures

involved with memory. MST has been used successfully in the treatment of depression in pilot studies but uptake of the method is slow and further development is required.¹²³

Drug Therapeutics

The use of antidepressants has been the most common method of managing depression since their emergence in the 1950s. In most cases they are the first port of call for a patient presenting with depression. Prescription of antidepressants has increased drastically since the release of the SSRIs in the early 1990s; however, the efficacy of current antidepressants is the subject of much debate. Remission rates are low for depression sufferers being treated with antidepressants. Only approximately 33% of patients show full remission from the disease, with a further 33% described as responding partially to treatment while maintaining significant retention of residual symptoms. The remainder of patients show minimal improvements¹²⁴ and this is the case even when optimal treatment of concurrent psychotherapy is considered.

The benefits of antidepressants far outweigh the drawbacks however. Modern drugs have much improved safety profiles than the original treatments and have enhanced the quality of life and outlook for countless patients of depression. The prospect of more efficacious therapeutics with similar safety profiles is a driving factor for much research as it is seen as the most likely route to successfully tackling the disease.

History and Development of Antidepressants

Prior to the discovery and marketing of the first antidepressants, patients with MDD or manic-depression (now known as bipolar disorder) were treated with stimulants such as amphetamines due to their ability to alleviate the symptoms of anhedonia in patients. Herbal remedies were also widely used, particularly St. John's Wort which was even cited by Hippocrates in ancient Greek times as a cure for "demonic possession".

As previously described ECT began to be employed in the 1930s for the treatment of patients with depression and dementia. Not only was it effective in improving motivation and mood in patients, it was also an important step in the development of

antidepressants as it provided evidence that mood could be influenced by external stimuli and gave weight to the neurological basis of depression.¹²⁵

The first antidepressant, iproniazid (Fig. 1.18), was serendipitously discovered while being used to treat tuberculosis. It was proven to be ineffective in treating tuberculosis, but it induced long spells of elevated mood in its patients. However, iproniazid was still considered a stimulant as it had similar side-effects to amphetamines, including insomnia, agitation, hyperactivity and paranoia,¹²⁶ and it was eventually removed from the market owing to this and its hepatic toxicity. Kline *et al.* ultimately characterised iproniazid as a MAOI (*vide infra*) and opened the door to more powerful and specific antidepressants of this type.

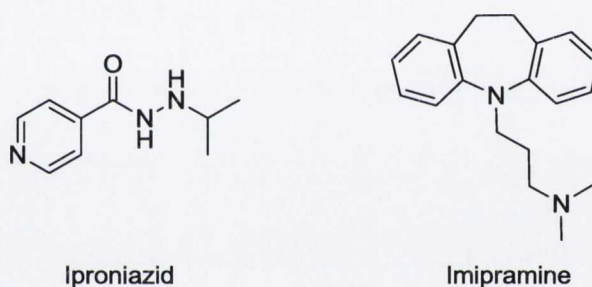


Fig. 1.18. Early antidepressants, iproniazid (*left*) and imipramine (*right*).

Around the same time another antidepressant was serendipitously discovered by Ronald Kuhn while seeking a treatment for schizophrenia at the Münsterlingen asylum in Switzerland. Imipramine (Fig. 1.18), a tricyclic compound, was shown to lack stimulant effects yet induced improved mood in patients with depression. Though Kuhn made many unsubstantiated claims (including suddenly curing patients who had been depressed for several years and converting a homosexual man to heterosexuality) his work was among the first to suggest that exogenous compounds could reverse the biochemical pathways of depression. He observed that cessation of treatment caused a depressive mood to return and that mania could be induced in healthy individuals on treatment with imipramine.¹²⁷

Imipramine was in the clinic in both Europe and the U.S.A. by the end of 1959. Through a series of modifications to its structure, it gave rise to amitriptyline and nortriptyline (Fig. 1.19) which were approved for use in 1961 and 1963 respectively, both before their mode of action was known. Further observations on their effects in the brain, and on the mode of action of other agents like reserpine, led to the first theory on the pathophysiology of depression, the monoamine theory described earlier.

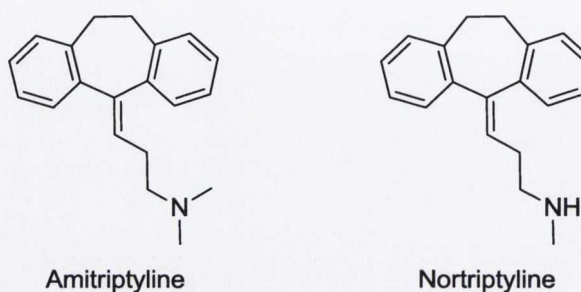


Fig. 1.19. The structures of amitriptyline (*left*) and nortriptyline (*right*) which arose from the success of imipramine.

While these TCAs gave relief from the symptoms of depression to many, they were also associated with several severe side-effects, including sluggishness, weight gain and occasionally death from overdose. Because of this, they often came with very strict administration and dosage regimes – a situation which is less than ideal for patients suffering from low motivation and self-worth. There were several reports of cardiovascular¹²⁸ as well as anticholinergic¹²⁹ side effects incurred by patients being treated with TCAs, while psychiatric effects were also reported. However, this did not stop them being used in the treatment of depression, and it was not until 1987 that a new class of antidepressants with improved safety profile reached the market.

Fluoxetine hydrochloride (Fig. 1.20), marketed as Prozac by Eli Lilly, was the first marketed SSRI. It provided relief for the same percentage of patients as its predecessors did, but was easier to prescribe without risking overdose and had fewer side effects. Because of this, Prozac was exceptionally successful; less than five years after its launch its annual sales had topped \$1 billion and it was estimated that 10 million different people worldwide had taken the drug.¹³⁰ The popularity of Prozac also meant that it was over-prescribed, often to people who were not in need of its effects, and it

has been suggested that this damaged the reputation of antidepressants. A study in the Archives of General Psychiatry found that patients were turned off seeking attention for their depression based on scepticism over the effectiveness of antidepressants, and a reluctance to take them.¹³¹ Prozac did, however, go some way to removing the stigma attached with depression and led to the disease being discussed more openly in society.

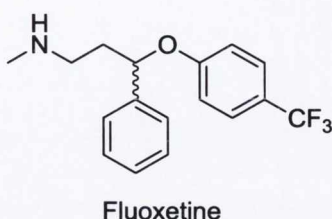


Fig. 1.20. Fluoxetine hydrochloride (Prozac) was the first SSRI antidepressant.

The antidepressant effects of Prozac are attributed to blocking SERT. This has the effect of increasing the extracellular concentration of 5-HT and, in turn, its activity at post-synaptic receptors. It has also been shown to be a potent antagonist of the post-synaptically located 5-HT_{2C} receptor which could be the source of antidepressant effects as activation of this receptor inhibits DA and NA release in several regions of the brain including the PFC, the hippocampus, the hypothalamus and the amygdala.¹³²

Prozac is not without its drawbacks however, and the most serious of these is without doubt the reported increase in suicidal behaviour during the first weeks of treatment with the drug. This has become such a concern that the Food and Drug Administration (FDA) now requires manufacturers of all antidepressants to include a warning termed the “black box” warning about the “increased risks of suicidal thinking and behaviour, known as suicidality, in young adults ages 18 to 24 during initial treatment” on the packaging of their drugs.¹³³

In the years after the introduction of Prozac several, SSRIs were released including sertraline, paroxetine and citalopram (Fig. 1.21). The SSRIs are still hugely popular and with the patents of many expired, several generics are now available.

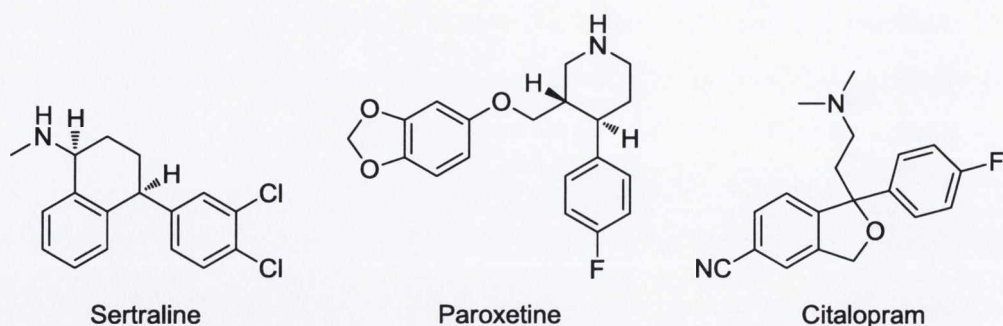


Fig. 1.21. Chemical structures of SSRIs marketed as Zoloft, Paxil and Celexa respectively (*left to right*).

During the 1990s a separate class of antidepressants was also introduced; the serotonin noradrenaline reuptake inhibitors (SNRIs). The theory behind this class of antidepressants was that they could maintain a similar safety profile to the SSRIs but recover some of the assumed loss in activity that went with only targeting the 5-HT system, compared to the previous TCAs which acted on both NA and 5-HT systems. This led to the release of venlafaxine and nefazodone (Fig. 1.22) in 1993 and 1994 respectively, which both act by blocking SERT and NET and are considered to be slightly more potent than the SSRIs.¹³⁴ It must be noted however that these drugs act by the same mechanism as the original TCAs, being more desirable only due to their reduced side-effect profiles.

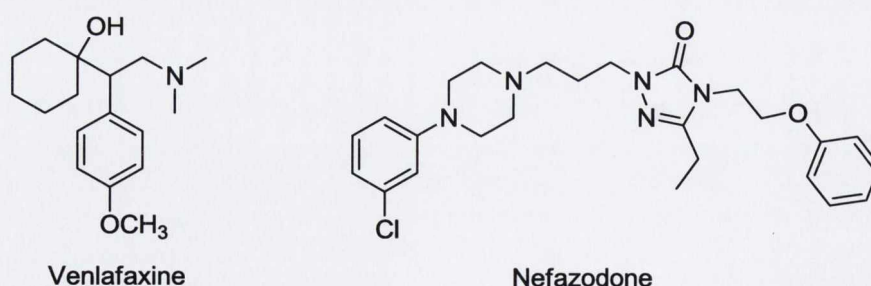


Fig. 1.22. Structures of SNRIs, venlafaxine (Effexor) and nefazodone (Serzone).

The popularity of the SNRIs meant that in 2009 Effexor and Serzone were the two top-selling antidepressants, with Escitalopram (the *S*-enantiomer of the SSRI Citalopram, Fig. 1.21) in third place.¹³⁵ In recent years, however, nine of the ten patented

antidepressants have reached the end of their patents and, with no new compounds emerging, the industry has turned to indicating older drugs for treating depression, as well as using combination therapies to augment pharmacological profiles of existing drugs. For example mirtazapine (discussed later) has been used in the treatment of depression since 1990 and is now being used in a combination therapy with the DA receptor agonist aripiprazole (Fig. 1.22) which is also indicated for the treatment of bipolar disorder, autism, and schizophrenia.¹³⁶

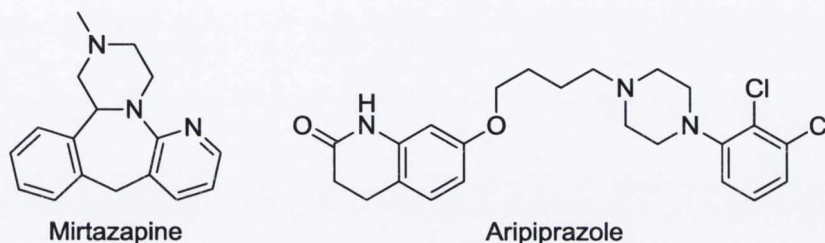


Fig. 1.22. Mirtazapine and aripiprazole are used in a combination therapy to treat depression, having both previously been used independently.

One illustrative fact of the progress made in antidepressant treatments since the 1950s is that several members of the original TCA and MAOI classes of antidepressants are still licensed for use today and prescribed for the treatment of the disease, despite their known limitations and serious side-effects.¹³⁷ Neither can it be ignored that the problem of slow onset of effects of currently available antidepressants is yet to be addressed and is almost seen as acceptable. Developments in the understanding of the processes and regions of the brain involved in depression have provided new targets which offer the hope that this problem could be overcome. One of the most promising of these is the α_2 -AR, the subject of this research project.

1.5. The α_2 Adrenoceptor, Noradrenaline and Depression

1.5.1. The Noradrenergic System in Depression

Disruptions of attention, memory, and sleep are often seen in major depression. These processes are regulated by the noradrenergic system (see Section 1.2.1.4) and are activated by stress; long term stress in particular is known to deplete levels of NA.¹³⁸ This has led to the belief that abnormal noradrenergic function could be responsible, at least in part, for the development of depression.

The depletion in NA levels is supported by several findings including that tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of NA, is up-regulated in the brains of victims of suicide.¹³⁹ Increased levels of this enzyme are consistent with low levels of NA and an effort to restore them. The fact that the NET protein has been found down-regulated in similar studies also suggests that NA is depleted extracellularly in cases of depression.¹⁴⁰

It follows that effects should be seen in the expression of the receptors for NA in cases of depression. Conflicting data exists for the β -ARs, though it is generally accepted that alterations to their densities are associated with schizophrenia more than with depression, a decrease in β_1 -ARs in the hippocampus in particular being observed.¹⁴¹ More significant, the α_2 -AR has consistently been found deregulated in cases of depression.

1.5.2. The α_2 -Adrenoceptor – Structure and Function

The α_2 -AR is a GPCR associated with the G_i protein. It is a member of the superfamily of rhodopsin-like G-protein coupled receptors and thus it has the familiar structural motif for this type of receptor, consisting of seven transmembrane α -helices, an intracellular domain and a ligand-binding domain (Fig. 1.23).

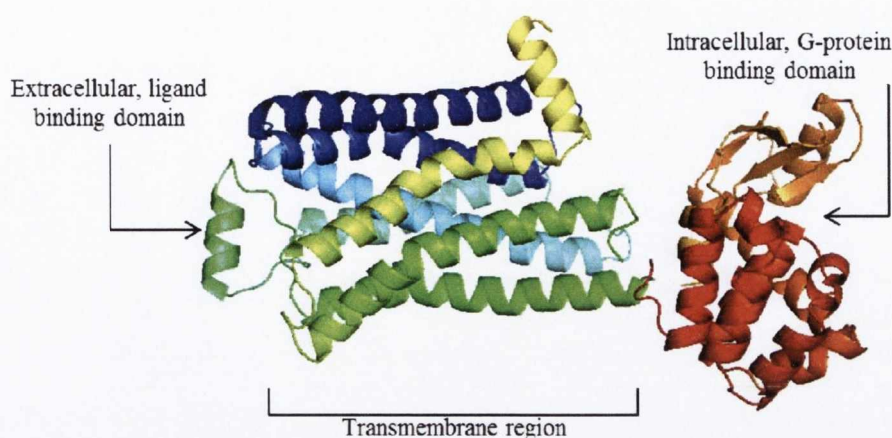


Fig. 1.23. General structure of GPCR transmembrane proteins.

At rest, the intracellular domain is associated with a heterotrimeric G-protein complex consisting of α , β and γ -subunits, the α -subunit being reversibly bound to guanosine diphosphate (GDP) (**1**, Fig. 1.24). The α_2 -AR is activated on binding of the endogenous ligand NA (**2**); binding induces a conformational change in the receptor and subsequent exchange of GDP with guanosine tri-phosphate (GTP) on the- α subunit. At this point the G-protein subunits dissociate from the receptor (**3**) as a GTP bound α -subunit and a β , γ -dimer that remains associated with the membrane. Both of these species can modulate the activity of intracellular proteins and once this has occurred two mechanisms act to return the receptor to its resting state (**4**). Firstly, the α -subunit has intrinsic GTP hydrolysis activity which can reinstate the GDP bound state and cause re-assembly of the three subunits at the intracellular domain and deactivation of the system. Secondly, many of the effector proteins acted on by the active α -subunit (including adenylyl cyclase) also have GTP-ase activity which can return the GPCR to its resting state.

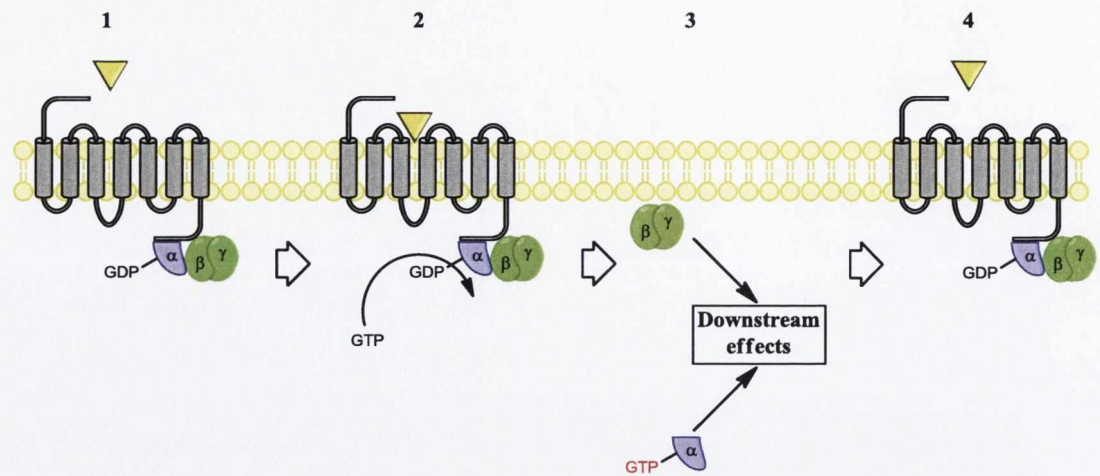


Fig. 1.24. General mechanism of action of GPCRs such as the α_2 -AR. The activating ligand NA is represented by the yellow triangle.

In the case of the α_2 -AR the α -subunit is the G_i protein which inhibits adenyl cyclase, the enzyme responsible for converting ATP to cAMP. This means that Protein Kinase A cannot be activated and, in the periphery, the downstream effect is decreased glycogen breakdown. When the α_2 -AR is activated in neurons it has the effect of activating the negative feedback loop that occurs during NA neurotransmission, preventing subsequent action potentials from releasing NA into the synaptic cleft (Fig. 1.25). This occurs again through the action of the G_i protein inhibiting adenyl cyclase, and shutting down the synthesis of cAMP from ATP. During an action potential cAMP is required to open the cyclic nucleotide-gated ion channels (CNGs) through which Ca^{2+} ions enter. These Ca^{2+} ions are required to activate calmodulin (Cal) which induces fusion and exocytosis of NA-containing vesicles with the membrane and subsequent release of NA into the synaptic cleft. Thus, the net effect of activation of the α_2 -AR and its signalling cascade is to prevent NA release during action potentials (Fig. 1.25).

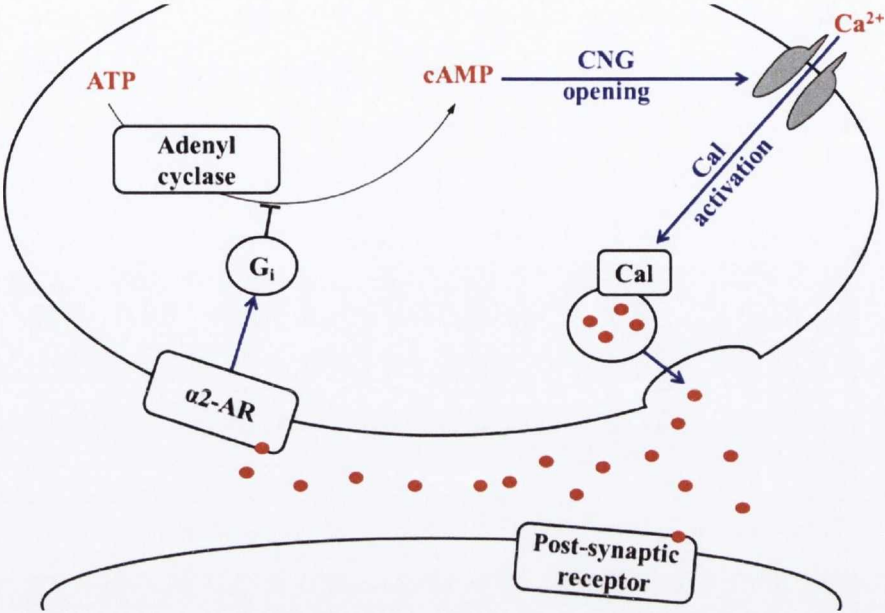


Fig. 1.25. Signalling cascade associated with the α_2 -AR. Red dots indicate NA, which starts the process by activating the α_2 -AR.

Due to its depletive effects on NA levels in the synaptic cleft, the α_2 -AR has been considered to play an important role in the pathophysiology of depression. The α_2 -ARs are divided into three subtypes in humans; α_{2A} , α_{2B} and α_{2C} . The distinct pharmacological roles of these subtypes are not well defined due to the high degree of homology between them and the lack of highly subtype-selective ligands.¹⁴² Knowledge of the role of the different subtypes in the brain and in behavioural disorders comes from their localisation in different regions of the brain, as well as alterations to their expression in depressed brains. For example, the α_{2A} -AR is closely associated with noradrenergic neurotransmission in the PFC and is also localised in the hippocampus. Several findings have led to the belief that the α_{2A} -AR is fundamentally involved in the pathophysiology of depression. While the functions of the α_{2B} -AR are not fully understood, it is thought to be less significant than the other subtypes as it is the least prevalent. Reports have suggested that it has a role in the sedative effects of α -AR agonist anti-hypertensives such as Clonidine (Fig. 1.10).

A selective increase in α_2 -AR expression has been observed in the brains of depressed suicide victims both in the PFC and hippocampus.¹⁴³ Furthermore, a study by our collaborators at the University of the Basque Country found that this increase was most

pronounced for the α_{2A} -AR subtype, with little change being observed in the α_{2C} -AR subtype. The same study also revealed that depressed suicide victims had a larger proportion of receptors in a high-affinity conformation for agonists in the PFC than controls (61% vs. 39%). This was shown using competition binding assays of the α_2 -AR antagonist radioligand [3 H]RX821002 and adrenaline.¹⁴⁴

This implicates the α_2 -AR in depression for several reasons. Its primary role is to act as an autoreceptor, activating the negative feedback loop during NA neurotransmission, preventing subsequent action potentials from releasing NA into the synaptic cleft and reducing the activity of NA at postsynaptic receptors. Over-expression and hypersensitivity of the α_2 -AR have been seen in patients with depression, suggesting that deregulation of the α_2 -AR exacerbates the effects of depression and could be the reason for the onset of the disease.

Several other factors lend credence to the idea that the α_2 -AR has a large part to play in depression, and since its hyperactivity is a problem, antagonists are of huge interest. Encouragingly, antagonists of the α_2 -AR have been shown to result in increases to levels of NA, 5-HT and DA in the synapse.¹⁴⁵ It has been posited that activation of post-synaptic α_2 -ARs by NA is required for the action of antidepressants; however, the functions of these receptors are unknown as yet and further evidence is required.¹⁴⁶

It has recently come to light that not only is NA involved in the potentiation of stress, it also has an influence on plasticity in areas of the brain where neurogenesis persists in the adult, notably the dentate gyrus of the hippocampus. Furthermore, the α_2 -AR, a regulator of NA activity, has been implicated in causing decreased cell proliferation in the hippocampus.¹⁴⁷ Not surprisingly antagonists of the α_2 -AR can reverse this, as was shown *in vivo* using dexefaroxan (Fig. 1.26).¹⁴⁸ Significantly, a concomitant increase in BDNF – seen as a key promoter of neurogenesis (see Fig. 1.17) – was also observed, indicating that the α_2 -AR is directly involved in the regulation of neurogenesis.¹⁴⁸ A simple postulation of the mechanism of this is that antagonists of the α_2 -AR increase levels of cAMP, leading to increased activation of protein kinase A. This kinase is known to act on CREB, activating it to CREB-P, which has the downstream effect of increased neurogenesis (see Fig. 1.17).

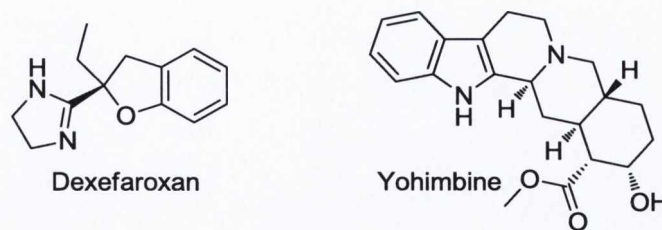


Fig. 1.26. Dexefaroxan and yohimbine are α_2 -AR antagonists used in research but not therapeutically.

However, the most significant finding in this area offers an explanation for the slow onset of effects of antidepressants. What was demonstrated in a recent study¹⁴⁹ was that agonists of the α_2 -AR decrease hippocampal neurogenesis through effects on the proliferation of progenitor cells. These effects were replicated in dopamine β -hydroxylase knockout mice (thus lacking NA) indicating that the effect could be mediated through α_2 -ARs expressed in the progenitor cells instead of the autoreceptors which switch off NA release. In the same study, it was shown that co-administration of the α_2 -AR antagonist yohimbine (Fig. 1.26) with the antidepressant imipramine (a TCA) significantly accelerates hippocampal progenitor proliferation and increases in BDNF expression.

The real therapeutic potential for α_2 -AR antagonists was demonstrated using animal behavioural models of depression where co-treatment of yohimbine and imipramine was three times faster at inducing behavioural improvements than treatment with imipramine alone.¹⁴⁹ This offers the hope that the greatest drawback with current antidepressants, which has led to their packaging displaying a black box warning of possible suicidal thoughts, could be overcome using α_2 -AR antagonists and that faster acting antidepressants could be developed.

1.5.3. α_2 -Adrenoceptor Antagonists

In the 1960s a search for tetracyclic analogues of the TCAs led to Mianserin (Fig. 1.27) being discovered as a compound with antidepressant effects, yet unknown mechanism. It was later discovered to be a potent antagonist of the α_2 -AR and its antidepressant effects are attributed to this, though Mianserin also displays some inhibition of NET.¹⁵⁰

It showed promising results, outperforming the classic TCAs imipramine and amitriptyline which were widely used at the time. However, it was shown to cause, among other side effects, agranulocytosis (depletion of white blood cells) in some patients and, hence, it is not used. The close analogue Mirtazapine was introduced to overcome these problems. It has been shown to be as effective as the SNRI Venlafaxine (the former best-selling antidepressant) and to have faster onset of action than SSRIs, acting as an antagonist of both the α_2 -AR and post-synaptic 5-HT₂ and 5-HT₃ receptors.¹⁵¹ It maintains side effects such as somnolence, weight gain and dizziness but has a much more favourable profile.

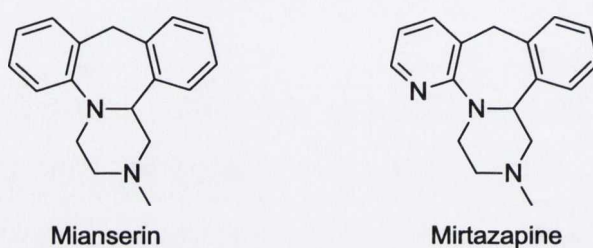


Fig. 1.27. Chemical structures of mianserin and mirtazapine.

Despite the huge promise that they have shown and the vast interest in obtaining them, Mianserin and Mirtazapine are the only α_2 -AR antagonists that have been approved to date. Compounds which have been investigated include analogues of the alkaloid yohimbine (Fig. 1.26), which are known to be potent compounds but none have proven specific enough to avoid severe side effects through interactions at further 5-HT and AR receptors.¹⁵² Owing to the success of the α_2 -AR agonist clonidine (Fig. 1.10, used clinically as an anti-hypertensive and an epidural analgesic) many imidazole and imidazoline containing compounds have also been investigated. In particular, compounds which incorporate bezodioxane and imidazoline moieties have been extensively studied (Fig. 1.28). As a result 3-methoxyidazoxan (RX821002) is used as a standard ligand in competitive binding experiments involving the α_2 -AR.



Fig. 1.28. Chemical structures of idazoxan and 3-methoxyidazoxan (RX821002).

1.5.4. Pharmacological Evaluation of α_2 -AR Antagonists

Several methods are utilised for the evaluation of potential α_2 -AR antagonists. It is essential to identify that a compound has both high affinity and antagonistic activity at the receptor using *in vitro* assays before any *in vivo* tests can be carried out. Thus, the sequence of tests performed for a promising compound is: binding affinity for the α_2 -AR (*in vitro*), activity at the α_2 -AR (*in vitro*), effects on extracellular NA concentrations (*in vivo*), antidepressant evaluation by behavioural testing (*in vivo*).

In Vitro Testing

The binding affinity of compounds for the α_2 -AR is measured using competition binding assays with the standard α_2 -AR radioligand [³H]RX821002 (Fig. 1.28) using human tissue from the PFC of depressed suicide victims. This assay involves isolating the membranes containing the α_2 -AR by centrifugation and incubating them with constant concentrations of [³H]RX821002, whose affinity for the α_2 -AR is known. Varying concentrations of the compound with unknown affinity are then added and displacement of the standard ligand can be measured using a scintillation counter after the incubation period. A plot of the percentage of bound [³H]RX821002 versus the negative logarithm of the concentration of the unknown-affinity compound (Fig. 1.29) can be used to determine the IC₅₀ – the concentration of compound which displaces 50% of the standard ligand.

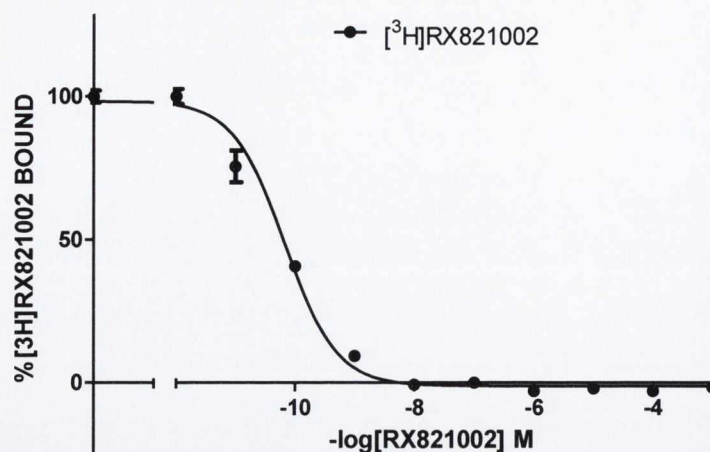


Fig. 1.29. Standard radioligand binding curve for $[^3\text{H}]\text{RX821002}$. In this case the competing ligand is un-labelled RX821002.

The IC_{50} is dependent on the concentration of membrane that was used. Thus it needs to be converted to the receptor-concentration-independent K_i . This can be achieved using the Cheng-Prusoff equation (Fig. 1.30) if the affinity of the radioligand (K_m) and the concentration of radioligand used $[S]$ are known. For the sake of convenience and ease of comparison, the pK_i ($-\log K_i$) is often used, and is solely discussed here.

$$K_i = \frac{\text{IC}_{50}}{1 + \frac{[S]}{K_m}}$$

Fig. 1.30. The Cheng-Prusoff equation used to convert IC_{50} values to concentration-independent K_i .

Those compounds with a binding affinity less than $1 \mu\text{M}$ ($K_i = 1 \times 10^{-6}$, $\text{pK}_i = 6$) were further examined for activity at the $\alpha_2\text{-AR}$ using functional $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ assays carried out in the same PFC tissue samples. This assay indicates the degree to which the receptor is activated by measuring the amount of labelled GTP (Fig. 1.31) taken up by the receptor in the presence of a compound. Activation of the $\alpha_2\text{-AR}$ involves exchange of a bound GDP molecule with GTP on the α -subunit of the receptor, while deactivation of the receptor involves hydrolysis of this GTP back to GDP (see Section 1.5.2) The radiolabelled $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ is not hydrolysable and so during this assay any activated α -

subunits bound to [^{35}S]GTP γ S will remain in this state and can be measured by a scintillation counter.

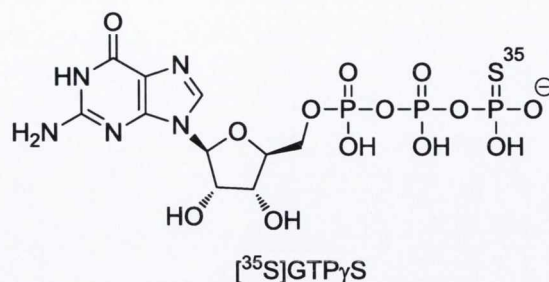


Fig. 1.31. Chemical structure of [^{35}S]GTP γ S. The γ -phosphate group is replaced by a radiolabelled thiophosphate group.

In this manner, an agonist activating the receptor will show an increase in radioactivity after incubation as the radiolabelled GTP is taken up. Antagonists lead to no change in radioactivity as they prevent activation of the receptor, while some compounds act as inverse agonists, turning down the basal activation of the receptor and displaying decreases in radioactivity. The test is carried out at a range of concentrations to get a profile of activity for the compound being tested. A plot of percentage bound [^{35}S]GTP γ S versus the negative logarithm of ligand concentration shows these effects (Fig. 1.32).

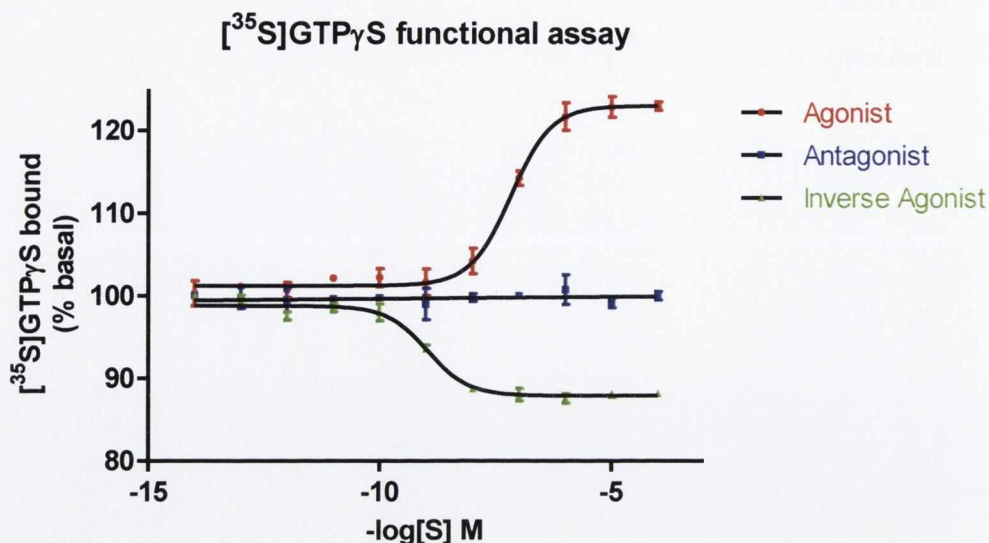


Fig. 1.32. Curves obtained for agonists, antagonists and inverse agonists in the $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ functional binding assay for a substrate S.

These plots can be used to measure EC_{50} values for agonists and inverse agonists – in the same way as IC_{50} values are calculated from competitive binding assays – as there is a change in the activation of the receptor. However, this cannot be obtained for antagonists as the activation state of the receptor is not altered. To measure their efficacy the effect on levels of bound $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ (or GPCR activation) can be measured in the presence of an agonist of the receptor. Decreases in bound $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ in this test indicate competitive antagonism, with larger decreases meaning greater potency.

In Vivo Testing

Microdialysis studies allow measurement of NA concentrations in a conscious mouse. This test works by inserting two catheters into the mouse brain; one which allows an aqueous solution mimicking the ionic composition of the surrounding tissue (perfusate) to enter, and one which allows solutes to cross a semi-permeable barrier by passive diffusion and leave the brain in a solution of extracellular fluid called the dialysate. This

dialysate is then analysed using HPLC, in the case of this testing for NA concentrations at different time points.

The microdialysis study has several applications, particularly for CNS-acting drugs. Firstly, their direct administration to the brain area of interest can demonstrate the effects of the drug on any NT concentration, and secondly, administration of the compounds in the periphery can indicate if the compound is capable of crossing the BBB; *i.e.* if the effects of direct administration are replicated.

Behavioural Testing

The tail suspension test (TST), introduced in 1985,¹⁵³ is a test in which a mouse is suspended by the tail from a lever and its movements monitored. The test is divided into periods of agitation and immobility, representing will to escape and despair respectively. Immobility times are measured and controls are compared with subjects treated with compound. While this test is crude in nature it has produced results which correlate well with human data for existing antidepressants and tests on its validity as a model of depression have returned favourable conclusions.¹⁵⁴

Antidepressant activity can only be concluded if results are replicable in multiple models and so the forced-swim test (FST) is often used in conjunction with the tail suspension test. In this test mice are placed in an inescapable tank that is filled with water and their escape-related mobility behaviour is measured.¹⁵⁵ After initial intense escape-directed behaviour, such as swimming and climbing, the mice stop struggling and show passive immobile behaviour. Reduced immobility time is observed when the mouse is treated with an antidepressant and replication of this with a new compound is an indication of antidepressant activity.

1.5.5. Previous Work in the Rozas group

Research within our group has described the synthesis and pharmacological evaluation of over 80 molecules to date in a series of papers describing structure-activity relationships at the α_2 -AR.^{156,157,158} These molecules have had the general form of a substituted aryl ring directly attached to either a guanidine or 2-iminoimidazolidine

group (Fig. 1.33) which are cationic at physiological pH due to the basicity of these groups and have been prepared as the corresponding hydrochlorides due to the water solubility of these salts which is desirable for pharmacological testing. Several dimeric molecules of the same type have been investigated as well, where the aryl rings are attached *via* a linker (X) through either both *para* carbons, or the *meta* carbon of one ring and the *para* carbon of the other. Some tricyclic derivatives were also synthesised (Fig. 1.33). All molecules were evaluated *in vitro* and the most successful from these tests were subjected to *in vivo* microdialysis and behavioural testing in mice.

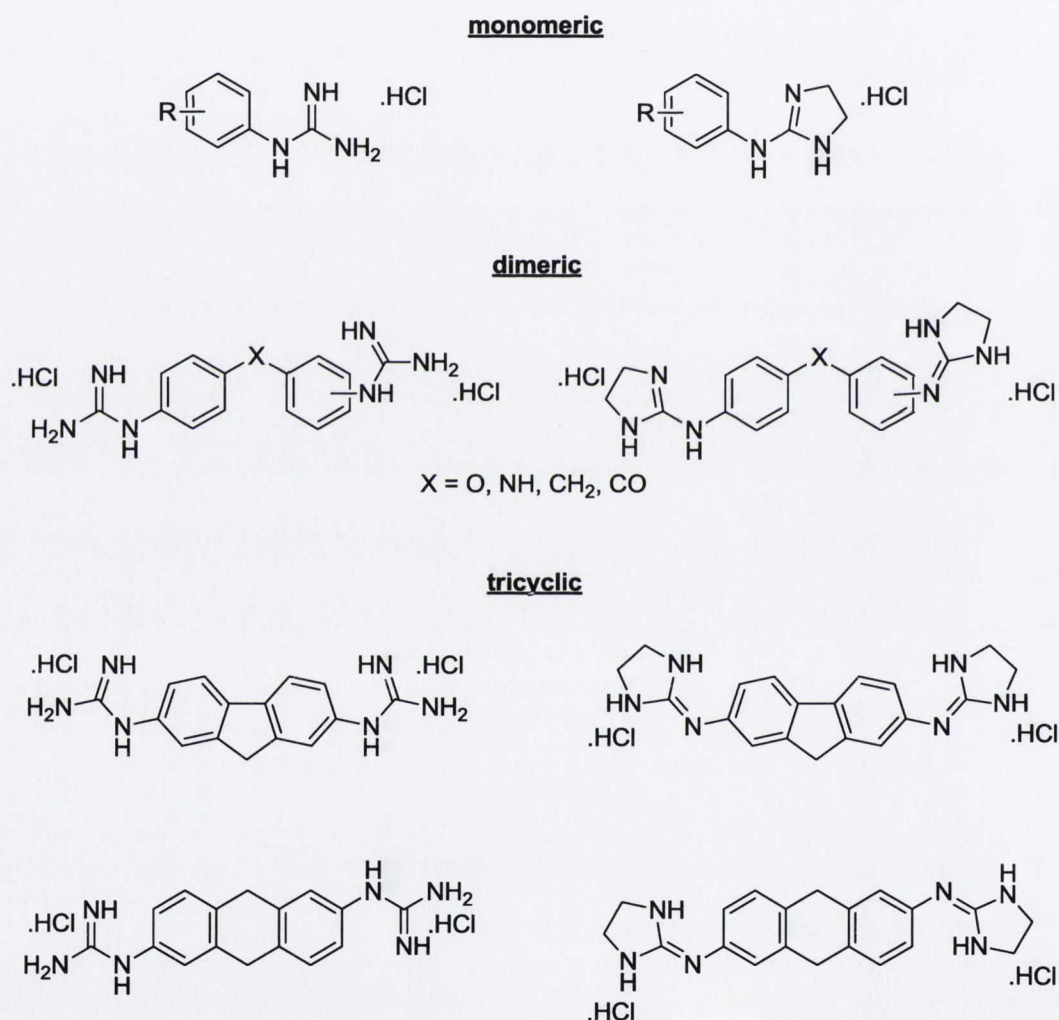


Fig. 1.33. General structures of compounds prepared previously in Rozas' group for activity as α_2 -AR antagonists. Monomeric (*top*), dimeric (*middle*) and tricyclic (*bottom*) guanidine and 2-iminoimidazolidine hydrochlorides have been investigated for a variety of X and R groups.

In Vitro Testing

Each compound was tested for binding affinity at the α_2 -AR in competition binding assays with the standard α_2 -AR radioligand [^3H]RX821002 (Fig. 1.28) using human tissue from the PFC of depressed suicide victims. This allows the binding affinity of any compound to be compared with another and also indicates if a compound is binding to the active site of the receptor as it is in competition with a ligand known to bind there. Those compounds with a binding affinity less than $1\ \mu\text{M}$ ($K_i = 1 \times 10^{-6}$, $\text{p}K_i = 6$) were further examined for their activity at the α_2 -AR using functional [^{35}S]GTP γ S assays carried out in the same tissue samples.

From these studies five high-affinity ($\text{p}K_i > 6.5$) antagonists of the α_2 -AR were identified (Fig. 1.34), encompassing each type of ligand tested; guanidines **1** and **2**, 2-iminoimidazolidines **3** and **4**, and dimeric *bis*-2-iminoimidazolidine **5**. Several extremely high affinity compounds were also obtained which had the undesired agonistic activity; these compounds had $\text{p}K_i$ values close to 9.0 indicating near nanomolar binding affinity.

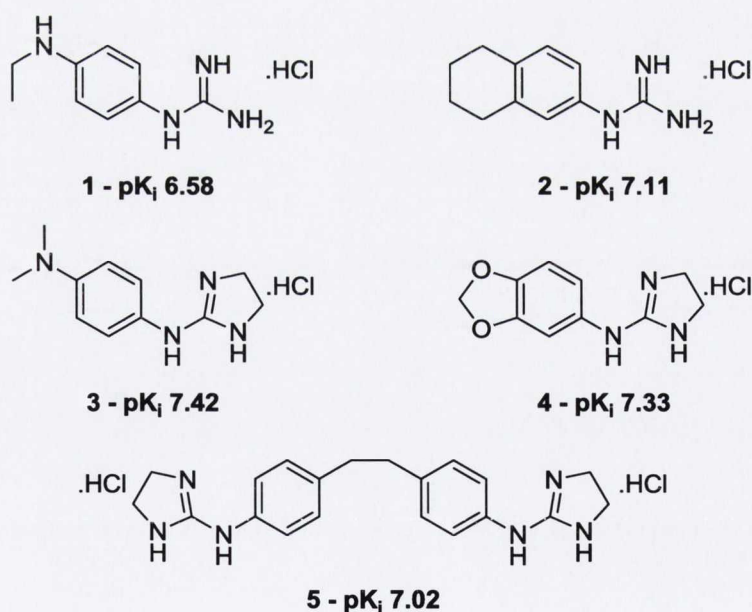


Fig. 1.34. High affinity α_2 -AR antagonists designed by the Rozas group.

Significantly, these five compounds have been examined for several properties using *in vivo* animal testing. Microdialysis studies measure NA concentrations in a conscious

mouse and this allowed two assertions to be made. Firstly, their direct administration to the PFC led to increased extracellular concentrations of NA and secondly, administration in the periphery led to the same enhancements, meaning crucially that these compounds can pass the BBB.

Having proven that these five compounds increase extracellular NA concentrations in mice, and that they possess high affinity and antagonistic activity at the α_2 -AR, they were subjected to behavioural tests to determine their antidepressant effects. Interestingly – and in a demonstration of the difficulties encountered in obtaining antidepressants – of the five compounds tested only **1** and **2** (Fig. 1.34) showed antidepressant effects in the TST and FST. Encouragingly, in data which has just been published,¹⁵⁹ these compounds outperformed fluoxetine hydrochloride in the tests. As a result of this, both **1** and **2** served as the lead compounds for this research.

Structure-Activity Relationships

Another striking feature of the *in vitro* testing of all compounds was that minor structural changes have huge effects on the binding affinity and activity of compounds, and rational structure-activity relationships (SARs) have been difficult to obtain. Overall, 2-iminoimidazolidine derivatives exhibit higher affinity for the α_2 -AR than their guanidine analogues. However, no 2-iminoimidazolidine compound gave antidepressant effects in the behavioural testing and the activity of closely related analogues of these compounds at the α_2 -AR is unpredictable. As an example of this, comparing compounds **2** and **3** with their direct analogues **6** and **7** (changing only the cationic moiety) leads to unexpected results (Fig. 1.35). For the tetrahydronaphthalene derivative **2** the guanidine is an antagonist, while the 2-iminoimidazolidine is an agonist. The opposite is the case for *para*-dimethylaminobenzene compound **3**, where the 2-iminoimidazolidine is an antagonist and the guanidine is an agonist.

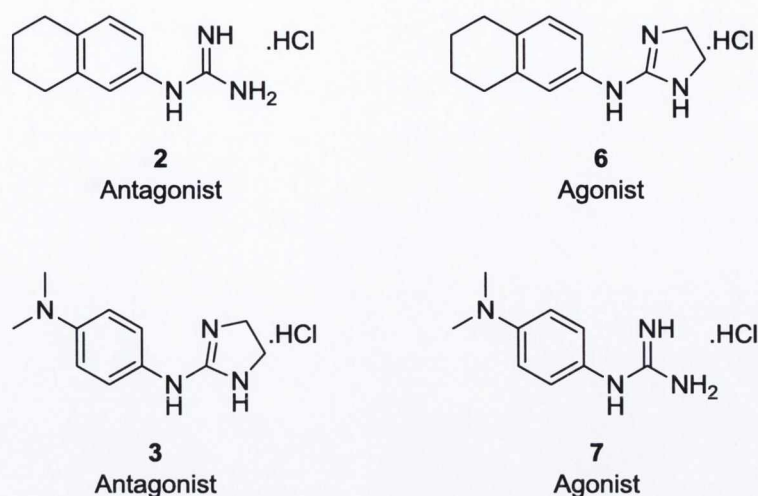


Fig. 1.35. Unpredictable activity has been obtained for chemically similar ligands at the α_2 -AR.

With a large library of compounds for which binding affinity and activity data are known, and two high-affinity antagonists with antidepressant activity, this project aimed to clarify some of the requirements for obtaining enhanced antagonists of the α_2 -AR and identifying potential new antidepressants.

1.6. Computational Chemistry Techniques

During the course of this project several computational methods were employed to gain structural information on the target compounds and to predict how they would behave in binding affinity and activity studies with the α_2 -AR. Initial families of compounds were studied to gain SAR information and aid in the choice of future ligands. The studies were carried out using four types of calculations. Density functional theory (DFT) was used to identify the optimised geometries of the compounds (or complexes, depending on the system being studied), the atoms in molecules (AIM) theory¹⁶⁰ was

used to describe the electron-density of the systems, the natural bond orbital (NBO) theory¹⁶¹ was used to investigate orbital interactions and charge transfers, and nucleus-independent chemical shifts (NICS)¹⁶² were used to probe the aromaticity of rings across series of compounds. Below is a brief description of the theory behind these computational chemistry techniques.

1.6.1. Density Functional Theory

Origins of Density Functional Theory

Density-functional theory is a quantum mechanical model in which all the information of a system is described using functionals of the electron density. The 1998 Nobel Prize in chemistry was awarded to Walter Kohn, who is credited with developing the theory, and John Pople who was instrumental in implementing DFT in computational chemistry.

It is known that the Schrödinger equation can be solved exactly for a hydrogen atom or any mono-electronic system in order to get the wave-function of that system. However it is impossible to solve the Schrödinger equation for a many-body system. The purpose of DFT is to solve the Schrödinger equation as accurately as possible for such many-body systems. An early method for approximately solving the Schrödinger equation of such systems was the Hartree-Fock (HF) method. It employs the Hartree potential which treats electrons as moving independently of each other, only feeling the average electrostatic field due to all the other electrons plus the field due to the nuclei. The HF method neglects correlation effects which consider electron-electron interactions.

The exchange interaction is a quantum mechanical effect discovered by both Heisenberg and Dirac that occurs due to wave-functions of indistinguishable particles overlapping. The result is that electrons which have spatially symmetrical wavefunctions (*i.e.* have parallel spins) are found at closer distances to each other than would be expected. Electronic correlation governs the Coulombic repulsion of electrons.

Within the HF method, the exchange interactions are added by forcing the anti-symmetry of the wavefunction to the Hartree potential; however, the correlation is

approximated such that Coulombic interactions are not properly taken into account, leading to a total electronic energy which differs from the exact solution of the Schrödinger equation. This energy is always higher than the exact solution, by an amount termed the correlation energy.

Density Functional Theory

Density Functional Theory is another method of obtaining an approximate solution to the Schrödinger equation of a many-body system. Firstly, it reduces the number of degrees of freedom of the system by applying the Born-Oppenheimer approximation, which accounts for the nuclear degrees of freedom only in the form of a potential energy $v(r)$ acting on the electrons. Thus the wavefunction depends solely on the electronic coordinates and the total wavefunction is broken into electronic and nuclear (rotational and vibrational) components (Fig. 1.36).

$$\Psi_{\text{total}} = \Psi_{\text{electronic}} \times \Psi_{\text{nuclear}}$$

Fig. 1.36. The generalised wavefunction (ψ) describing systems in DFT.

Then, DFT uses functionals of the electron-density to describe the properties of the many-body system. This greatly speeds up calculations relative to HF which deals directly with the wavefunction as opposed to the electron-density. This is because the many-body electronic wavefunction is a function of $3N$ variables (the coordinates of all N atoms in the system), while the electron density is only a function of the three variables x , y and z .

The development of DFT was greatly helped by the Hohenberg-Kohn theorem which states that the electron-density of any system determines all ground-state properties of that system. In this case the total ground state energy of a many-electron system is a functional of the density, so it follows that a knowledge of the electron-density functional leads to the total energy of the system. Using the electron-density it becomes possible to solve effective one-electron-type Schrödinger equations successively, resulting in the total energy of the system.

In DFT calculations these electronic Schrödinger equations are first solved while the nuclei are fixed in a certain configuration (the equilibrium configuration). The electronic energies calculated therein consist of kinetic energies, inter-electronic repulsions and electron-nuclear attractions. In a second step, this wavefunction serves as a potential energy acting on the nuclei in solving a Schrödinger equation containing only the nuclei. The combination serves as the total wavefunction describing the system.

These time-independent Schrödinger equations (Fig. 1.37) are applied and solved by setting up the Hamiltonian operator ($\hat{H}$) for the system – *i.e.* the kinetic and potential energy of the particles in the system – and solving the resulting differential equation for the wavefunction.

$$E\Psi = \hat{H}\Psi$$

$$E\Psi(r) = \frac{-\hbar^2}{2m} \nabla^2\Psi(r) + V(r)\Psi(r)$$

Fig. 1.37. The time-independent Schrödinger equation in general form (*top*) and in specific nonrelativistic form (*bottom*).

The general form of the Schrödinger equation, where Ψ is the wavefunction and $\hat{H}$ is the Hamiltonian operator which characterises the total energy of the wavefunction, states that when the $\hat{H}$ operates on the wavefunction Ψ the result is proportional to the same wavefunction, and that when Ψ is a stationary state the proportionality constant, E , is the energy of the state Ψ . This is an eigenvalue equation which can be solved for E . The specific nonrelativistic form of the Schrödinger equation (Fig. 1.37, *bottom*), for a single particle moving in an electric field, describes the energy in terms of its constituent kinetic (first term) and potential (second term) energies. Here, $\hbar$ is the reduced Planck constant, m is the particle's mass, ∇ is the Laplacian and V is the potential energy.

The Kohn-Sham equations are concerned with calculating the kinetic energy terms of this equation and incorporating electron-electron interactions into DFT calculations. Under this model electrons are considered as non-interacting particles moving in an

effective potential which incorporates an external potential and the effects of Coulombic interactions between the electrons, *i.e.* the exchange and correlation interactions. Thus, the problem of a system of interacting electrons has been mapped onto that of a system of non-interacting electrons moving in an effective potential.

An approximation of the exchange-correlation term is used in DFT called the local density approximation (LDA) in which the exchange-correlation energy that is modelled for a particular region of the many-body system is not the exact one; it is replaced by an energy taken from a homogeneous electron gas whose density is the same as the local density around the electron.

However, the improper treatment of exchange and correlation interactions is seen as the major drawback of DFT methods, particularly as they are critical to the description of weak interactions like van der Waals forces. Many functionals have been designed to better allow for this. These “hybrid functionals” are a class of approximations of the exchange and correlation energies which incorporate portions of exact exchange from Hartree-Fock (HF) theory with further exchange and correlation parameters from other *ab-initio* methods.

One of the most commonly used hybrid functionals is B3LYP (Becke, 3-parameter, Lee-Yang-Parr), named after its developers, principally Axel Becke.¹⁶³ According to Becke it combines the benefits of HF and DFT. Hartree-Fock theory benefits from exact treatment of exchange but suffers from the necessity to include post HF techniques to account for correlation. Such methods (*e.g.* Moller-Plesset perturbation theory) are computationally expensive and cannot be readily applied to large systems. DFT correlation approximations can be evaluated very easily as they are based on the integration of functionals which depend only on total electron density. Thus, combining these two theories allows for superior accuracy at similarly low computational cost to DFT methods. The resulting close agreement with experimental data, coupled with its relatively low computational cost, makes it one of the most popular quantum mechanical approaches to matter.

When weak interactions are of principal interest to a system, the M05-2X and M06-2X functionals introduced by Truhlar are popular as they are known to describe these interactions well. They are discussed further in Section 3.1, where they were applied.

1.6.2. Atoms in Molecules Theory

The AIM theory¹⁶⁰ developed by Richard Bader is an intuitive way of dividing molecules into atoms and describing their properties. It defines an atom based on its electronic charge density. The theory was founded on the recognition that the electron density $\rho(r)$ plays a critical role in explaining and understanding the experimental observations of chemistry. It is often known as the Quantum Theory of Atoms in Molecules (QTAIM) today, in recognition of the fact that it is rigorously based on quantum mechanics. The theory relates features such as chemical structure, chemical bonding, functional group behaviour and chemical reactivity to the topology of the $\rho(r)$ distribution.¹⁶⁴

In the AIM approach space is divided into atomic volumes containing exactly one nucleus, which acts as a local attractor of the electron density. Thus, molecular structure is given by the stationary points of $\rho(r)$, with nuclei acting as point attractors immersed in this cloud of negative charge. The electron density describes the manner in which the electronic charge is distributed throughout real space. It is a maximum at the position of each nucleus and decays rapidly moving away from these positions. Thus, the cloud of negative charge is seen to be most dense at the nuclear positions and to become more diffuse moving away from these centres of attraction. The presence of local maxima at the positions of the nuclei is the dominant topological property of $\rho(r)$.

Gradient vector maps may be obtained by tracing the trajectory of $\nabla \rho(r)$, the change in electron-density, through space (Fig. 1.38). Since the density exhibits a maximum at the position of each nucleus, sets of trajectories terminate at nuclei on these maps. Because of this property, the space of the molecule becomes partitioned into basins, and since a single attractor (nucleus) is associated with each basin, an atom is defined as the union of an attractor and its basin.

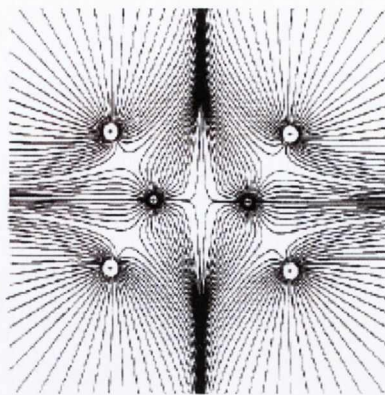


Fig 1.38. Maps of the gradient vector field of $\rho(r)$ of ethane for the plane containing the nuclei. Each line represents a trajectory traced out by the vector $\nabla\rho(r)$.¹⁶⁰

Molecular structure is given by the stationary points – or critical points – of $\rho(r)$. These critical points denote an extreme in $\rho(r)$, a point where $\nabla\rho(r) = 0$. Associated with each such critical point is a set of trajectories that start at infinity and terminate at the critical point. Only two of these originate at the critical point and terminate, one each, at the neighbouring nuclei. They define a line through space along which $\rho(r)$ is a maximum. This type of critical point is called a bond critical point (BCP) and these two trajectories define the bond path. The remaining trajectories from this point define an interatomic surface. In an equilibrium geometry the bond path – or line of maximum electron density – faithfully reproduces the chemical bonds assigned on the basis of chemical considerations.

While the topology of $\rho(r)$ maps well the concepts of atoms, bonds and structure, it provides no indication of maxima in $\rho(r)$ corresponding to the electron pairs of the Lewis model. The Laplacian, the scalar derivative of the gradient vector field of the electron-density ($\nabla^2\rho$), determines where electronic charge is locally concentrated ($\nabla^2\rho < 0$) and depleted ($\nabla^2\rho > 0$), mapping the electron pairs of the Lewis and Valence Shell Electron Pair Repulsion (VSEPR) models.

In this manner four types of critical points are used to describe molecules in AIM (Fig. 1.39):

1. *(3,-3) critical point*; a point at which $\rho(r)$ decreases in all three perpendicular directions of space. This is a local maximum of $\rho(r)$ and occurs at the location of an atom. It is also called an atomic critical point (ACP).

2. *(3,-1) critical point*; a point at which $\rho(r)$ falls down in two perpendicular directions of space and rises in the third direction. This is a saddle point of $\rho(r)$ and occurs between two neighbouring atoms, defining a bond (or interaction) between them. It is thus called a bond critical point (BCP).
3. *(3,+1) critical point*; a point at which $\rho(r)$ falls in one spatial direction and rises in the other two directions. This type of critical point is found in the middle of bonds forming a ring and is termed a ring critical point (RCP).
4. *(3,+3) critical point*; This is a local minimum, at which $\rho(r)$ rises in all three directions of space. It occurs when several rings form a cage and is therefore called a cage critical point (CCP).

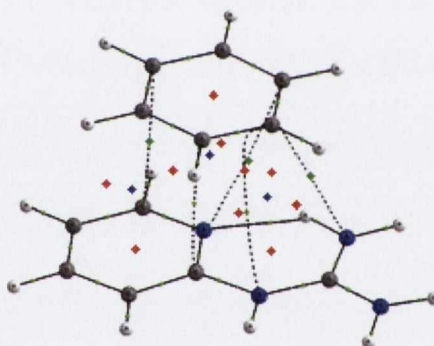


Fig. 1.39. Sample output from the AIM analysis of a complex from this project. Critical points are shown in coloured dots; ACPs are located on atoms, green = BCP, red = RCP, blue = CCP.

AIM analysis has become very popular due to its ability to accurately describe experimental properties, and especially so since the formulation of modern DFT methods which have allowed electron-density maps of high quality to be obtained rapidly.

1.6.3. Natural Bond Orbitals

The Natural Bond Orbital (NBO) method was conceived as a “chemist’s basis set” that would correspond closely to the picture of localised bonds and lone pairs as basic units of molecular structure, yet ideally maintaining the accuracy of quantum chemistry analyses.¹⁶¹ Such NBOs are one of a sequence of natural localised orbital sets used to calculate the distribution of electron-density in atoms and in bonds between atoms.

The NBO method translates accurate calculations into chemical insights such as Lewis diagrams, hybrid descriptors, bond orders and charge transfer. The charges of the constituent atoms are calculated and Lewis diagrams are constructed in terms of bonding and lone-pair electrons. Bond orbitals are created in terms of their type (σ , π), coefficients (for example hybrid composition of s and p orbitals) and occupancy, bond orders are described as the total of their ionic and covalent contribution.

The results detail charge transfers from filled to empty orbitals, which are described by type of orbital interaction and stabilisation gained (in terms of the Second Order Perturbation Theory estimate). This feature makes NBO useful for finding the relative contribution of resonance structures to the overall structure of a system and for describing intermolecular interactions where correlation effects are important.

The NBO theory formulates a localised criterion for orbitals which maintain maximum-occupancy (natural) character in localised one and two centre regions of the molecule (lone-pair and bond respectively). Because the maximum occupancy of an orbital is inherently limited to a pair of electrons by the Pauli exclusion-principle, one and two centre orbitals with occupancies sufficiently close to 2.000 can serve as well as more delocalised molecular orbitals for describing the wave-function (Ψ), while they are vastly more intuitive and applicable to organic chemistry.

These one and two-centre regions map perfectly to the localised orbitals of near double occupancy proposed by Lewis in his model of molecular structure.¹⁶⁵ Such NBOs are one of a sequence of natural localised orbital sets (Fig. 1.40) which are intermediate between basis atomic orbitals (AOs) and molecular orbitals (MOs), including natural atomic (NAOs), hybrid (NHOs) and semi-localised molecular orbital (NLMO) sets.

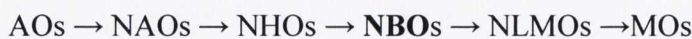


Fig. 1.40. Natural localised orbital sets.

Each bonding NBO σ_{AB} can be written in terms of two valence hybrids (NHOs) h_A , h_B on atoms A and B with corresponding polarisation coefficients c_A and c_B which vary from covalent ($c_A = c_B$) to ionic ($c_A \gg c_B$) (Fig. 1.41). Similar to molecular orbital theory, each bonding NBO must be paired with a corresponding antibonding NBO.

$$\sigma_{AB} = c_A h_A + c_B h_B$$

$$\sigma_{AB}^* = c_A h_A - c_B h_B$$

Fig. 1.41. Description of bonding and antibonding NBOs arising from a combination of NHOs.

So the Lewis-type donor NBOs are complemented by non-Lewis-type acceptor NBO^{*}s which are formally empty in the Lewis picture. The NBO approach can take account of stabilisation gained through interaction of these donor-acceptor orbitals ($\sigma \rightarrow \sigma^*$), which are estimated using the second-order perturbation theory which, for the interaction $\sigma_i \rightarrow \sigma_j^*$ can be expressed in terms of the energy of the interacting orbitals and the orbital Hamiltonian F (Fig. 1.42).

$$\Delta E_{i \rightarrow j^*} = -2 \frac{\langle \sigma_i | F | \sigma_j^* \rangle^2}{\epsilon_{j^*} - \epsilon_i}$$

Fig. 1.42. The second order perturbation energy expression, where F is the orbital Hamiltonian and ϵ_i and ϵ_j^* are the respective orbital energies of the donor and acceptor NBOs.

This leads to an extension of Lewis structure concepts to include delocalisation. A further consequence is that the doubly occupied bonding NBOs have some antibonding character, being a linear combination of the NBO σ_i (coefficient $c_i \cong 1$) and the antibonding NBO σ_i^* (coefficient $c_i \cong 0$).

It is important to note that a Slater determinant of these doubly occupied NBOs (an expression that describes the wavefunction of a system that satisfies anti-symmetry requirements and the Pauli-exclusion principle) is equivalent to that of the usual MO wavefunction, meaning no significant loss of accuracy occurs.¹⁶¹ Thus the benefit of NBO theory is that it maintains accuracy while being vastly more intuitive.

1.6.4. Nucleus-Independent Chemical Shift (NICS)

Historically, aromaticity has been demonstrated, and attempts have been made at quantifying it, using experimental techniques. Exaltation of magnetic susceptibility (Λ),¹⁶⁶ NMR chemical shifts of aromatic ring nuclei, aromatic stabilisation energies (ASEs) and bond-length measurements have all been used. However, these are often difficult to measure and are highly dependent on ring size.

For example, the NMR spectrum of benzene demonstrates that the protons experience de-shielding as the induced magnetic field has the same direction as the external field, and this is revealed in the characteristic high shift of the benzene protons (7.3 ppm in CDCl_3). An interesting phenomenon, applied in NICS theory, is that protons at the centre of an aromatic ring, such as those in [18]annulene, experience a large shielding effect (Fig. 1.43), as the induced magnetic field opposes the external field. In fact, the six inner protons of [18]annulene appear at -3.0 ppm (in CDCl_3). As the rings of most aromatic systems are too small to accommodate atoms internally, the NMR chemical shift of lithium ions (δLi^+) complexed with small aromatic rings has been used to replicate this effect.¹⁶²



Fig. 1.43. The characteristic NMR chemical shifts of [18]annulene (*left*) demonstrate the ring currents of aromatic systems. The shift of lithium ions (δLi^+) complexed to aryl rings is used to replicate this for rings too small to accommodate internal *H* atoms.

The NICS^{167,168} is also based on the fact that an external magnetic field induces a diatropic π ring current. It calculates the values of absolute magnetic shielding at the centre of rings and at both 1 or 2 Å above or below the molecular plane (Fig. 1.44), NICS(0), NICS(1) or NICS(2) respectively. It has the advantage that it can be calculated for all ring sizes as it does not require a proton inside the ring. Thus, we can now define the NICS as an index of aromaticity, which computationally calculates the absolute magnetic shielding at the centre of a ring taken with reversed sign. Hence, negative NICS values indicate aromaticity and positive values indicate antiaromaticity (cyclic molecules which have alternating single and double bonds, but whose π -electron energy is higher than that of its open-chain analogue).¹⁶⁷

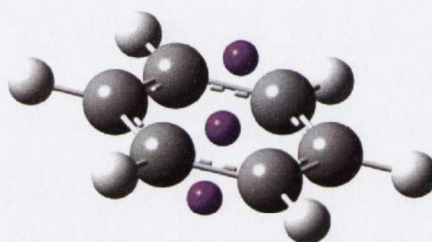


Fig. 1.44. The values of absolute magnetic shieldings are calculated at “dummy atoms” placed at the ring centre [NICS(0)] and 1 Å above/below the ring plane [NICS(1)].

The NICS(1) values computed 1 Å above the ring centres are considered to better reflect the π -electron effects due to ring-currents as NICS(0) values are dominated by the effects of the σ -bonding electrons of the ring, particularly for small rings.¹⁶⁹ In some cases NICS(2) values at 2 Å above the ring centre are calculated to enhance this selectivity for measuring π -electron effects. The NICS is now considered to be a better criterion of aromaticity than exaltation of magnetic susceptibility, aromatic stabilisation energies and NMR shifts.¹⁷⁰ It is computationally inexpensive and highly accurate, giving good indication of relative aromaticity and perturbations to the system by external forces.

In practise, a molecule's geometry is optimised and the NMR shift of dummy atoms placed at the points of interest is calculated using the gauge-independent atomic orbital (GIAO) method, under the same method and basis set conditions as the optimisation.

Chapter 2 - Objectives

Over 80 compounds with similar structural features have been previously prepared and tested for their binding affinity and functional activity at the α_2 -AR in Rozas' group. Pharmacological results have often been surprising, with small structural changes resulting in significant variations in the profile of compounds, both in terms of binding affinity and functional activity. The most significant problem has been that very similar molecules have given rise to opposing agonistic or antagonistic activity at the α_2 -AR. Thus, a primary objective of this project is to elucidate molecular features which will confer the desired antagonistic activity to ligands. If this can be attained then efforts will concentrate on obtaining high affinity binding at the α_2 -AR. In an effort to gain SARs, compounds will be studied using a variety of computational methods and spectroscopic techniques, and the results therein will be correlated with pharmacological results.

2.1. Computational Chemistry

The initial target derivatives will be studied theoretically to gain an understanding of their conformational and electronic features, and to predict how they will behave in the aqueous media of the pharmacological tests. A DFT analysis of target compounds will allow assessment of the features leading to favourable pharmacological actions at the α_2 -AR. A comparison of the actions of different families will provide information on favourable molecular features, while studying the results from compounds within each family will allow SARs to be suggested within families. On synthesis of these derivatives, NMR spectroscopy and X-ray crystallography will be employed to substantiate any claims made on the structure of molecules during the theoretical study. Compounds will be pharmacologically evaluated at the earliest stage possible so that subsequent ligands can be guided by the results therein.

Furthermore, the proposed π -cation interaction between guanidinium and a phenylalanine residue in the α_2 -AR active site will be investigated *in-silico* and its posited importance to the binding of ligands to the receptor will be evaluated. Benzene

will be employed as a model for phenylalanine and all possible complexes between benzene and our ligands will be compared in terms of their interaction energies. Thus, DFT optimisations, AIM calculations on electron density, NBO investigations of charge transfers and orbital interactions, and NICS evaluations of aromaticity will all be employed to achieve this. For optimal modelling of the biological environment, all calculations will be carried out in PCM-water solvation. An understanding of this type of interaction, particularly in conjunction with pharmacological data, would give valuable information on the relevance of π -cation complexes for binding to the α_2 -AR, and would guide the design of future antagonists of the receptor.

2.2. Synthetic Chemistry

Synthesis of Pharmacological Targets

Synthetic targets were subject to change throughout the course of the project, based on the results of pharmacological testing and computational studies. Initial targets were set out as pyridin-2-yl and pyridin-3-yl guanidine hydrochlorides. Synthesis of these compounds was planned to employ established synthetic methods where possible; mercury(II) chloride promoted guanidylation of aryl amines with thiourea derivatives was the method of choice. However, several new synthetic methodologies were required to obtain these derivatives due to their differing chemical reactivities, including nucleophilic aromatic substitution with guanidine, which will be described in the Results and Discussion section. Several of the pyridylamines also had to be synthesised as they were not commercially available.

According to computational and pharmacological results from within this project and elsewhere in Rozas' group, 2-iminoimidazolidine hydrochlorides, *N,N'*-di-substituted guanidine hydrochlorides and *N*-substituted-1,4-dihydroquinazolin-2-amine hydrochlorides emerged as promising synthetic targets for the development of improved α_2 -AR antagonists. Synthesis of these compounds employed either methodologies being developed in our group or literature procedures as appropriate, with an emphasis on using functional, clean and high yielding reactions.

New Methods for the Synthesis of Guanidines and Guanidine Derivatives

As the main synthetic route to the compounds of interest involves toxic mercury(II) chloride, efforts are on-going to achieve greener, more efficient and more scalable methods for their preparation. Furthermore, the reactivity patterns of some reactants in this project required that new approaches had to be used for their synthesis. Thus, a constant objective was to investigate new routes to aryl guanidines and their derivatives, in particular routes from the corresponding aryl amines which are widely available.

2.3. Pharmacology

All compounds prepared during this project will be pharmacologically evaluated using competitive binding assays to evaluate their binding affinity at the α_2 -AR. Those compounds with values of $pK_i > 6.00$ will be further analysed using [35 S]GTP γ S assays to assess their activity as either agonists, antagonists or inverse agonists at the receptor. These tests will be carried out in human PFC tissue obtained from the brains of depressed suicide victims to obtain results in the most relevant *in vitro* model of human depression available. Compounds with promising *in vitro* profiles will be subjected to microdialysis studies in mice, which measure changes in NT concentrations in real time in the presence of an administered compound. Only if compounds still display encouraging results will behavioural tests in mice be considered to evaluate their antidepressant effects.

2.4. Achieving Outlined Objectives

During the course of this project six families of compounds were investigated as α_2 -AR antagonists. The progression from the lead compounds to the final family is outlined in Fig. 2.1, along with a brief explanation of the reasoning behind each family.

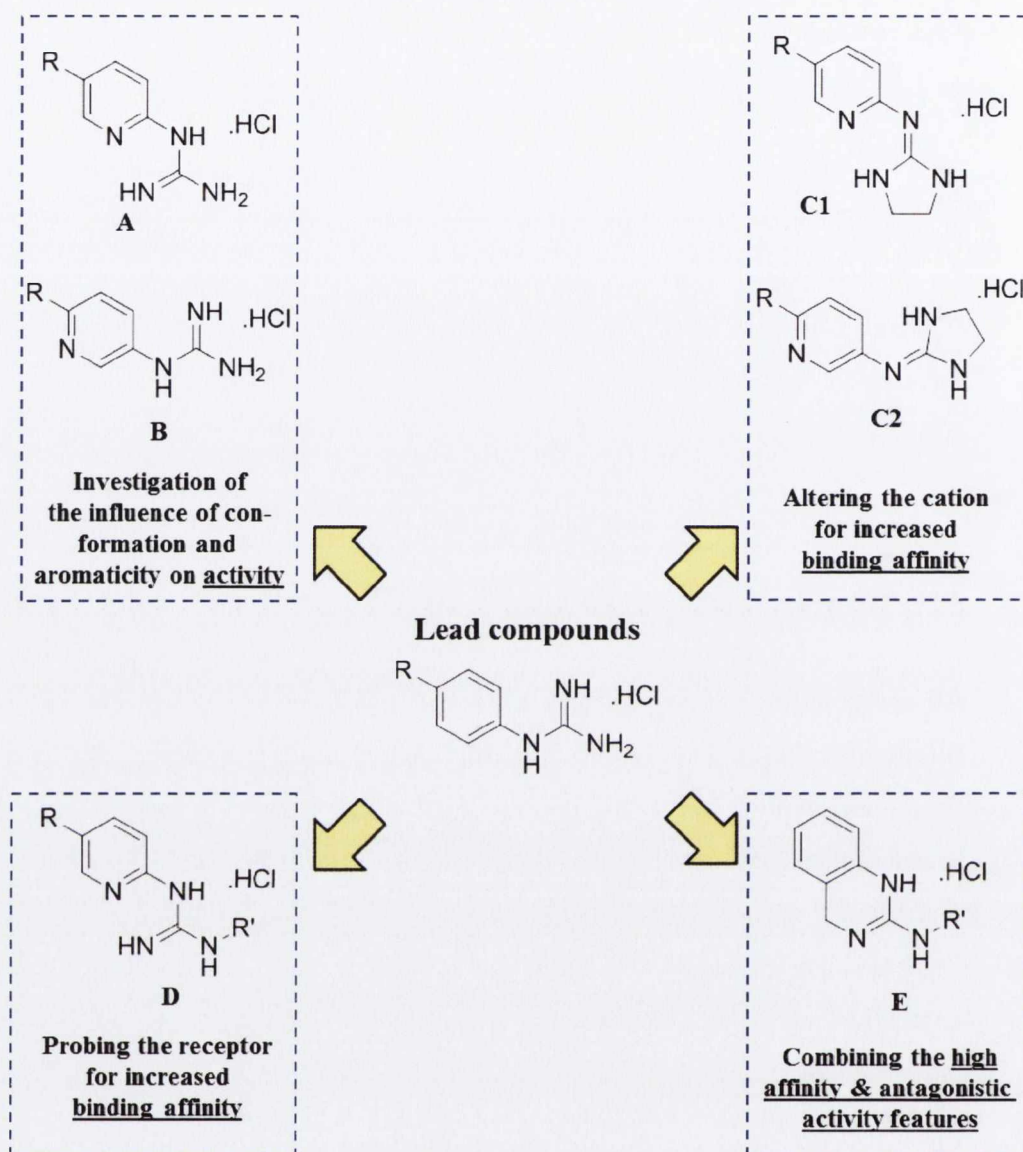


Fig. 2.1. Families of compounds investigated during this project.

As all families of compounds resulted in either the desired antagonistic or inverse agonistic activity at the α_2 -AR, efforts concentrated on increasing the binding affinity of compounds to the receptor. The design, synthesis and pharmacological evaluation of these compounds are discussed in the next sections.

Chapter 3 – Results and Discussion

3.1. Computational Study of the Potential Interactions of Pyridin-2-ylguanidiniums with the Active Site of the α_2 -Adrenoceptor

Despite the recent elucidation of the crystal structure of the human β_2 -AR,¹⁷¹ the structure of the α_2 -AR remains elusive, and though the new information on the β_2 -AR has allowed for higher quality homology models of the α_2 -AR to be constructed, they are not comprehensive. For example, the degree of homology between the human α_{2A} -AR and the crystallised human β_2 -AR is 36% according to a BLAST (Basic Local Alignment Search Tool) search and it is generally desirable to have sequence alignment of over 50% to build a reliable homology model.¹⁷²

The lack of a crystal structure of either the receptor alone or a ligand-receptor complex precludes employing a structure-based ligand design strategy. However, due to the large diversity of molecules which are known to interact with the α_2 -AR a ligand-based strategy can be undertaken. Through site directed mutagenesis and homology modelling studies, putative interactions between ligands and amino acid residues in the active site have been suggested, mainly for the endogenous ligand NA (Fig. 3.1.1). Thus, Ser200 and Ser204 are proposed to be involved in hydrogen bonding with the catechol hydroxyl groups,¹⁷³ Asp113 forms a strong salt bridge with the protonated nitrogen (a common feature in ligands of adrenoceptors),¹⁷⁴ Phe412 undergoes π - π stacking interactions with the catechol ring, and either Asp113 or Ser90 interact with the β -hydroxy group through hydrogen bonds (HBs).¹⁷⁵

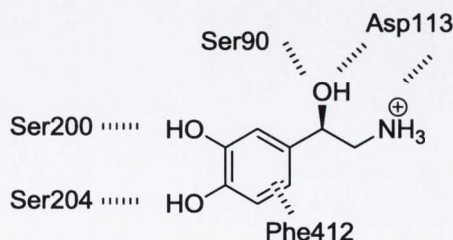


Fig. 3.1.1. Suggested interactions of NA with amino acid residues in the α_2 -AR.

Interestingly, it is also reported that agonists and antagonists may bind the α_2 -AR through different interactions.¹⁷⁶ In a study by Xhaard *et al.*, a homology modelling approach was used to examine the preferred docking mode of 12 structurally diverse antagonists of the α_2 -AR. One interaction which was consistently repeated was that between a positively charged nitrogen on the ligand (protonated or quaternary) and a Phe residue in the receptor binding site, illustrated for Yohimbine in Fig. 3.1.2. The authors note that fewer deviations from the only known structure of a GPCR at the time – bovine rhodopsin – were required for this binding mode and that their binding scores agreed with experimental data for the α_2 -AR more than alternative binding models.

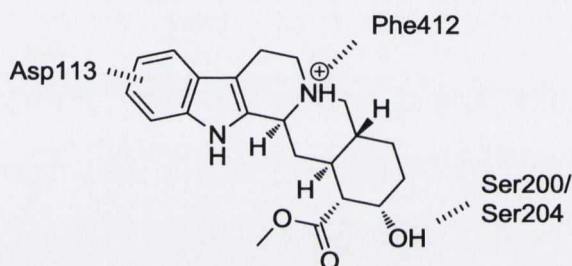


Fig. 3.1.2. Xhaard *et al.* proposed mode of antagonist binding to the α_2 -AR, shown for Yohimbine. Only the recurring interactions are shown.

This suggested mode of binding for antagonists gave encouragement for our aromatic guanidine type molecules as guanidinium, which is the ionic state of guanidine at physiological pH, is known to undergo strong cation- π interactions with aromatic rings, such as that of the Phe412 side chain.

With this in mind we undertook to computationally characterise the interactions available to pyridin-2-ylguanidiniums – our first family of compounds proposed – and benzene, as a model for Phe.

3.1.1. Cation- π Complexes of Guanidinium with Aromatic Systems

Experimental precedence for the existence of cation- π complexes involving guanidinium came from the crystal structure of 5-methylpyridin-2-ylguanidinium chloride obtained during the synthesis of family **A** (Fig. 3.1.3, *left*). The monomer is planar due to a HB between the pyridine nitrogen lone-pair and a guanidinium N-H. The supermolecular structure exhibits stacked cation- π interactions between the pyridine ring of one monomer and the guanidinium group of another, piling up to four units alternating the aromatic and cationic moieties to optimise the contacts.

The asymmetry introduced by the pyridine nitrogen means there are two possible cation- π stacked dimers available, with the pyridine nitrogens being either on the same or opposite side of the dimer (Fig. 3.1.3, *middle* and *right*). We performed a theoretical study of the cation- π interactions available to both of these dimers in gas phase and PCM-water model using the M05-2X functional with the 6-311+G(d,p) basis set. This functional is known to accurately describe weak interactions, taking into account dispersion forces, such as the HB and cation- π ones, important in these systems.

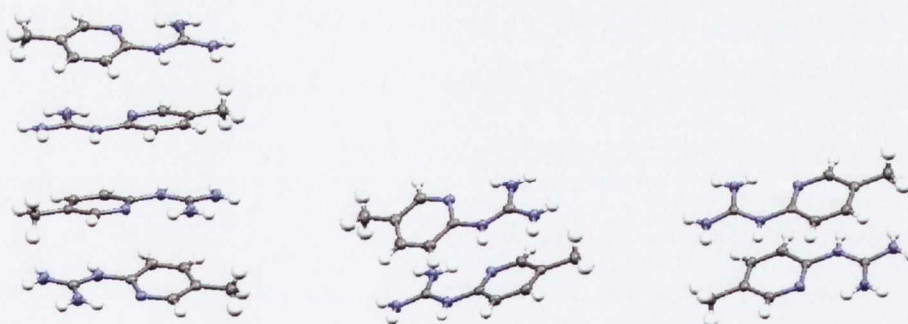


Fig. 3.1.3. Crystal structure of 5-methylpyridin-2-ylguanidinium chloride (counterion omitted for clarity) showing repeating tetramer unit (*left*), and dimers with pyridine nitrogens on the same side (*middle*) and opposite sides (*right*).

Furthermore, we examined the complexes of guanidinium cation with simple aromatic systems such as benzene, naphthalene and pyridine in gas phase and PCM-water using the same level of theory.¹⁷⁷

Gas Phase Calculations

When all of these complexes were examined in the gas phase only hydrogen bonding interactions were observed. For the dimers from the crystal structure no complexes were obtained due to repulsion of the cationic groups. Even those complexes between guanidinium and aromatic systems which had stacked complex starting orientations evolved to hydrogen bonded complexes in which the NHs of guanidinium were interacting with the π -electrons of the aromatic ring (Fig. 3.1.4). For naphthalene, one complex where these guanidinium NHs were directly over the ring centres and one in which they were directly over C atoms of the rings were observed.

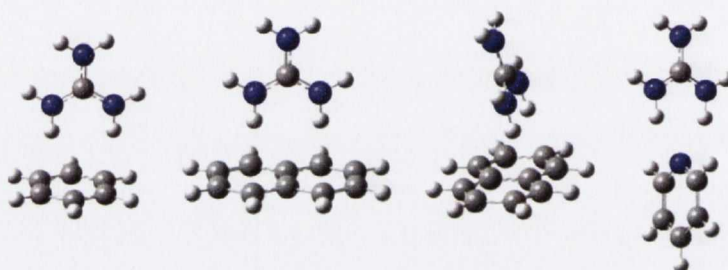


Fig. 3.1.4. Summary of the main hydrogen bonding complex observed for guanidinium with (*left-right*) benzene, naphthalene and pyridine.

These systems were comprehensively characterised in gas phase using the AIM and NBO theories; however, as the main purpose of this work is biological activity the results from the PCM-water model calculations will be discussed in more detail.

PCM-Water Calculations

A striking difference in the preferred complexes of these systems was observed when the PCM-water solvation model was used. Though all geometries examined in gas phase were studied in PCM-water (including the varied staggered and eclipsed orientations introduced by the pyridine ring in its complexes), a move away from hydrogen bonded complexes occurred and a number of cation- π stacked complexes were optimised, shown in Fig. 3.1.5. Benzene formed two stable stacked complexes with guanidinium; one termed ‘staggered’ in which the central carbon atom of

guanidinium is over the C-C bonds of benzene, and one termed ‘eclipsed’ in which it is over a carbon atom of the benzene ring. The most favoured complex for guanidinium with naphthalene incorporated the guanidinium carbon atom directly over a carbon atom from the C9-C10 bond. Pyridine formed two stable stacked complexes with guanidinium; one in which the guanidinium carbon is located over the centre of the ring (staggered) and one in which it is located on top of C3 of the pyridine ring (eclipsed), understandably as this is the most electron-rich position of the pyridine ring.

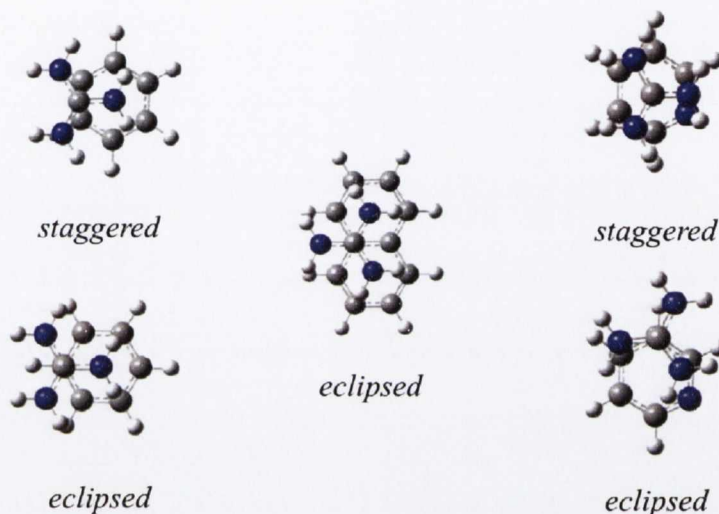


Fig. 3.1.5. Most stable stacked complexes for guanidinium with benzene (*left*), naphthalene (*middle*) and pyridine (*right*).

The interaction energy for these complexes was calculated as the difference between the supermolecule's (complex) energy and the sum of the energies of the isolated monomers in their minimum energy configuration ($E_I = E_{\text{complex}} - [E_{\text{mon}} + E_{\text{mon}}]$). In this way the relative strengths of the complexes of different systems could be evaluated. First of all, comparing the interaction energies of the complexes obtained in gas phase with those obtained in aqueous solution, the interactions in gas phase are always stronger by around 40-60 kJ mol⁻¹. This was expected considering the large influence that solvation has on the interactions established by the guanidinium cation and other molecules. A second explanation is that the introduction of solvent weakens the hydrogen bonded interactions, through lower HB donor ability for the guanidinium NHs, to such an extent that it allows for other weak interactions – such as π -cation

stacking interactions – to be observed, and prevents evolution of stacked complexes to hydrogen bonded ones.

Within the π -cation complexes optimised in PCM, the most stable was that formed with naphthalene (Table 3.1.1), probably due to the increased electron-density of this system compared to either benzene or pyridine, which can effectively stabilise the positive charge of guanidinium. The difference in energy between the staggered and eclipsed complexes of guanidinium with both benzene and pyridine are minimal.

Table 3.1.1. Interaction energies of complexes optimised at the M05-2X/6-311+g(d,p) level in PCM-water solvent model.

Aromatic	Complex with guanidinium	Interaction Energy (kJ mol ⁻¹) ^a
Benzene	<i>staggered</i>	-9.8
	<i>eclipsed</i>	-9.0
Naphthalene	<i>eclipsed</i>	-18.8
Pyridine	<i>staggered</i>	-5.4
	<i>eclipsed</i>	-6.0

^a Defined as the difference between the supermolecule's energy and the sum of the energies of the isolated monomers in their minimum energy configuration.

3.1.2. Complexes of Pyridin-2-ylguanidinium Derivatives and Aromatic Systems

Considering the knowledge gained during our study on the interactions of guanidinium with aromatic systems in PCM-water solvent model, and the importance of such interactions in ligand-receptor binding, we wanted to examine if the preferred complexes of our potential α_2 -AR antagonists would coincide with their pharmacological profile, and in particular if we could draw structure-activity relationships based on these properties.

Aside from our interest in the binding of ligands to the α_2 -AR π -cation interactions have attracted widespread attention elsewhere. Dougherty *et al.* performed a protein database

survey which demonstrated that cation-stabilisation plays an important role in protein structure and function and that the guanidinium-containing Arg is the residue that binds to aromatic residues most often.¹⁷⁸ The π -cation interaction is described for the side chains of the aromatic amino acids (Phe, Tyr, Trp), which provide a surface of negative electrostatic potential that can bind to cations through a predominantly electrostatic interaction. It has also been reported that these aromatic amino acids prefer stacking to hydrogen bonding interactions,¹⁷⁹ indicating that in our molecules competition would exist between cation- π and π - π complexes.

Thus, continuing with the theoretical characterisation of these cation- π interactions a full computational study of the possible π -cation and π - π interactions established by different 5-substituted derivatives of pyridin-2-ylguanidiniums (family A) with benzene and hexafluorobenzene – as examples of electron-rich and electron-depleted aromatic systems – was carried out, employing geometry optimisations, NBO theory, AIM theory and NICS studies. A family of 5-substituted pyridin-2-ylguanidiniums with electronically diverse groups in the 5-position (H, NO₂, CN, Cl, Br, CH₃, ⁱPr) were considered to examine their effects on the complexes formed with benzene. We also investigated the dimers between pyridin-2-ylguanidinium and hexafluorobenzene to see if particular complexes could be induced by controlling the aromatic system.

For this study, based on our previous results which indicate that correct description of these types of interactions requires the inclusion of solvation effects, only PCM-water solvation computations were considered. Initial calculations were performed using the M05-2X functional with the 6-31+G(d,p) basis set and, subsequently, with the higher level M06-2X functional at the 6-311++G(d,p) level of theory, both functionals known to describe weak interactions well. The AIM theory was utilised to analyse the electron-density of the complexes, NBO theory was used to investigate the charge transferred and the perturbation stabilisations involved in the complexes, and NICS was used to probe the effect of complexation on the aromaticity of the pyridine, benzene and hexafluorobenzene rings upon complexation.¹⁸⁰

3.1.2.1. Geometry Optimisations

For this study four types of complex were optimised (Fig. 3.1.6); two π - π stacking complexes with the benzene and pyridine rings either staggered or eclipsed, and two π -cation complexes with the benzene atoms either staggered or eclipsed in relation to the guanidinium nitrogen atoms.

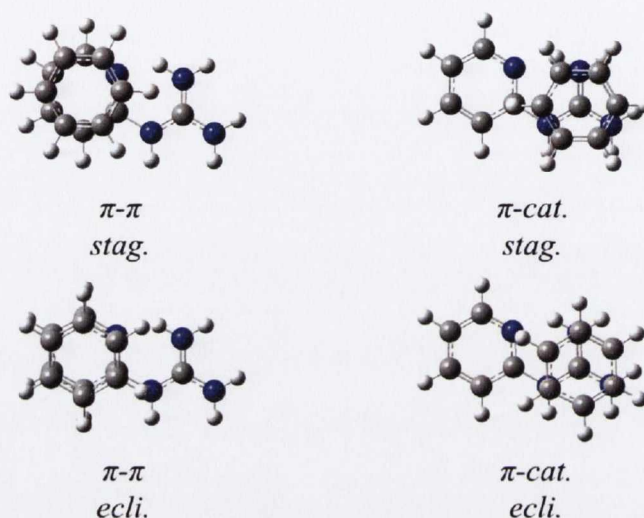


Fig. 3.1.6. Input geometries of the four types of complex examined in this study shown for pyridin-2-ylguanidinium interacting with benzene.

Firstly, the complexes were optimised using the Gaussian09 package¹⁸¹ at the M05-2X and M06-2X computational levels with the 6-31+G(d,p) and the 6-311++G(d,p) basis sets respectively. Frequency calculations were performed at both computational levels to verify that optimised structures were energetic minima. Effects of water solvation were included by means of the SCRF-PCM approach including dispersing, repulsing and cavitating energy terms for the solvent in the optimisation.

In all cases, a slightly distorted eclipsed configuration was the most stable π - π optimised complex whereas the staggered orientation was the most stable of the π -cation interactions (Table. 3.1.2), hence, the discussion will be centred on these more stable complexes. Furthermore, the same patterns were visible at both levels of theory (the M06-2X functional giving slightly larger interaction energies) and so only results

from the higher M06-2X level will be discussed. The interaction energies (E_I) for all complexes are presented in Table 3.1.2.

Table 3.1.2. Geometries of optimised π -cation [π -(+)] and π - π complexes and associated interaction energies (kJ mol^{-1}). Most stable complexes are shown in bold.



Complex	Interaction type	E_I (kJ mol^{-1})
C_6H_6 :5-H-PyrGua	π - π	-19.8
	π -(+)	-21.6
C_6F_6 :5-H-PyrGua	π - π	-35.4
	π -(+)	-24.2
C_6H_6 :5- NO_2 -PyrGua	π - π	-22.3
	π -(+)	-19.1
C_6H_6 :5-CN-PyrGua	π - π	-23.0
	π -(+)	-19.9
C_6H_6 :5-Cl-PyrGua	π - π	-23.0
	π -(+)	-20.8
C_6H_6 :5-Br-PyrGua	π - π	-22.2
	π -(+)	-20.3
C_6H_6 :5- CH_3 -PyrGua	π - π	-19.3
	π -(+)	-21.4
C_6H_6 :5- ^iPr -PyrGua	π - π	-19.2
	π -(+)	-22.3

The nature of the substituent in the five position of the pyridine ring is decisive in the type of complex (either π - π or π -cation) favoured between the pyridin-2-ylguanidinium and benzene. All pyridin-2-ylguanidiniums with inductive electron-withdrawing

substituents in the 5-position (NO_2 , CN , Cl , Br) favoured the π - π interaction of benzene with the pyridine ring over the π -cation interaction with guanidinium. This occurs because these substituents electronically deplete the current ring in pyridine making the interaction with an electron rich ring, such as benzene, more favourable. On the contrary, the unsubstituted pyridine and those with inductive electron-donating substituents (H , CH_3 , ^iPr) in the five position of the pyridine ring favoured complexes with benzene forming π -cation interactions with guanidinium.

The complex formed between the unsubstituted pyridin-2-ylguanidinium and benzene is more stable when benzene is located over the guanidinium cation, with a π -cation interaction that is around 2.0 kJ mol^{-1} stronger than the π - π interaction between benzene and the pyridine moiety. However, when an electron-poor aromatic ring such as hexafluorobenzene is considered, the most stable interaction is the π - π one formed over the pyridine ring, by 11.2 kJ mol^{-1} . This indicates that electron-rich aromatic rings (benzene) prefer π -cation interactions with pyridin-2-ylguanidiniums to π - π ones, whereas electron-poor aromatic systems (hexafluorobenzene) favour π - π interactions with electron-rich systems such as pyridine, a point which is encouraging as all native aromatic amino acids are electron rich. It also has to be pointed out that while the π -cation interactions position the aromatic ring (either benzene or hexafluorobenzene) directly over the guanidinium group, in the π - π complexes the aromatic ring centre is located over the C3-C4 bond of pyridine and not exactly on top of the pyridine moiety, indicating that some π -cation character could exist in these complexes.

It must be pointed out that the difference in energy between π - π and π -cation complexes is not substantial when considering the contributions of such interactions to the binding affinity of a compound to a receptor (preferences for adopting either complex-type were in the range of 1.8 - 3.2 kJ mol^{-1}). However, this is dependent on the functional and the basis set being used and is more instructive in identifying trends than quantitative differences (for example, the *ab initio* MP2 method with an augmented Dunning basis set was tested for some complexes and gave significantly higher interaction energies, though it was too computationally expensive). Thus, it was reasonable for us to expect differences in the pharmacological profiles of these compounds based on their relative abilities to form π -cation interactions with the aromatic amino acids thought to be involved in ligand binding in the active site of the α_2 -AR. The results of those tests are presented in section 3.2.4.

3.1.2.2. Analysis of the Electron Density

The electron density of the complexes was analysed within the AIM theory using the AIMAll program. There are several points of interest to be examined using this approach. The numbering scheme used throughout this and the following sections is shown in Fig. 3.1.7.

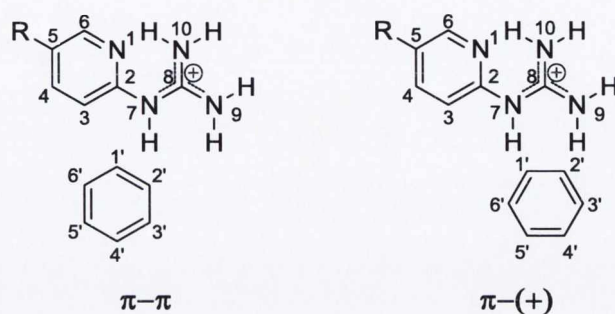


Fig. 3.1.7. Numbering scheme used for $\pi-\pi$ (left) and π -cation (right) complexes.

Effects on the Intramolecular Hydrogen Bond

Firstly, the intramolecular hydrogen bond (IMHB) between the pyridine nitrogen and a guanidinium N-H confers a planar conformation to pyridin-2-ylguanidiniums and the effects of complexation on the strength of this interaction are relevant to how these molecules will behave while interacting with the α_2 -AR (Fig. 3.1.7). The results of a study of this IMHB in both monomers and complexes, presented in Table 3.1.3, show that it is maintained upon complexation. The HB distances are relatively unchanged upon complexation and, in all cases, the electron density (ρ) as well as its Laplacian ($\nabla\rho^2$) at the bond critical point (BCP) are in agreement with a HB interaction, confirming that it is formed both in pyridin-2-ylguanidiniums and in their complexes.

Table 3.1.3. Interaction distances ($\text{\AA}$) and AIM results (ρ and $\nabla\rho^2$ at the BCPs, a.u.) of the IMHB present in pyridin-2-ylguanidinium, its 5-substituted derivatives and the complexes studied at the M06-2X/6-311++G(d,p) level in PCM (water solvent).

	System	d(N $\cdots$ HN)	ρ	$\nabla\rho^2$
PyrGua	<i>monomer</i>	1.91	0.0338	0.1138
C₆H₆:PyrGua	<i>π-π complex</i>	1.92	0.0325	0.1107
	<i>π-(+) complex</i>	1.90	0.0345	0.1156
C₆F₆:PyrGua	<i>π-π complex</i>	1.90	0.0343	0.1154
	<i>π-(+) complex</i>	1.90	0.0345	0.1156
5-NO₂-PyrGua	<i>monomer</i>	1.91	0.0338	0.1162
C₆H₆:5-NO₂-PyrGua	<i>π-π complex</i>	1.91	0.0333	0.1151
	<i>π-(+) complex</i>	1.93	0.0319	0.1119
5-CN-PyrGua	<i>monomer</i>	1.91	0.0339	0.1165
C₆H₆:5-CN-PyrGua	<i>π-π complex</i>	1.91	0.0333	0.1146
	<i>π-(+) complex</i>	1.93	0.0322	0.1124
5-Cl-PyrGua	<i>monomer</i>	1.90	0.0342	0.1161
C₆H₆:5-Cl-PyrGua	<i>π-π complex</i>	1.91	0.0335	0.1144
	<i>π-(+) complex</i>	1.94	0.0313	0.1096
5-Br-PyrGua	<i>monomer</i>	1.90	0.0341	0.1158
C₆H₆:5-Br-PyrGua	<i>π-π complex</i>	1.91	0.0338	0.1152
	<i>π-(+) complex</i>	1.96	0.0302	0.1064
5-CH₃-PyrGua	<i>monomer</i>	1.89	0.0353	0.1165
C₆H₆:5-CH₃-PyrGua	<i>π-π complex</i>	1.92	0.0332	0.1121
	<i>π-(+) complex</i>	1.92	0.0327	0.1110
5-ⁱPr-PyrGua	<i>monomer</i>	1.90	0.0350	0.1158
C₆H₆:5-ⁱPr-PyrGua	<i>π-π complex</i>	1.90	0.0343	0.1140
	<i>π-(+) complex</i>	1.90	0.0341	0.1141

For the pyridin-2-ylguanidinium monomers, electron-donating substituents in the 5-position increase the ρ (BCP) value, indicating a stronger HB interaction, while electron-withdrawing substituents in this position show little change from the unsubstituted case. Across all pyridin-2-ylguanidiniums, complexation has minimal effect on the IMHB distance, with changes in the range of 0.00-0.06 Å. However, decreases in the strength of the IMHB [indicated by decreases in ρ (BCP)] are observed upon formation of π - π complexes, most notably for electron-withdrawing group

substituted pyridin-2-ylguanidiniums. No clear pattern is observed for the value of $\rho(\text{BCP})$ in π -cation complexes, though it can be said that electron-withdrawing substituents in the 5-position lead to decreased values of $\rho(\text{BCP})$.

When comparing the $\rho(\text{BCP})$ values calculated for both types of complexes, the strength of the IMHB in the π -cation complexes of electron-withdrawing substituents is significantly weaker than in the corresponding π - π complexes. On the contrary, a very small decrease of $\rho(\text{BCP})$ (around -0.0005 a.u.) for the IMHB in the π -cation complexes involving electron-donating substituents is observed compared to the corresponding π - π complexes. In the particular case of the unsubstituted pyridine cation- π complex the $\rho(\text{BCP})$ value at the IMHB was found to be larger than in any of the other complexes.

These results indicate that there is a degree of flexibility in the IMHB on complexation, particularly in the π -cation complexes. This is borne out by the observed geometry (Fig. 3.1.8) which displays a deviation from planarity in the pyridin-2-ylguanidiniums to facilitate the stacking interaction with benzene.

Intermolecular Interactions

Further to these intramolecular effects BCPs have been found between the interacting moieties, *i.e.* the aromatic system (either benzene or hexafluorobenzene) and either the pyridine or guanidinium of the pyridin-2-ylguanidinium derivatives. As well, a number of ring and cage critical points (RCP and CCP) were found between both molecular planes (*i.e.* guanidinium/pyridine and aromatic systems).

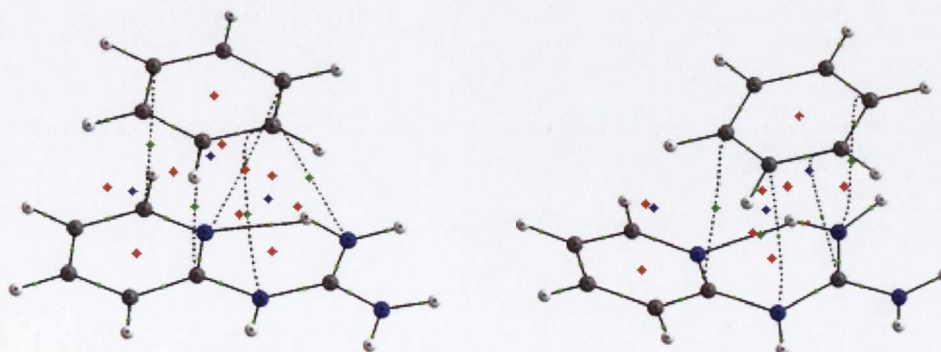


Fig. 3.1.8. Molecular graph (AIM) of the complexes formed between benzene and pyridin-2-ylguanidinium; π - π (*left*) and π -cation (*right*). The IMHB is marked with a solid line. BCPs are in green, RCPs are in red and CCPs are in blue.

In all complexes, the values of the electron density and the Laplacian of the electron density for the BCPs (Table 3.1.4) correspond to weak closed-shell interactions (indicated by positive values).

Table 3.1.4. AIM analysis (interaction type, ρ and $\nabla\rho^2$ at the BCPs, a.u.) of the π - π and π -cation complexes studied at the M06-2X/6-311++G(d,p) level in PCM.

Complexes	Type	Interaction	ρ	$\nabla\rho^2$
C₆H₆:PyrGua	π - π	C2' – N1	0.0059	0.0171
		C3' – N10	0.0074	0.0213
		C6' – C6	0.0076	0.0224
		C3' – N7	0.0076	0.0226
	π -(+)	C4' – C2	0.0086	0.0271
		C3' – N10	0.0075	0.0225
		C4' – C8	0.0075	0.0256
		C5' – N7	0.0077	0.0231
C₆F₆:PyrGua	π - π	C3' – N10	0.0075	0.0232
		F5' – C4	0.0075	0.0282
		C6' – C6	0.0087	0.0261
		F3' – C8	0.0093	0.0420
	π -(+)	C4' – C2	0.0102	0.0328
		C3' – N10	0.0072	0.0207
		C5' – N7	0.0089	0.0285
		F6' – N1	0.0099	0.0413
C₆H₆:5-NO₂-PyrGua	π - π	C1' – N1	0.0060	0.0174
		C1' – N10	0.0076	0.0227
		C2' – N7	0.0080	0.0237
		C4' – C6	0.0083	0.0250
	π -(+)	C3' – C2	0.0087	0.0264
		C1' – N10	0.0068	0.0212
		C3' – N10	0.0076	0.0232
		C5' – C8	0.0080	0.0270
C₆H₆:5-CN-PyrGua	π - π	C6' – N1	0.0086	0.0265
		C3' – N10	0.0070	0.0201

	π -(+)	C6' – C6	0.0082	0.0245
		C4' – C2	0.0084	0.0263
		C3' – N10	0.0073	0.0211
		C4' – C8	0.0074	0.0255
		C5' – N7	0.0084	0.0246
		C6' – N1	0.0089	0.0284
C₆H₆:5-Cl-PyrGua	π - π	C2' – N1	0.0059	0.0170
		C3' – N10	0.0069	0.0193
		C4' – C2	0.0084	0.0259
	π -(+)	C6' – C6	0.0084	0.0251
		C3' – N10	0.0071	0.0209
		C5' – N7	0.0082	0.0246
C₆H₆:5-Br-PyrGua	π - π	C2' – N1	0.0060	0.0174
		C3' – N10	0.0066	0.0184
		C4' – C2	0.0083	0.0253
	π -(+)	C6' – C6	0.0091	0.0278
		C3' – N10	0.0070	0.0209
		C5' – N7	0.0078	0.0239
C₆H₆:5-CH₃-PyrGua	π - π	C6' – C2	0.0092	0.0302
		C3' – N10	0.0062	0.0189
		C3' – N1	0.0083	0.0244
	π -(+)	C5' – C5	0.0085	0.0246
		C3' – N10	0.0073	0.0219
		C5' – N7	0.0083	0.0253
C₆H₆:5-ⁱPr-PyrGua	π - π	C6' – N1	0.0089	0.0292
		H6' – H(ⁱ Pr)	0.0036	0.0119
		C3' – N10	0.0074	0.0224
	π -(+)	C4' – C2	0.0083	0.0254
		C6' – C6	0.0092	0.0272
		C3' – N10	0.0071	0.0205
		C5' – N7	0.0084	0.0252
		C6' – N1	0.0092	0.0301

In general more interactions were found for the π - π complexes than the π -cation complexes, however BCPs were found between the guanidinium central C atom (C8) and the N atoms nearer to the pyridine ring (N7 and N10), and the aromatic C atoms of benzene. The values both of the electron density ρ at the BCPs and its Laplacian $\nabla\rho^2$ are in agreement with weak closed-shell interactions.

In addition, electron density difference maps were calculated for several complexes (Fig. 3.1.9). These maps describe the electronic displacement resulting from the complexation relative to the electronic distribution of the isolated monomers. In

general, the π -cation complexes show large areas of depleted electron density (yellow) under the benzene ring and areas of gained electron density over the guanidinium moiety. Little interaction with the pyridine ring is found; however, more is observed for the pyridine rings substituted with electron-donating groups. The π - π complexes of both electron-donating and electron-withdrawing group substituted pyridines show increased electron density above the pyridine ring. For the 5-NO₂ substituted case significant electron density depletion is observed under the benzene ring whereas, in contrast, the 5-CH₃ substituted pyridine displays more depletion from under the pyridine ring.

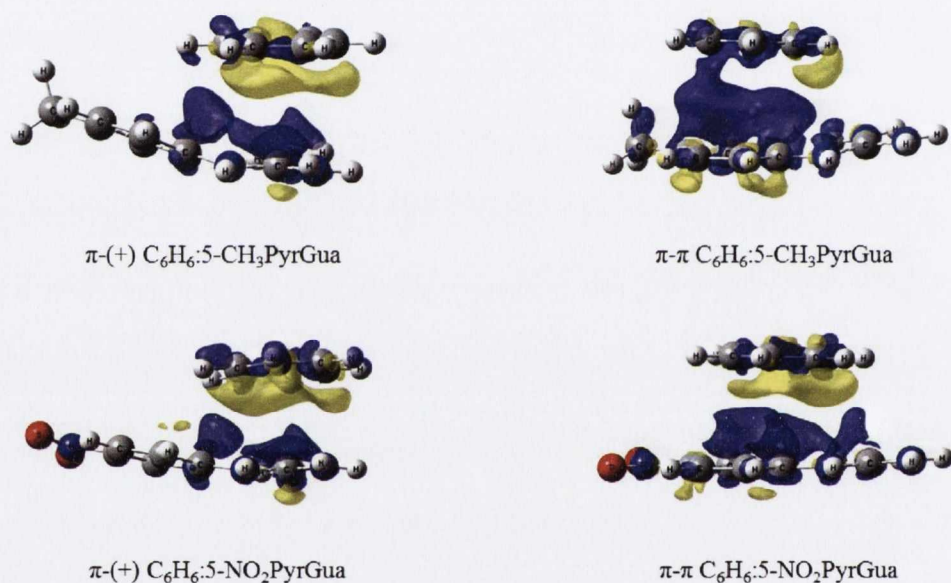


Fig. 3.1.9. Examples of electron density difference maps of π -cation and π - π complexes of benzene with 5-CH₃ (*top*) and 5-NO₂ (*bottom*) pyridin-2-ylguanidiniums. Blue isosurfaces represent gain of electron density and yellow isosurfaces represent loss of electron density on complexation relative to the isolated monomers. Contours shown are 0.0002 e per a.u. calculated at the M06-2X/6-311++G(d,p) level.

Thus, it can be concluded that benzene is acting as a donor of electron density in all π -cation complexes, while in π - π complexes this donation is significantly more pronounced for pyridine rings substituted with electron-withdrawing groups in the 5-position.

3.1.2.3. Natural Bond Orbital Analysis

The NBO analysis of these complexes was carried out to assess the charge transferred and orbital interactions established and the main results are presented in Table 3.1.5. The study of the NBO charges shows that in both π - π and π -cation complexes either none or insignificant charge transfer takes place in any direction, with the exception of both hexafluorobenzene complexes where there is a charge transfer from the pyridin-2-ylguanidinium system (e^- donor) to the electron poor ring (e^- acceptor) as indicated by the tabulated positive values.

Also presented in Table 3.1.5 are the second order perturbation energies [$E(2)$] which describe the stabilisation gained through interaction of donor and acceptor orbitals (BD = bonding orbital, BD* = antibonding orbital, LP = lone-pair, LP* = empty lone-pair).

Table 3.1.5. Orbital energy [$E(2)$, kJ mol⁻¹] and charge transfer (aromatic to pyridin-2-ylguanidinium, positive values).

Complexes	Type ^a		$E(2)$	Charge
C₆H₆:PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	3.9	-0.001
		BD* CC $\leftarrow$ BD* CC	13.4	
	π -(+)	BD CC $\rightarrow$ LP* C _G	5.8	-0.003
		BD* CC $\leftarrow$ BD NC _P	2.2	
C₆F₆:PyrGua	π - π	BD* CC $\rightarrow$ BD* NC _P	27.4	0.321
		BD* CC $\leftarrow$ BDCC	2.0	
	π -(+)	BD CC $\rightarrow$ LP* C _G	4.3	0.319
		BD* CC $\leftarrow$ BD NC _P	3.3	
C₆H₆:5-NO₂-PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	4.8	0.001
		BD* CC $\leftarrow$ BD* CC	6.3	
	π -(+)	BD CC $\rightarrow$ LP* C _G	13.8	0.001
		BD* CC $\leftarrow$ BD* NC _P	3.0	
C₆H₆:5-CN-PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	3.5	0.004
		BD* CC $\leftarrow$ BD* CC	7.4	
	π -(+)	BD CC $\rightarrow$ LP* C _G	8.0	-0.002
		BD* CC $\leftarrow$ BD NC _P	2.1	
C₆H₆:5-Cl-PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	3.6	0.000
		BD* CC $\leftarrow$ BD* CC	6.6	
	π -(+)	BD CC $\rightarrow$ LP* C _G	17.1	-0.003
		BD* CC $\leftarrow$ BD* NC _P	5.1	
C₆H₆:5-Br-PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	3.2	0.001
		BD* CC $\leftarrow$ BD* CC	7.7	
	π -(+)	BD CC $\rightarrow$ LP* C _G	6.9	-0.002

		BD* CC $\leftarrow$ BD* NC _P	2.4	
C₆H₆:5-CH₃-PyrGua	π - π	BD CC $\rightarrow$ BD* CC	3.3	0.003
		BD* CC $\leftarrow$ BD* CC	3.4	
	π -(+)	BD CC $\rightarrow$ LP* C _G	6.9	-0.003
		BD* CC $\leftarrow$ BD NC _P	2.4	
C₆H₆:5-ⁱPr-PyrGua	π - π	BD CC $\rightarrow$ LP* C _G	2.9	-0.001
		BD* CC $\leftarrow$ BD* NC _P	6.6	
	π -(+)	BD CC $\rightarrow$ LP* C _G	6.3	-0.004
		BD* CC $\leftarrow$ BD NC _P	2.4	

^a $\rightarrow$ = From aromatic to pyridin-2-ylguanidinium, $\leftarrow$ = from pyridin-2-ylguanidinium to aromatic.

In the benzene π -cation complexes the most important orbital interactions found are those from the C-C bonding orbitals of benzene to an “empty” lone-pair of the guanidinium C atom (BD CC $\rightarrow$ LP* C_G), indicating a donation from benzene to guanidinium. In the benzene π - π complexes the largest $E(2)$ values observed correspond to the interaction between C-C antibonding orbitals of the pyridine moiety to C-C antibonding orbitals of the benzene ring (BD* CC $\rightarrow$ BD* CC). In these benzene π - π complexes the BD CC $\rightarrow$ LP* C_G interaction is also observed but with a less important contribution.

The complexes of hexafluorobenzene with pyridin-2-ylguanidinium showed the same principal interactions; however, for the π - π complex, the most significant $E(2)$ value was for the interaction between an antibonding C-C orbital of hexafluorobenzene and an antibonding N-C orbital of the pyridine moiety (BD* CC $\rightarrow$ BD* NC_P). Interestingly, as previously observed by us in this type of system, interactions involving donation from orbitals of the guanidinium group towards the aromatic systems are also observed in almost all the complexes studied.

3.1.2.4. Aromaticity – Nucleus Independent Chemical Shift Analysis

The theoretical NICS values were calculated using the GIAO method on the optimised geometries. These corresponding NICS(0), NICS(1) and NICS(2) values (at the ring centre and at 1 Å and 2 Å above the plane respectively) were calculated for the

monomers and the complexes to assess how the aromaticity and electronic ring currents of pyridine, benzene and hexafluorobenzene are affected upon complexation. For the complexes, the NICS(1) and NICS(2) were calculated on the opposite side of the ring to the intermolecular interaction for best representation of the changes to aromaticity (Fig. 3.1.10).

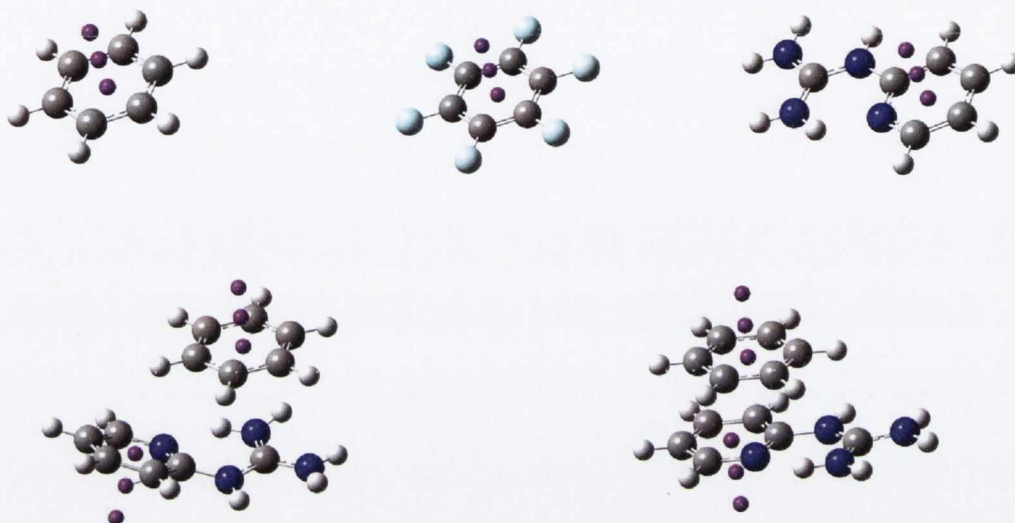


Fig. 3.1.10. Representation of the NICS(0), 1 and 2 in monomers (*top*; C₆H₆, C₆F₆ and pyridin-2-ylguanidinium) and the C₆H₆:PyrGua π -cation (*bottom left*) and π - π (*bottom right*) complexes calculated at the M06-2X/6-311++G(d,p) level in gas phase.

The effect of complexation on the aromaticity of the benzene and hexafluorobenzene rings is presented in Table 3.1.6. The calculated NICS(1) values of the complexes are shown in the first column, followed by the change in this value on complexation with the pyridin-2-ylguanidinium relative to the monomer.

Table 3.1.6. NICS(1) of the aromatic moiety (C₆H₆ and C₆F₆)^a calculated at the M06-2X/6-311++G(d,p) level in gas phase. NICS(1) differences between the monomer and complexes ($\Delta_{\text{NICS(1)}}$) for C₆H₆ and C₆F₆ are also indicated.

	π -(+)	π -(+)	π - π	π - π
	NICS(1)	$\Delta_{\text{NICS(1)}}$	NICS(1)	$\Delta_{\text{NICS(1)}}$
C ₆ H ₆ :PyrGua	-10.65	-0.10	-10.75	-0.20

C₆F₆:PyrGua	-12.19	0.27	-12.65	-0.19
C₆H₆:5-NO₂-PyrGua	-10.55	-0.00	-10.96	-0.41
C₆H₆:5-CN-PyrGua	-10.55	-0.00	-10.81	-0.26
C₆H₆:5-Cl-PyrGua	-10.56	-0.01	-10.77	-0.22
C₆H₆:5-Br-PyrGua	-10.64	-0.09	-10.72	-0.17
C₆H₆:5-CH₃-PyrGua	-10.64	-0.09	-10.72	-0.17
C₆H₆:5-ⁱPr-PyrGua	-10.70	-0.15	-10.85	-0.30

^a NICS(1) index for C₆H₆ is -10.55 and for C₆F₆ is -12.46.

In the π - π complexes with unsubstituted pyridin-2-ylguanidinium, both benzene and hexafluorobenzene slightly increase in aromaticity (move to larger negative value) upon complexation. For the π -cation complexes a similar increase in the aromaticity of benzene is observed; however, a decrease occurs for hexafluorobenzene. This seems counter intuitive to the NBO results which showed that the hexafluorobenzene donates a lesser amount than benzene does in its orbital interactions [$E(2)$] with the guanidinium, suggesting lesser effects on its electronic properties such as aromaticity. The AIM results coincide with the observed changes in NICS(1), however. At the BCPs between the aromatic ring and guanidinium of the π -cation complexes, larger ρ values are observed for the hexafluorobenzene complex than the benzene complex, indicating stronger interactions which could disrupt the aromaticity of hexafluorobenzene.

For the π -cation complexes of benzene with 5-substituted pyridin-2-ylguanidiniums, little or no change is observed in the NICS(1) values on complexation relative to the benzene monomer. However, in the π - π complexes of these subunits a clear increase is observed in the aromaticity of the benzene ring.

The changes to the aromaticity of the pyridine ring in the complexes of pyridin-2-ylguanidiniums with benzene and hexafluorobenzene relative to the monomers are presented in Table 3.1.7. These results show that, in the π -cation complexes, a decrease in the aromaticity of the pyridine ring is observed, indicating that the interaction between the attached guanidinium and the aromatic cycle disrupts its aromaticity. In contrast, the π - π interactions between pyridine and both aromatic systems have a

synergistic effect in all complexes studied resulting always in increased aromaticity of the pyridine moiety.

Table 3.1.7. NICS(1) for the pyridine ring calculated at the M06-2X/6-311++G(d,p) level in gas phase. NICS(1) differences between the monomer and complexes ($\Delta_{\text{NICS(1)}}$) for the pyridine ring are also indicated.

	Pyr. mon. NICS(1)	π -(+) NICS(1)	π -(+) $\Delta_{\text{NICS(1)}}$	π - π NICS(1)	π - π $\Delta_{\text{NICS(1)}}$
C₆H₆:PyrGua	-10.24	-10.11	0.13	-10.78	-0.54
C₆F₆:PyrGua	-10.24	-10.17	0.07	-10.34	-0.10
C₆H₆:5-NO₂-PyrGua	-10.36	-10.24	0.12	-10.99	-0.63
C₆H₆:5-CN-PyrGua	-10.21	-10.04	0.17	-10.73	-0.52
C₆H₆:5-Cl-PyrGua	-10.01	-9.95	0.06	-10.47	-0.46
C₆H₆:5-Br-PyrGua	-9.88	-9.71	0.17	-10.24	-0.36
C₆H₆:5-CH₃-PyrGua	-10.22	-10.13	0.09	-10.79	-0.57
C₆H₆:5-ⁱPr-PyrGua	-10.58	-10.16	0.42	-10.61	-0.03

3.1.2.5. Conclusions

The computed interaction energies (E_i) show that electron-rich aromatic rings such as benzene prefer π -cation interactions with pyridin-2-ylguanidiniums over π - π ones, whereas electron-poor aromatic systems like hexafluorobenzene favour π - π interactions with the pyridine moiety of these systems. They also indicate that more electron-rich pyridin-2-ylguanidiniums favour π -cation complexes over π - π complexes, while the opposite is true for electron-poor pyridin-2-ylguanidiniums.

The AIM analysis of the interactions indicates that the characteristics and strength of the IMHB formed between the guanidinium moiety and the pyridine N atom is affected but maintained upon complexation with the aromatic rings. Additionally, a number of BCPs were found between the stacking systems indicating that either π - π or π -cation complexes are being formed. The flow of electron density between the monomers of

some of the complexes studied, visualised by means of electron density difference maps, shows that benzene acts as a donor of electron density, particularly in π -cation complexes.

Natural Bond Orbital analysis provided an understanding of the nature of the orbital interactions between the π and cation systems, finding that in the benzene π -cation complexes $\text{BD CC} \rightarrow \text{LP}^* \text{C}_G$ are the most important orbital interactions – indicative of a donation from the aromatic system to guanidinium – while the most important interaction in the benzene π - π complexes is $\text{BD}^* \text{CC} \rightarrow \text{CC}^* \text{CC}$, corresponding to stabilisation due to overlap of antibonding orbitals from both units of the complex.

The NICS calculations show that, in π - π complexes, benzene, hexafluorobenzene and pyridine synergistically increase in aromaticity upon complexation. On the contrary, in π -cation complexes an increase in the aromaticity of benzene is observed whereas a decrease is found in the pyridine moiety and perfluorinated ring.

With an understanding of these complexes it was hoped to gain structure-activity relationships on synthesis and pharmacological testing of these compounds, and to determine if π -cation interactions are likely to play a part in ligand binding to the α_2 -AR. A discussion on the pharmacological profile of these molecules and their relationship to this data is presented in Section 3.2.

3.2. Design, Synthesis and Pharmacological Evaluation of Pyridin-2-yl and Pyridin-3-ylguanidine Hydrochlorides (families A and B, respectively)

Based on the pharmacological results previously obtained for compounds in Rozas' group^{156,157,158} a family of pyridin-2-yl and pyridin-3-yl guanidine hydrochlorides with coherent variations in the substituent *para* to the guanidine was decided upon (Fig. 3.2.1). Pyridine analogues of the pharmacologically successful tetrahydronaphthalenyl and *N*-ethylphenyl guanidine hydrochlorides, as well as the unsubstituted, *para*-methyl and *para*-chloro pyridin-yl guanidine hydrochlorides were chosen. With these families it was hoped that several pieces of SAR information could be learned. Firstly, the effects of introducing the pyridine ring could be inferred by comparison of their pharmacological profile to that of the phenyl guanidine hydrochlorides. Pyridine has altered aromaticity, as well as the possibility of HB acceptor interactions. Secondly, replication of these effects could be tested for by varying the nature of the *para* substituent; hence, an inductive electron-withdrawing (Cl) and electron-donating (CH₃) substituents were initially chosen to screen this. Any pharmacological effects based on the *para* substituent could also be concluded from this. Thirdly, the effects of molecular conformation on pharmacological properties could be examined due to the differing conformational preferences of pyridin-2-yl and pyridin-3-yl guanidine hydrochlorides.

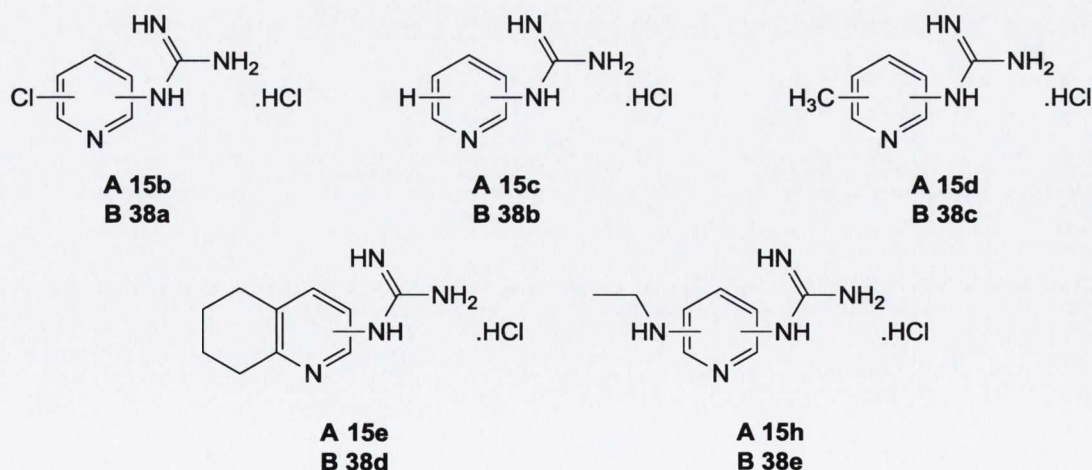


Fig. 3.2.1. Original target compounds; family **A** – 5-substituted pyridin-2-ylguanidine hydrochlorides, family **B** – 6-substituted pyridin-3-ylguanidine hydrochlorides.

As the pK_{aH} of aryl guanidines is in the region of 11-13, it can be assumed that they will be protonated at physiological pH and will exist in the guanidinium form. Thus, when considering the interactions of these compounds with the active site of the α_2 -AR, they must be considered in terms of a guanidinium instead of a guanidine. Within pyridin-2-ylguanidiniums the possibility exists for an IMHB between the pyridine nitrogen and an NH of the guanidinium, forming a *pseudo* six-membered ring (Fig. 3.2.2, *left*) and inducing a coplanar arrangement of the pyridine ring and the guanidinium moiety. In pyridin-3-ylguanidiniums, the opposite is the case; a coplanar arrangement is unfavoured as it would bring the guanidinium NH in close proximity to an aromatic proton. Thus, as is the case for phenyl guanidiniums, an out of plane arrangement of pyridine ring and guanidinium is adopted to minimise this repulsion (Fig. 3.2.2, *right*). Undoubtedly, this will be the conformation adopted in aqueous solution; however, in order to prove that pyridin-2-ylguanidiniums would maintain their coplanar conformation in the aqueous environment of the pharmacological tests a full structural study was conducted involving DFT calculations, nuclear magnetic resonance (NMR) spectroscopy studies and X-ray crystallography (see Section 3.2.2).

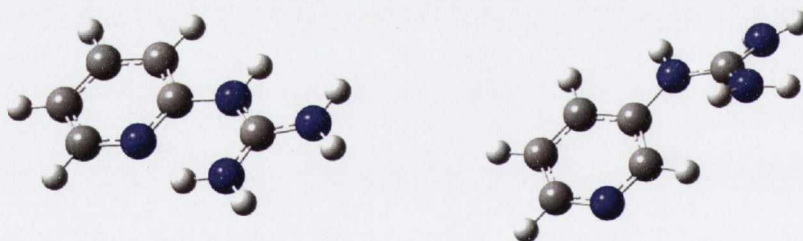


Fig. 3.2.2. Conformational effects between pyridin-2-yl (**A**, left) and pyridin-3-yl (**B**, right) guanidiniums occur due to interactions between guanidinium NHs and the pyridine ring.

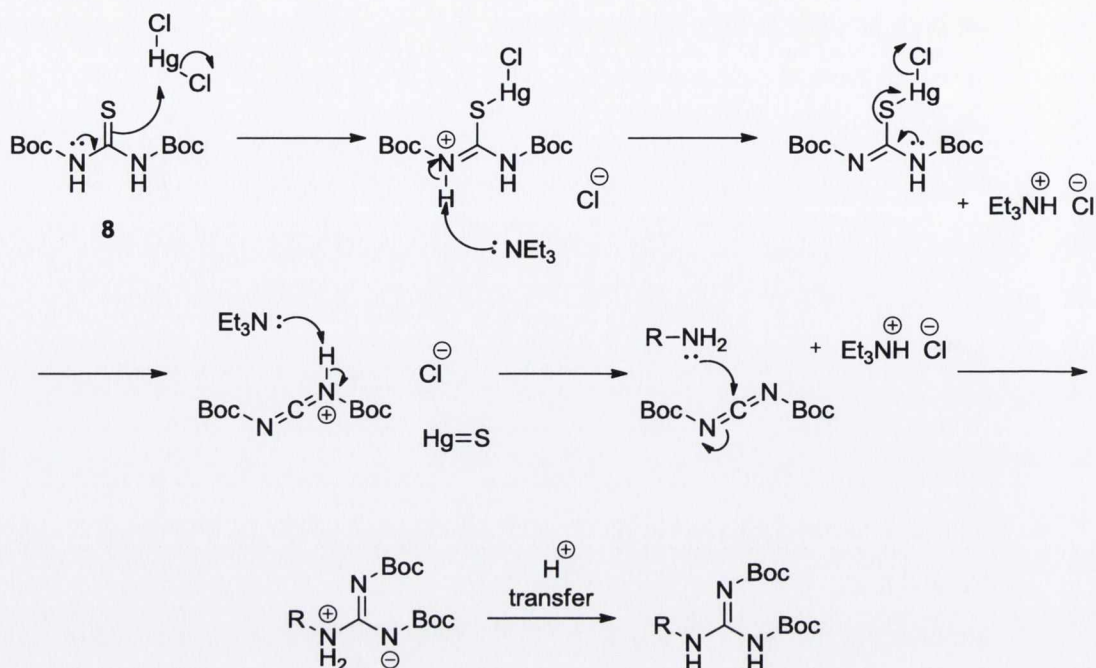
We expected family **A** to have superior pharmacological profile to family **B** due to these phenomena. Making ligands with restrained conformations is a well exploited strategy for obtaining antagonists of receptors as these molecules can often bind competitively to the active site but cannot undergo the receptor-induced movements which lead to receptor activation. Thus family **B** was preserved to the initial target compounds; however, family **A** was extended to include 5-Br, 5-NO₂ and 5-NH₂

pyridin-2-ylguanidine hydrochlorides so that further information on the requirements for high binding affinity and antagonistic activity at the α_2 -AR could be obtained. These members were also added to family **A** to assist in the structural study of such pyridin-2-ylguanidiniums (see Section 3.2.2).

3.2.1. Synthesis of pyridin-2-yl and pyridin-3-ylguanidines

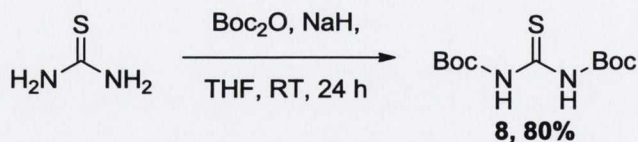
The majority of molecules from families **A** and **B** were synthesised using a method developed by Kim and Qian¹⁸² for conversion of amines to guanidines using thiourea derivatives and mercury(II) chloride. In this procedure the amine is reacted with *N,N'*-bis-(*tert*-butoxycarbonyl)thiourea **8** in the presence of mercury(II) chloride and triethylamine to generate *bis*-Boc-protected guanidines. The Boc groups on the thiourea are necessary to increase the electrophilicity of the thiourea carbon, making this method very suitable for poorly reactive aryl amines. In the original Kim and Qian paper describing this, it was suggested that the reaction proceeds through a carbodiimide intermediate, formed after desulfurisation of the thiourea derivative. This carbodiimide is then thought to undergo nucleophilic attack by the amine, leading to the *bis*-Boc protected guanidine (Scheme 3.2.1) on proton exchange.

Scheme 3.2.1.



This procedure requires the preparation of **8** by treatment of thiourea with di-*tert*-butyldicarbonate and sodium hydride (Scheme 3.2.2). This is a straightforward and high yielding reaction; however, the commercially available *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea **9** (Table 3.2.1) can be used in its place with no detrimental effect to the guanidylation, and thus, it is employed in our lab where possible.

Scheme 3.2.2.



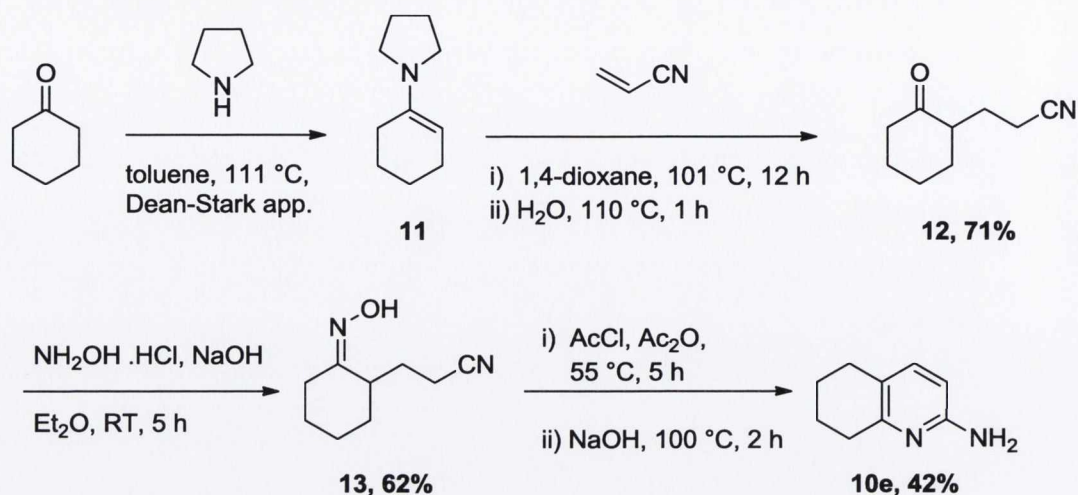
While the use of toxic mercury-containing reagents – and the storage and disposal problems this entails – is undesirable, the utility and efficiency of the reaction cannot be denied. Experimentally the reaction conditions are mild and the workup is straightforward; the mercury sulfide and any remaining mercury(II) chloride are removed by filtration through a bed of celite, aqueous workup and flash column chromatography then afford the product in reasonable to high yield.

This method is particularly useful for the guanidylation of deactivated aryl amines, where other methods fail. Its reliability means that it is still the method of choice for guanidylation of sensitive amines (for a full discussion see Section 3.6, where; new synthetic methodologies for the guanidylation of amines are presented). Another advantage of the synthesis is that the removal of the Boc groups to reveal the biologically relevant guanidine hydrochlorides is well studied and several methods exist which allow the deprotection of sensitive guanidines which are prone to decomposition (see below).

Pyridin-2-ylguanidine Hydrochlorides – family A

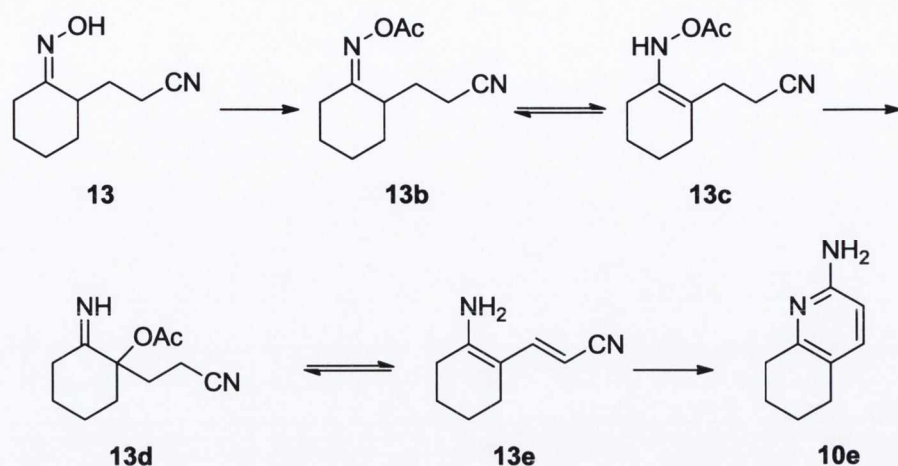
Thus, the mercury(II) chloride promoted synthesis of *bis*-Boc protected guanidines was employed for the synthesis of the majority of family **A** from the corresponding amines **10a-e**. Unsubstituted 2-aminopyridine and those substituted in the 5-position with chlorine, bromine and methyl groups (**10a-d**) were commercially available, while 5,6,7,8-tetrahydroquinoline **10e** was synthesised using a literature procedure (Scheme 3.2.3).¹⁸³ The pyrrolidine enamine of cyclohexanone **11** was synthesised in a reflux condenser fitted with a Dean-Stark water separator and immediately reacted – without purification – with acrylonitrile to give **12** after hydrolysis of the enamine back to the carbonyl. This was then converted to its oxime **13** by reaction with hydroxylamine hydrochloride free-based using sodium hydroxide. In the key step to the synthesis, **13** was reacted with acetyl chloride and acetic anhydride (as solvent) to form *N*-acyl-5,6,7,8-tetrahydroquinolin-2-amine, which was subsequently hydrolysed *in situ* in aqueous sodium hydroxide to give **10e** in low overall yield, similar to the published results.

Scheme 3.2.3.

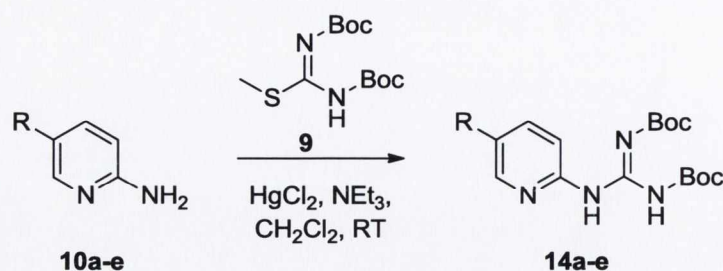


The mechanism for this key step is thought to go by initial acetylation of **13** to give the *O*-acetyl oximino nitrile **13b**, which is in tautomeric equilibrium with *N*-acetoxy enamine **13c** (Scheme 3.2.4). This enamine is then set up to undergo a sigmatropic rearrangement to yield α -acetoxy imine **13d**. Elimination of acetic acid and isomerisation give the more stable enamine nitrile **13e**, which can cyclise *via* a 6-exo dig mechanism when the *Z,Z*-diene geometry is adopted (isomerism is possible due to the presence of HCl in the mixture), the enamine nitrogen nucleophilically attacking the nitrile carbon. Excess acetyl chloride or acetic anhydride acetylates the resulting amine, which can then be hydrolysed as described to regenerate pyridin-2-amine **10e**.

Scheme 3.2.4.

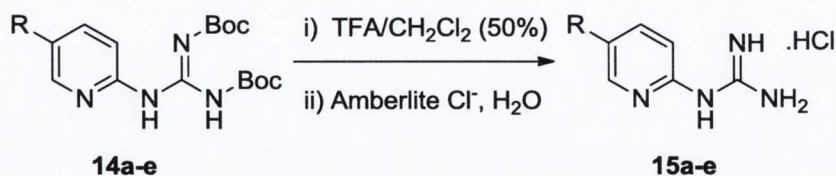


The mercury(II) chloride promoted guanidylation of these amines was affected in good to excellent yield (Table 3.2.1), with variations based on the reactivity of the starting amine; more reactive amines gave higher yields while less reactive amines gave poorer yields. The reaction was performed in dichloromethane using the commercially available isothiurea **9** and triethylamine as base to afford the corresponding *N,N'*-bis-Boc-protected intermediates **14** (Table 3.2.1). It should be noted that the yields below for **10a**, **10b** and **10c** are for material purified by silica gel chromatography and recrystallisation from hexane:EtOAc, which was necessary to attain the necessary purity for generating the guanidine hydrochlorides **15** (Table 3.2.2).

Table 3.2.1. Synthesis of *bis*-Boc pyridin-2-ylguanidines **14a-e**.

Compound	R	Yield (%)
14a	Br	80
14b	Cl	35
14c	H	79
14d	CH ₃	71
14e	5,6-(-CH ₂ -) ₄	90

The removal of the Boc groups (Table 3.2.2) was performed using a 25% (v/v) solution of trifluoroacetic acid (TFA) in dichloromethane with a large excess of TFA (approximately 30 equivalents). This yielded the guanidine trifluoroacetate salts, which are generally not water-soluble, and thus, ion exchange was necessary to obtain the chloride salt, a more suitable compound for pharmacological testing. This was completed by stirring the salt in water and Amberlite® IRA-400 resin – a polystyrene bead – in its chloride activated form (available from Sigma-Aldrich). After 24 h removal of the resin by filtration generally yielded the guanidinium chloride salt, though in some cases a subsequent stirring in the resin was required for full conversion of the trifluoroacetate salt. This was checked for using ¹⁹F NMR spectroscopy, the absence of any peaks in this spectrum confirming full conversion.

Table 3.2.2. Generation of Pyridin-2-ylguanidine hydrochlorides **15**.

Compound	R	Yield (%)
15a	Br	74
15b	Cl	85
15c	H	71
15d	CH ₃	84
15e	5,6-(-CH ₂ -) ₄	87

Once all traces of the trifluoroacetate were removed, the crude hydrochloride salt was purified using small scale reverse phase chromatography with C-8 silica. Using 100% water as mobile phase was normally sufficient to elute the hydrochloride salt; where necessary solutions of water and acetonitrile (90:10) were used to elute less polar derivatives. Compounds were recovered pure after reverse phase chromatography; however, recrystallisation was often performed to ensure purity and gain crystals for X-ray analysis.

Recrystallisation of guanidinium salts is notoriously difficult due to their highly hygroscopic nature; however, several specimens suitable for X-ray analysis were obtained and all yields in Table 3.2.2 are for recrystallised material. The most successful technique to obtain crystals proved to be a slow diffusion of diethyl ether into a conical flask containing the salt dissolved in a minimum amount of either methanol or isopropanol in a sealed jar.

5-N-substituted Pyridin-2-ylguanidine Hydrochlorides

Not all members of family **A** were attainable using this method. Those compounds which were substituted with either NO₂, NH₂, or NH₂Et in the 5-position of the pyridine

ring all proved problematic. While the corresponding 2-aminopyridine for each of these substituents could be synthesised (see Section 3.3.2), the guanidylation was unsuccessful in each case. For 2-amino-5-nitropyridine, no reaction occurred on exposure to our guanidylation conditions. The electron-withdrawing nature of the nitro functionality deactivated the amine in the 2-position to such an extent that no product formation was observed.

A different problem prevented the formation of the amino derivative. The amine in the 5-position of the pyridine ring of **16** is much more reactive than the amine in the 2-position and substitution occurred solely at the 5-position. This occurs because the amine in the 2-position is in conjugation with the pyridine nitrogen (Fig. 3.2.3). The 2-aminopyridine tautomer is much more favoured, with predominance over the imino tautomer of 1000:1;¹⁸⁴ however, this delocalisation is enough to reduce the nucleophilicity of the amine. In particular, compared to the amine in the 5-position, which is not in conjugation with the pyridine nitrogen, it is much less reactive.

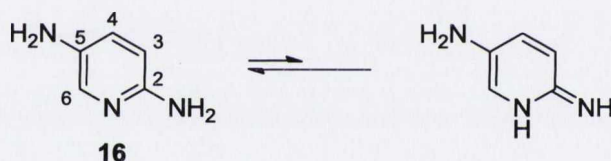
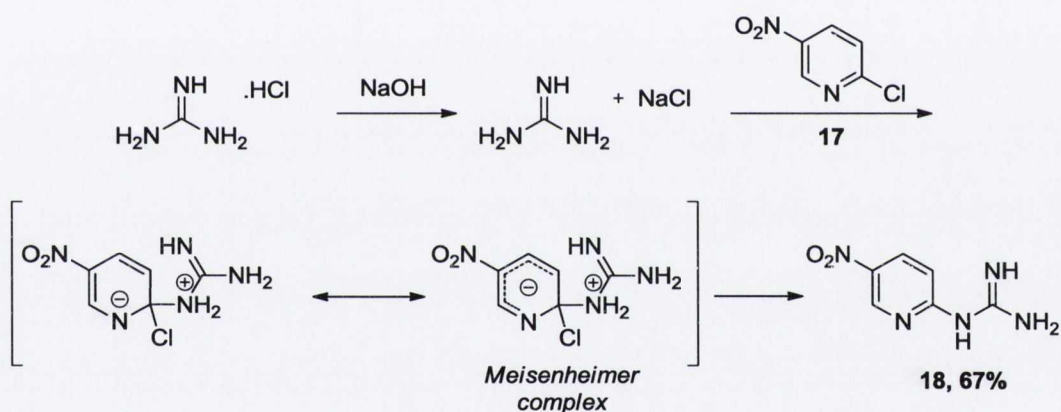


Fig. 3.2.3. Tautomerism between the amino and imino isomers reduces the reactivity of the amine in the 2-position, directing electrophilic substitution to the 5-position.

The possibility of buffering the reaction conditions to direct substitution to the amine in the 2-position was investigated, however this was unsuccessful. Despite being more nucleophilic, the amine in the 5-position is less basic (pK_{aH} 6.03) than that in the 2 position (pK_{aH} 6.71)¹⁸⁵ due to stabilisation of the conjugate acid by delocalisation into the pyridine ring. In any case, the difference in pK_{aH} values is quite small meaning no selectivity would likely be obtained.

Alternative Route to 5-N-substituted Pyridin-2-ylguanidine Hydrochlorides

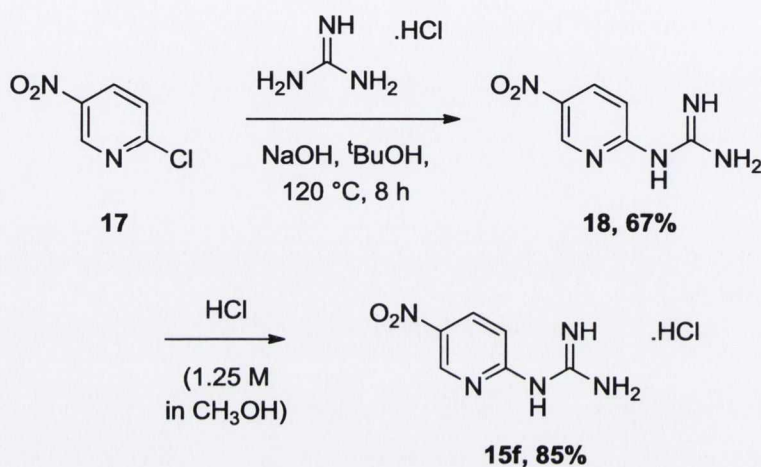
Taking advantage of the electrophilicity of the 2-position of pyridines, we hoped to introduce the guanidine functionality using nucleophilic aromatic substitution (NAS). In our experience, the free-base of guanidine is poorly nucleophilic (no reaction was observed in attempts at NAS on 4-nitro-fluoroaniline). However, using 2-chloro-5-nitropyridine it was expected that at least some reaction would occur (Scheme 3.2.5). Both the pyridine nitrogen and the nitro substituent *para* to the electrophilic site are ideal for stabilising the Meisenheimer complex that would be formed on addition of guanidine to the carbon in the 2-position (Scheme 3.2.5).

Scheme 3.2.5

Performing the reaction at 83 °C in *tert*-butanol, guanidine hydrochloride and sodium hydroxide were mixed together. Sodium hydroxide is adequately basic to deprotonate the guanidinium cation (pK_{aH} 13.6) and to liberate free guanidine. After 30 minutes 2-chloro-5-nitropyridine **17** was added and none of this starting material was visible by TLC after six hours (Scheme 3.2.6). The highly polar nature of the product meant that a mixture of isopropanol and dichloromethane (20% v/v) was required to extract the product and only water basified with sodium hydroxide was used to wash the organic layer. Even with these precautions product was without doubt lost to the aqueous layer and the yield for neutral guanidine **18** was variable (best yield 73%). Furthermore, purification proved unsuccessful (not being amenable to any of recrystallisation, normal phase or reverse phase chromatography) and it was eventually decided to form the

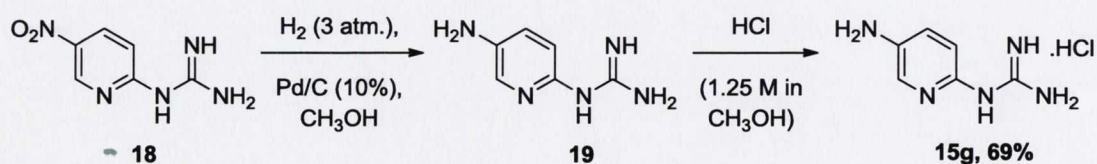
hydrochloride salt **15f** first and then purify it. Thus, **18** was stirred in an excess of hydrochloric acid (1.25 M solution in methanol) and purified using reverse phase silica chromatography to yield the guanidinium salt of biological interest (Scheme 3.2.6).

Scheme 3.2.6.



A similar strategy was employed for the preparation of 5-amino-pyridin-2-ylguanidine hydrochloride **15g** (Scheme 3.2.7); un-purified neutral guanidine **18** was directly hydrogenated at three atmospheres in methanol using catalytic amounts of palladium on carbon. After full conversion of starting material, removal of palladium and carbon by filtration and removal of methanol under reduced pressure yielded the crude product which was stirred in excess hydrochloric acid (1.25 M in methanol) to generate guanidine hydrochloride **15g**. This was subsequently purified by reverse phase chromatography and recrystallisation from methanol/diethyl ether.

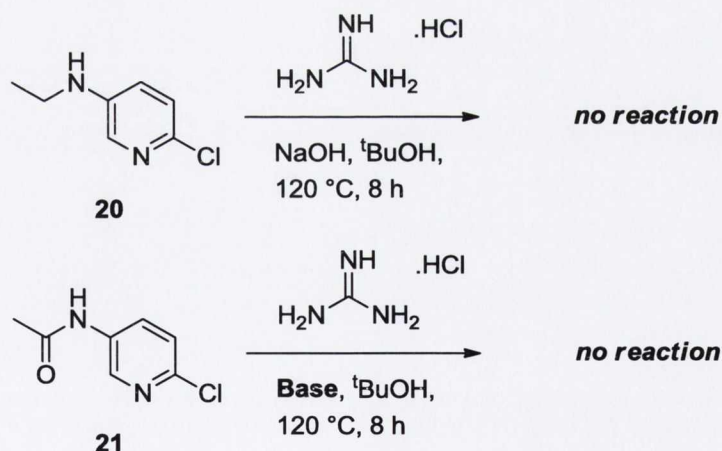
Scheme 3.2.7.



One of the principal target compounds 5-(*N*-ethylamino)pyridin-2-ylguanidine hydrochloride **15h** proved the most difficult member of family **A** to attain. Initial attempts went through 2,5-diaminopyridine **16** and alkylation of the amine in the 5-position which successfully led to 2-amino-5-(*N*-ethylamino)pyridine (see Section 3.3). However, even with a secondary amine in the 5-position, the reactivity pattern of 2,5-diaminopyridines was maintained, and exposure to our guanidylation conditions led exclusively to substitution of the amine in the 5-position.

Subsequent attempts aimed to exploit the success achieved with NAS reactions of 2-chloropyridines. Substitution of both 2-chloro-5-(*N*-ethylamino)pyridine **20** and 2-chloro-5-(*N*-acetyl)pyridine **21** – having in place either the *N*-ethylamino group or a masked version respectively – proved fruitless (Scheme 3.2.8); it seems that the electron-withdrawing nitro substituent in the 5-position is a decisive factor for NAS to occur. Hydrolysis of the amide of **21** was observed when sodium hydroxide was used; however, changing the base to either potassium *tert*-butoxide, sodium hydride or sodium hexamethyldisilazide gave no reaction.

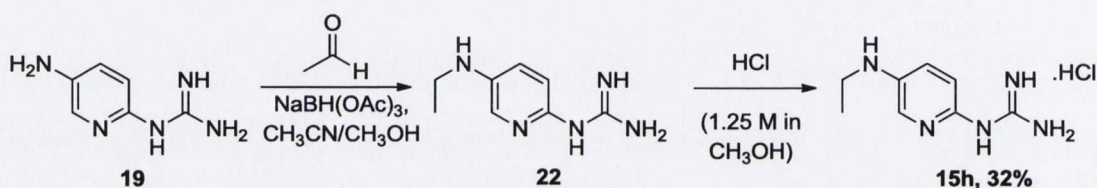
Scheme 3.2.8.



Eventually, reductive alkylation of the amine of neutral guanidine **19**, followed by protonation in hydrochloric acid yielded the desired product **15h** (Scheme 3.2.9), though in poor yield. Under an argon atmosphere the neutral, unpurified, amino guanidine **19** was reacted with acetaldehyde and triacetoxyborohydride in a mixture of

acetonitrile and methanol (5:1), which was used to aid solubility. After extraction with isopropanol/dichloromethane (20%) and washing with saturated sodium hydrogen carbonate the crude residue **22** was stirred in excess hydrochloric acid (1.25 M in methanol) to yield a red solid identified as a mixture of *mono* and di-alkylated ammonium salts. Normal phase silica gel chromatography using a gradient of dichloromethane to dichloromethane/[chloroform:methanol:ammonia, 80:20:3] (50%) successfully separated these salts to afford **15h** after re-stirring in hydrochloric acid (1.25 M in methanol) to ensure full protonation of the guanidine.

Scheme 3.2.9.



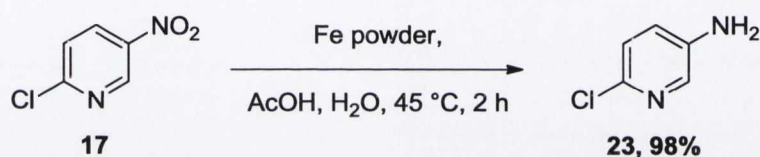
The extent of manipulation meant that only 32% yield was obtained. Some of this can be attributed to di alkylation of the amine (which occurred even with 1.0 equivalents of acetaldehyde); however, it seems that **15h** is inherently unstable and that degradation is an issue. Nevertheless, sufficient material was obtained to carry out the pharmacological tests.

Pyridin-3-ylguanidine Hydrochlorides – Family B

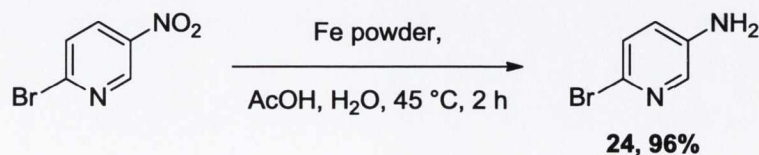
Pyridin-3-ylguanidines were synthesised in a similar fashion. In this case all relevant pyridin-3-ylamines were tolerant to the mercury(II) chloride promoted conditions of guanidylation. Only 3-aminopyridine was available commercially for a reasonable price so synthesis of the four remaining pyridin-3-amines was undertaken.

6-Chloro-3-aminopyridine, 23

Having used 2-chloro-5-nitropyridine **17** as a substrate for NAS in the synthesis of **15f-15h**, this compound was available in large quantities and would afford **23** on reduction of the nitro group. Catalytic hydrogenation, the preferred method of reduction, was not an option as it would almost certainly cleave the C-Cl bond as well, leading to 3-aminopyridine. Thus, a dissolving metal reduction using iron powder and acetic acid in water was employed (Scheme 3.2.10). The reaction was extremely clean and gave **23** after aqueous workup, without the need for further purification, in almost quantitative yield (98%).

Scheme 3.2.10.*6-Bromo-3-aminopyridine, 24*

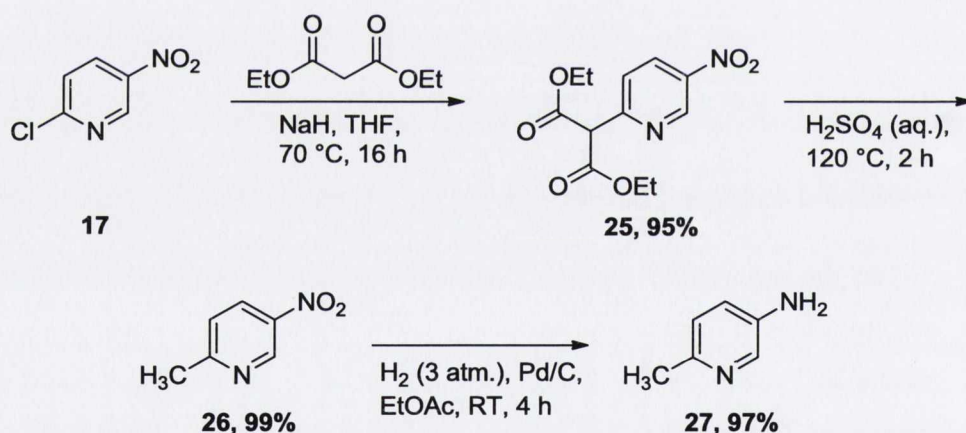
Given the success of this reaction and the commercial availability of 2-bromo-5-nitropyridine, the same approach was taken for the synthesis of 6-bromo-pyridin-3-amine **24**. Previously, the substrate had degraded when zinc powder and acetic acid had been used. However the iron/acetic acid reaction was equally effective for this substrate as it had been for the chloro-derivative and **24** was afforded in 96% yield (Scheme 3.2.11).

Scheme 3.2.11.

6-Methyl-3-aminopyridine, 27

In this case, the nitro derivative was not an option as a precursor; hence, NAS of diethylmalonate on 2-chloro-5-nitropyridine **17** was employed to introduce a group which could reveal the methyl group to the pyridine ring on decarboxylation. For this reaction (Scheme 3.2.12), sodium hydride was used as base to avoid hydrolysis of the esters and produce deprotonation of the α -carbon (pK_a 12.9). The reaction proceeded in extremely high yield (after 16 h at 70 °C in tetrahydrofuran), a 96% yield of **25** being obtained after purification by silica gel chromatography. Subsequent reflux in concentrated aqueous sulfuric acid yielded 2-methyl-5-nitropyridine **26** in 99% yield, which was stable enough to be reduced using catalytic hydrogenation in ethyl acetate to afford **27** in excellent yield.

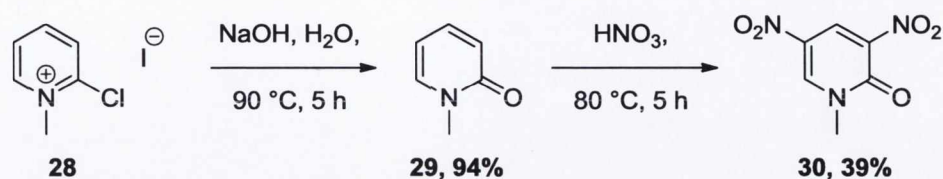
Scheme 3.2.12.

*5,6,7,8-Tetrahydroquinolin-3-amine, 32*

A synthesis of 5,6,7,8-tetrahydroquinolin-3-amine **32** was reported by Tohda *et al.* from 3,5-dinitro-*N*-methylpyridin-2-one **30**. This compound was highly expensive but could be synthesised from Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide, **28**) following alkaline hydrolysis and nitration (Scheme 3.2.13). Hydrolysis of **28** in aqueous sodium hydroxide led to *N*-methylpyridin-2-one **29** in high yield; however, the nitration was affected in poor yield (39%). It is assumed that the first nitration is relatively easy as compound **29** behaves like an electron-rich pyridine system and EAS

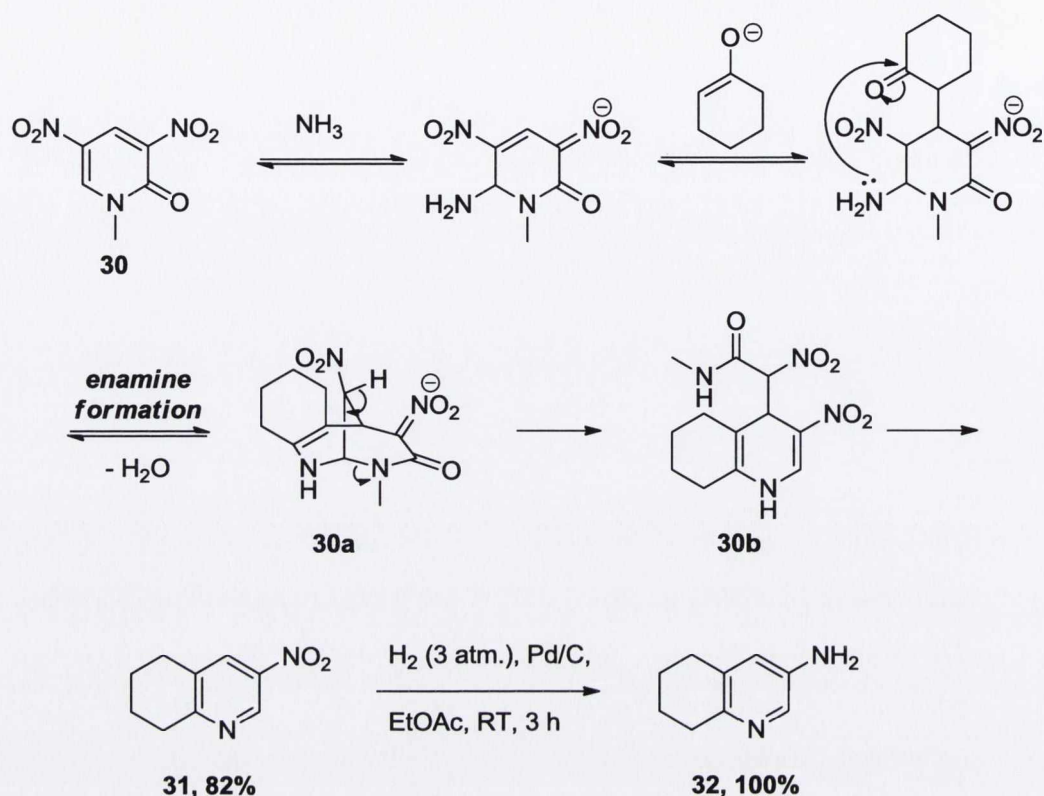
is reasonably favoured. The second nitration is slow, even in fuming nitric acid at 80 °C; however, it does lead to **30**, the substrate for the synthesis of **32**.

Scheme 3.2.13.



The synthesis described by Tohda *et al.*¹⁸⁶ combines **30** with cyclohexanone and ammonia in methanol to give 3-nitro-5,6,7,8-tetrahydroquinoline **31** (Scheme 3.2.14). The proposed mechanism involves initial conjugate-addition of ammonia at the 6-position of **30**, followed by a second conjugate addition of the enolate of cyclohexanone (generated by a second molecule of ammonia acting as base) to the 4-position to form intermediate **30a**, which can ring open with loss of an acetamide leaving group to dihydropyridine **30b** that aromatises to **31** on loss of the stabilised anion of *N*-methyl- α -nitroacetamide. This step was completed in 82% yield, consistent with the reported results. Subsequent reduction of the nitro group using catalytic hydrogenation gave **32** in quantitative yield.

Scheme 3.2.14.

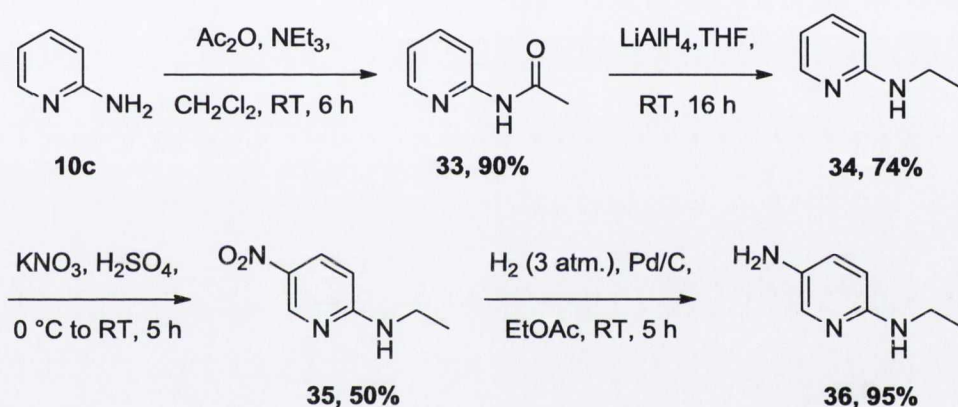


6-(*N*-Ethylamino)-3-aminopyridine, 36

In this case the reactivity pattern of amino pyridines worked in favour of forming 6-(*N*-ethylamino)pyridin-3-ylguanidine hydrochloride **38e** from the corresponding di-amino pyridine **36**. Thus, it was synthesised in four steps from 2-aminopyridine (Scheme 3.2.15). Firstly, 2-aminopyridine was acetylated using acetic anhydride and triethylamine, in 90% yield. The resulting amide **33** was then reduced using lithium aluminium hydride to 2-(*N*-ethylamino)pyridine **34** in moderate 74% yield as a red liquid. This two-step introduction of the *N*-ethylamino group to aryl amines has proven successful in our group, where alkylating agents have caused problems. Nitration of **34** using potassium nitrate and sulfuric acid was directed almost exclusively to the 5-position to give **35** due to the conjugative electron-donating effects of the amino-substituent and its steric hindrance of the 3-position, but went in poor yield (50%). The nitro group of **35** was then reduced using catalytic hydrogenation in ethyl acetate. Diamine **36** proved very sensitive however; filtering through celite to remove the

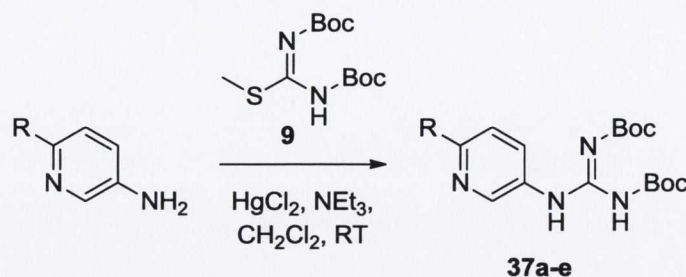
palladium and carbon led to degradation and loss of compound, and exposure to water during attempts at aqueous workup led to inseparable mixtures and product was very difficult to extract. Thus, the most successful procedure for the reduction of **35** was to perform the reaction in ethyl acetate, then simply remove the palladium and carbon by filtration through filter paper and concentrate the residue to give diamine **36**. This was quite effective, affording **36** in 95% yield, and high purity. It was assumed that any residual impurities would not interfere with the guanidylation reaction and could be removed after that stage of the synthesis.

Scheme 3.2.15.



Guanidylation of Pyridin-3-amines

The guanidylation of these amines was achieved in high yield, higher than the corresponding pyridin-2-ylamines (Table 3.2.3), due to the reactive nature of the amine in the 3-position of pyridines.

Table 3.2.3. Synthesis of *bis*-Boc pyridin-3-ylguanidines.

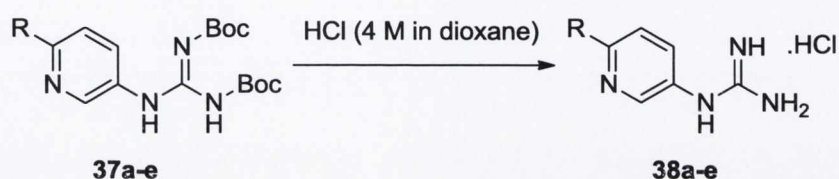
Compound	R	Yield (%)
37a	Cl	79
37b	H	89
37c	CH ₃	88
37d	5,6-(-CH ₂ -) ₄	87
37e	NHEt	91

At this point investigations were underway into removing the Boc groups of intermediates **37** using solutions of hydrochloric acid so that the guanidine hydrochloride could be obtained directly from the deprotection. Three solutions were screened; 1 M HCl in diethyl ether, 1.25 M HCl in methanol and 4 M HCl in 1,4-dioxane. In general, the *bis*-Boc intermediates were not soluble enough in diethyl ether for full conversion to occur using 1 M HCl in this solvent, while using 1.25 M HCl in methanol led to problems of degradation. The presence of nucleophilic methanol – and the unavoidable presence of water therein – meant that hydrolysis of the protonated guanidine could occur, and the ammonium salt of the pyridin-3-ylamine was often recovered from the reaction.

The most successful reagent proved to be 4 M HCl in 1,4-dioxane. The dioxane was capable of dissolving the *bis*-Boc intermediates and the dry conditions meant that hydrolysis of the guanidine was not an issue. Based on an article found in the literature on the mechanism of *N*-Boc cleavage, which elucidated that the rate of reaction was second order with respect to acid concentration, optimal conditions were found to be six equivalents of HCl per Boc group and an overall reaction concentration of 0.2 M with

respect to starting material.¹⁸⁷ This concentration was obtained by adding dichloromethane or, where solubility was an issue, a 1:1 mixture of dichloromethane and isopropanol. Using this method, pyridin-3-ylguanidine hydrochlorides **38a-e** were obtained in excellent yields (Table 3.2.4). In most cases, recrystallisation was not necessary, purification by reverse-phase silica chromatography being sufficient; it seems likely that Amberlite was at fault for introducing impurities previously.

Table 3.2.4. Generation of pyridin-2-ylguanidine hydrochlorides **38a-e**.



Compound	R	Yield (%)
38a	Cl	92
38b	H	85 ^a
38c	CH ₃	82
38d	5,6-(-CH ₂ -) ₄	87
38e	NHEt	100

^a Formed using TFA and ion exchange with Amberlite Cl⁻ as described in Table 3.2.2.

3.2.2. Structural Study of Pyridin-2-ylguanidines

The structure of guanidine in the solid state was recently resolved, revealing its high tendency to form HB interactions, 148 years after it was first synthesised.¹⁸⁸ Further studies have investigated tautomerism in guanidines,¹⁸⁹ as well as the conformational preferences of guanidines and acylguanidines.¹⁹⁰ Our group has been interested in characterising the π -cation interactions which guanidines are known to undergo (see Section 3.1). Yet despite these studies, and the huge interest in aryl guanidines for biological activity, their ligand-receptor interactions are poorly understood and a better knowledge of their conformational preferences would be of great benefit. Pyridin-2-ylguanidines had not previously been studied and, thus, we undertook an experimental and computational study of their structure and properties.¹⁹¹

For the purpose of this study, pyridin-2-ylamines **10a-e**, *bis*-Boc-pyridin-2-ylguanidines **14a-e** and pyridin-2-ylguanidine hydrochlorides **15a-e** were considered. Compounds were synthesised as described in Tables 3.2.1 and 3.2.2, and it was noticeable that there was a striking difference in the ^1H NMR chemical shifts of proton H^3 in the *bis*-Boc protected guanidines compared to the corresponding proton in the 2-aminopyridines and pyridin-2-ylguanidine hydrochlorides (Fig. 3.2.4); it was seen to be broadened and greatly shifted to high field. This difference was preserved across all R groups and in both deuterated chloroform (CDCl_3) and deuterated dimethylsulfoxide ($\text{DMSO-}d_6$), though this discussion will concentrate on spectra obtained in $\text{DMSO-}d_6$ as it is the only solvent in which all compounds were soluble.

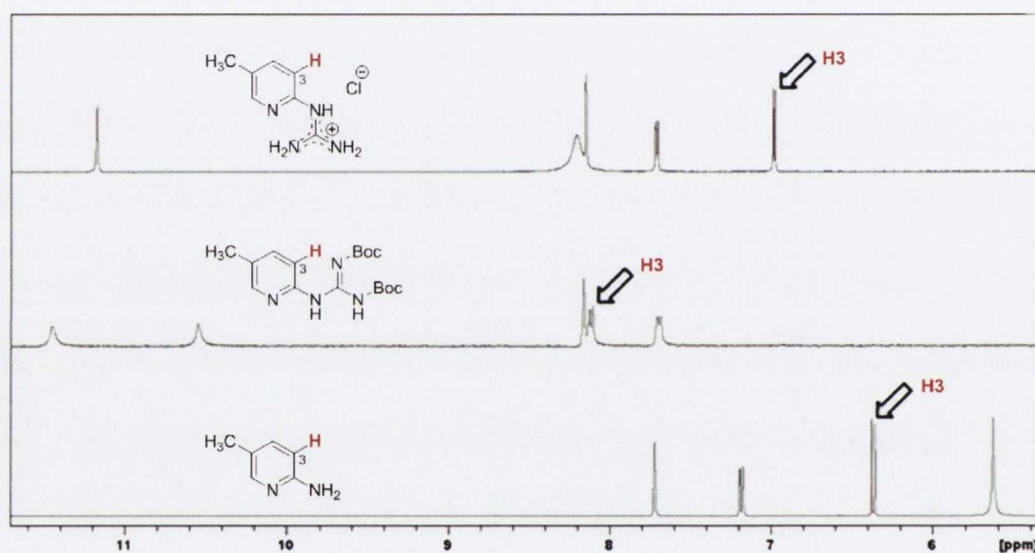


Fig. 3.2.4. Overlaid ^1H NMR spectra of **10d** (*bottom*), **14d** (*middle*) and **15d** (*top*), recorded in $\text{DMSO-}d_6$.

Theoretical Study of the Conformation of 14 and 15.

Considering that this type of shift in NMR spectroscopy is indicative of hydrogen bonding we investigated the possibility of this occurrence using DFT calculations. Considering the conformation of pyridin-2-ylguanidines in terms of the $\text{N}^1\text{-C}^2\text{-C}^{1'}\text{-N}^{2'}$ dihedral angle, two extreme possibilities arise that can be labelled *anti* and *syn* (Fig. 3.2.5).

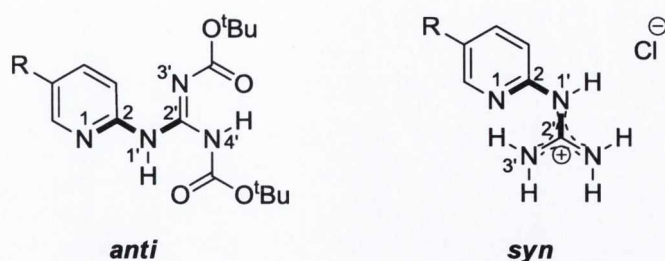


Fig. 3.2.5. *Anti* and *syn* conformations shown for *bis*-Boc-pyridin-2-ylguanidines and pyridin-2-ylguanidinium chlorides respectively.

For guanidiniums **15**, a quantum theoretical intrinsic reaction coordinate (IRC) study¹⁹² of the $N^1-C^2-N^{1'}-C^{2'}$ rotation process was carried out in gas phase – using the B3LYP functional and the 6-31+G(d,p) basis set – resulting in two energetic minima and two transition states. The global minimum of the guanidinium series corresponds to the *syn*-conformer, which features a coplanar arrangement of pyridine and guanidinium moieties and a $LP-N^1 \cdots H^{3'}$ IMHB (**Fig 3.2.7**). The *anti*-conformer is disfavoured by steric constraints ($H^3 \cdots H^{3'}$ repulsion). A local minimum exists which minimises this repulsion though it is 33.9 kJ mol^{-1} less stable than the global minimum. The two transition states correspond to the transition between the *syn* and *anti*-states and the transition between the two *anti*-states. These barriers are not sufficient to prevent the rotation process though they suggest that an almost quantitative prevalence of the *syn*-conformation will exist.

A similar treatment of *bis*-Boc-pyridin-2-ylguanidines is more complicated as the guanidine is now neutral, meaning the double bond is localised and the possibility of tautomerism exists. Further issues to consider are *E/Z* isomerism with respect to the imine and rotation of the secondary amines adjacent to this double bond. However, the problem could be simplified when the Boc groups were further examined. The fact that the methyl groups of the two Boc groups appear separately in the ^1H NMR spectrum suggests that they are in different environments, and that the imine double bond is localised to one of these nitrogens. If this is the case two IMHBs are available between the Boc CO and guanidine NHs ($O \cdots H^{1'}$ and $O \cdots H^{4'}$), and the ^1H NMR spectrum again suggests this to be the case, both of these NH signals appearing above 10 ppm.

Thus the *N,N'*-bis-Boc-substituted guanidine system could be considered as a single “pseudo bicyclic” structure held in place by a network of IMHBs and the issue of conformation could again be reduced to rotation around the $N^1-C^2-N^{1'}-C^{2'}$ dihedral angle. These compounds exhibit a clear preference for the *anti*-conformer as the *syn*-conformer now places the lone-pairs of N^1 and $N^{3'}$ in close proximity and, furthermore, a third stabilising IMHB between H^3 and $N^{3'}$ is possible (Fig. 3.26), resulting in the *anti*-conformer being 30.1 kJ mol^{-1} more stable than the *syn*-conformer. This type of $C^{Ar}H-N$ IMHB has been described for compounds such as 2,2-bipyridine among others.¹⁹³

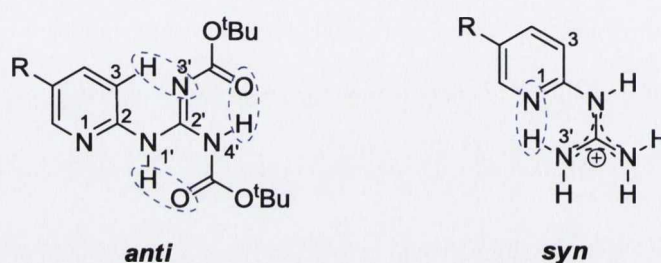


Fig. 3.2.6. Preferred conformations of *bis*-Boc-pyridin-2-ylguanidines (*left*) and pyridin-2-ylguanidiniums (*right*) and the IMHBs that confer these conformations (indicated as dashed ellipses).

It should be noted that a screen of the possible isomers resulting from *E/Z* isomerism with respect to the imine and rotation of the secondary amines adjacent to this double bond was performed for the computationally less costly di-acyl-pyridin-2-ylguanidines; however, the *anti*-conformer was consistently observed to be the most stable isomer.

Experimental Study of the Conformation of Compounds 14 and 15.

During the synthesis of **14** and **15** several representative crystal structures were obtained (Fig. 3.2.7) which corroborated our theoretical results and suggested that the hydrogen bonding networks are maintained in the solid phase. Slow recrystallisation from a mixture of hexane/ethyl acetate provided a crystal structure for **14b**, which displays a $180^\circ N^1-C^2-N^{1'}-C^{2'}$ dihedral angle, consistent with the *anti*-conformation. This crystal structure also reveals an extensive IMHB network involving the Boc CO

and N^{3'} lone pairs as HB acceptors and H^{1'}, H^{4'} and H³ as donors respectively, as previously described. The HB distance found for the CO $\cdots$ H^{1'} interaction (1.90 Å) is in agreement with a very strong HB, while those distances found for the N^{3'} $\cdots$ H³ (2.30 Å) and CO $\cdots$ H^{4'} (2.11 Å) contacts correspond to medium-weak HB interactions.

Crystals for two pyridin-2-ylguanidinium chlorides **15d** and **15e** were also obtained using a slow diffusion of diethyl ether in a cold methanolic solution in a sealed jar. These structures both exhibit the *syn* conformation featuring pyridine/guanidine coplanarity and an IMHB between N¹ and H^{3'}. The IMHB distances suggest strong interactions; the N¹ $\cdots$ H^{3'} distance is 2.09 Å in both cases.

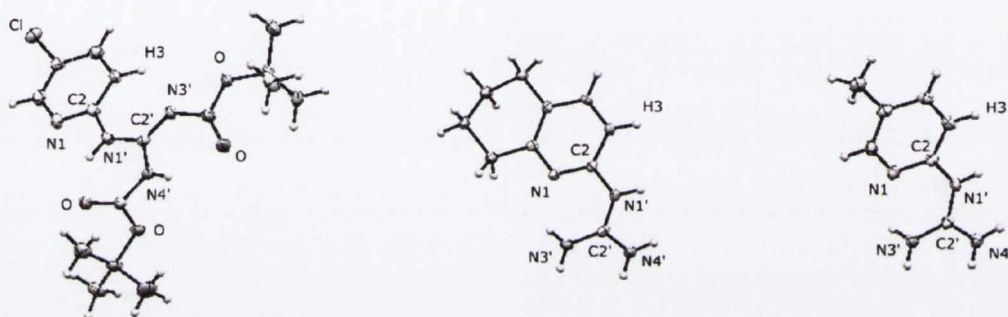


Fig. 3.2.7. Crystal structures of **14b** (CCDC 822416), **15e** (CCDC 822187) and **15d** (CCDC 822417) in ORTEP thermal ellipsoid representation. Chloride ions are omitted for clarity.

Significantly, NMR spectroscopic experiments in solution supported the persistence of the conformational effects that were theoretically predicted (DFT calculations) and experimentally observed in the solid state by X-ray crystallographic studies. In agreement with the *anti*-conformation, the existence of an IMHB between N^{3'} and H³ in the *bis*-Boc-protected series **14** is strongly supported by the ¹H NMR spectroscopic data recorded for proton H³ (Table 3.2.5). Throughout series **14**, this signal is broadened and strongly shifted to high field (7.93–8.27 ppm). In contrast, the H³ signal recorded for the guanidinium salt series **15** consistently appears as a sharp doublet at 6.80–7.13 ppm, which is much closer to that observed for the corresponding 2-aminopyridines **10**.

Table 3.2.5. ^1H NMR shifts of series **14** and **15** obtained in DMSO- D_6 - d_6 . The difference in chemical shift with respect to the 2-aminopyridines (**10**) is shown in parentheses for H^3 .

Compound	R	δH^3	$\delta\text{H}^{1'}$	$\delta\text{H}^{4'}$ ^a
10a	Br	6.42	6.16	
10b	Cl	6.45	6.14	
10c	H	6.41	5.85	
10d	CH_3	6.37	5.63	
10e	$-(\text{-CH}_2\text{-})_4$	6.20	5.53	
14a	Br	8.22 (+1.80)	11.36	10.64
14b	Cl	8.27 (+1.82)	11.36	10.65
14c	H	8.22 (+1.81)	11.44	10.59
14d	CH_3	8.11 (+1.74)	11.46	10.54
14e	$-(\text{-CH}_2\text{-})_4$	7.93 (+1.73)	11.53	10.49
15a	Br	7.06 (+0.64)	11.53	8.23
15b	Cl	7.13 (+0.68)	11.70	8.28
15c	H	7.07 (+0.66)	11.39	8.30
15d	CH_3	6.98 (+0.61)	11.17	8.20
15e	$-(\text{-CH}_2\text{-})_4$	6.80 (+0.60)	11.08	8.22

^a $\text{H}^{3'}$ and $\text{H}^{4'}$ are observed as a single signal for **15**.

To ensure that the effects on the chemical shift of H^3 were as a result of an IMHB and not electronic effects, the average change in the chemical shift of all aromatic protons with respect to the 2-aminopyridine series **10** was investigated (Fig. 3.2.8). The electronic effects of the *bis*-Boc guanidine and the guanidinium are similar; the results showed that protons H^4 and H^6 were shifted a similar amount by each with respect to the 2-aminopyridines (+0.57/0.56 for H^4 and +0.47/0.46 for H^6). However, in the case of H^3 , the average change in de-shielding is considerably higher for the Boc-protected series (+1.78) than for the guanidinium series (+0.64), a discrepancy that can be explained by the effects of an IMHB interaction to the guanidine imine lone-pair.

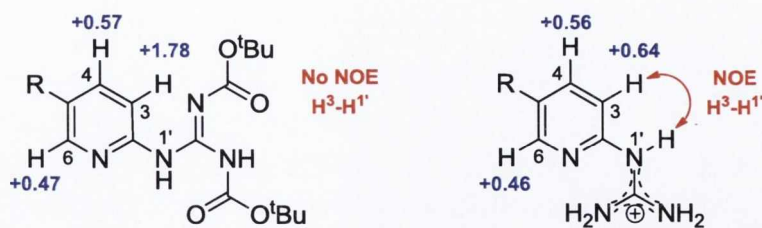


Fig 3.2.8. Average change in the value of ¹H NMR chemical shifts for H³, H⁴ and H⁶ in series **14** and **15** with respect to 2-aminopyridines **10** (blue). NOE interaction indicative of IMHB is also shown (red).

Nuclear Overhauser effect (NOE) experiments further supported the postulated conformational preferences. A through-space interaction was observed between H³ and H¹ in the pyridin-2-ylguanidinium chlorides (Fig. 3.2.8), in agreement with the *syn* conformation. Conversely, the absence of a NOE signal for these same protons in the *bis*-Boc protected series suggests that they are not nearby in space, supporting the prevalence of the *anti*-conformation.

Furthermore, variable temperature ¹H NMR spectroscopic experiments were carried out on compounds **14d** and **15d** to identify if equilibrium exists between the possible conformations in both series. Spectra were recorded in DMSO-D₆-d₆ at 10 °C increments from room temperature to 80 °C, and a final spectrum was recorded on re-cooling the sample to room temperature. Neither compound showed significant changes in its spectra, indicating in each case that in fact a single conformer exists experimentally in solution, confirming our predictions. The characteristic downfield shift and broad nature of H³ in compound **14d** was maintained throughout, as was the high field shift of the NH protons at 10.5 and 11.4 ppm. Some degradation was observed at 80 °C, which could be attributed to thermally induced loss of the Boc groups. The same spectrum was observed on cooling to room temperature, confirming that this was not the result of conformational exchange. The spectra of compound **15d** showed no observable changes on heating. In particular, the high shift seen for the NH signals was maintained and no degradation of the compound was observed in this case. This leads us to conclude that the *syn*-conformation for compounds **15** dominates in solution as well as in the solid state.

Conformational Control in N-Boc-N'-alkylpyridin-2-ylguanidines

It was hypothesised that the *anti*-conformation was conferred by the rigid network of IMHBs in compounds **14** and that disrupting this would induce a change to the *syn*-conformation. Thus one Boc group was replaced by an alkyl group (*n*-propyl) in compounds **39a** and **39b** (Fig. 3.2.9). A systematic conformational analysis of both compounds was performed at the B3LYP/6-31+G(d,p) level showing that the *syn*-conformation is indeed 25.9 kJ mol^{-1} more stable than the most stable *anti*-conformer (Fig. 3.2.9). However, because the symmetry of the guanidine subunit has now been broken, two energetic minima arose for both the *anti* and *syn* isomers, which had to be analysed. Both of these incorporate two IMHBs and were vastly more stable than any alternatives in each case.

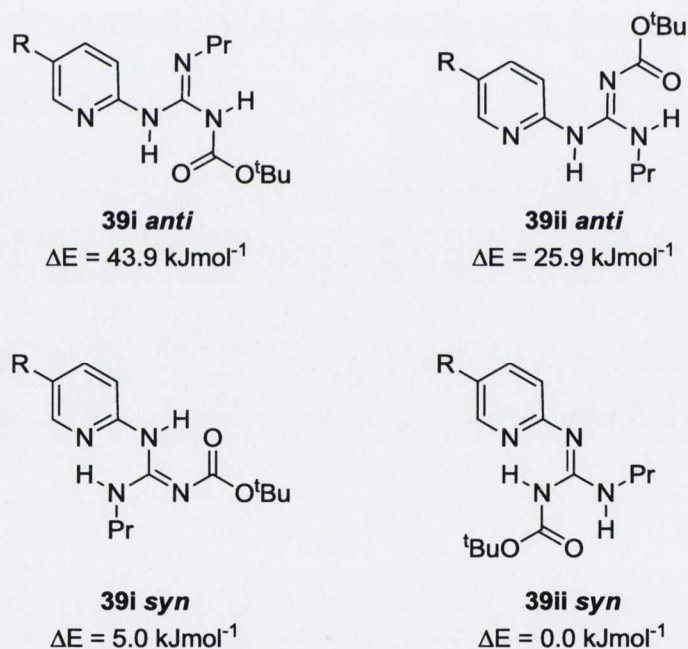


Fig. 3.2.9. The relative energies of the two best *anti* (top) and *syn* (bottom) energetic minima for compounds **39a** (R = Cl) and **39b** (R = CH₃), calculated at B3LYP/6-31+G(d,p) level.

These results suggested that the two *syn*-isomers shown are very close in energy and synthesis (see Section 3.4) proved this to be the case, a near 1:1 mixture of two isomers being obtained; for R = Cl, a 3:4 mixture of i:ii was produced, while for R = CH₃, a 6:5

mixture of i:ii was obtained (as adjudged by relative integration in the ^1H NMR spectrum). Neither ^1H NMR spectrum showed the high shift and broadening of H^3 characteristic of the IMHB that would exist in either *anti*-isomer. Further evidence of the *syn*-conformation for isomer i came from an NOE signal between H^3 and $\text{H}^{1'}$. Such a signal was not observed for isomer ii, which is not conclusive evidence for its existence but, in combination with the other evidence, strongly suggests that the second component of the mixture was ii.

From this family, it could be concluded that conformational control in pyridin-2-ylguanidines is firmly rooted in the IMHB interactions established between the guanidine and the pyridine subunits. As expected, removal of the Boc group from compounds **39a** and **39b** led to a single isomer, which adopts the *syn*-conformation as in guanidinium salts **15**. This information can be applied to the design of acylated guanidines with defined conformational space.

3.2.3. Determination of Purity

As with all hydrochloride salts in this project, purity was assessed using reverse phase HPLC with a diode-array detector scanning wavelengths from 200-950 nm. The method developed for this type of compound which gave optimum retention times used a gradient from 100% aqueous formate buffer (30 mM, pH 3.0) to 85% formate-buffered methanol (30 mM, pH 3.0) and 15% aqueous formate buffer. A minimum purity of 95% was set for compounds to be tested pharmacologically.

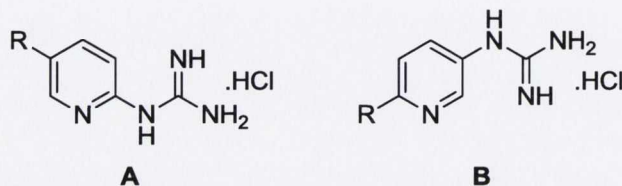
3.2.4. Pharmacological Testing of Families A and B

Compounds from families **A** and **B** were subjected to the competitive binding and [^{35}S]GTP γS functional assays to assess their binding affinity and activity at the α_2 -AR, as described in Section 1.5.4.

Binding Affinity (pK_i determination)

Affinities were measured in PFC tissue using a competitive binding assay with the α_2 -AR selective radioligand [^3H]RX821002 at 2 nM concentration. The results of these experiments are presented in Table 3.2.6, where binding affinities are expressed in pK_i values.

Table 3.2.6. Binding affinities (pK_i) of families A and B.



Compound	Family	R	pK_i
RX821002	-	-	8.72
15a	A	5-Br	5.69
15b	A	5-Cl	5.77
15c	A	5-H	6.25
15d	A	5-CH ₃	6.09
15e	A	5,6-(-CH ₂ -) ₄	6.84
15f	A	5-NO ₂	5.06
15g	A	5-NH ₂	5.06
15h	A	5-NHEt	4.10
38a	B	6-Cl	5.24
38b	B	6-H	3.85
38c	B	6-CH ₃	5.04
38d	B	5,6-(-CH ₂ -) ₄	5.55
38e	B	6-NHEt	6.34

The most striking piece of information from this Table is that pyridin-2-ylguanidine hydrochlorides **A** have higher pK_i values than almost all of their pyridin-3-ylguanidine

hydrochlorides **B**. The only exception to this is the *N*-ethylamino substituted compounds, though the very low pK_i of **15h** can be explained by its poor stability, which was observed during its synthesis. This exception aside, it seems that pyridin-2-ylguanidines bind more favourably to the α_2 -AR, by an average of 1.32 orders of magnitude.

There are a number of different features in **A** and **B** which could explain this. Taking that members of **A** adopt a planar geometry and members of **B** exist in an out-of-plane arrangement, it is possible that the conformation of **A** is more suitable for binding to the active site. However, as the conformation of compounds **B** is expected to be similar to that of their phenyl analogues which display much higher pK_i , it seems likely that either the changes in aromaticity introduced by the pyridine ring, or the free nitrogen lone-pair are the cause of the low pK_i values of family **B**. Changes to the basicity of the guanidine are thought to be unimportant as it will almost certainly be protonated at physiological pH (The pK_{aH} of phenyl guanidine is 10.88).

Within family **A** there is a pattern in pK_i values based on the effects of the *para* substituent of the pyridine ring on its electron density. The reason for the selection of the 5-nitro, 5-chloro and 5-bromo derivatives was to investigate this effect; the more electron-withdrawing in nature the *para* substituent is, the lower the pK_i value is (5.06-5.77). For unsubstituted, 5-methyl and 5,6- $(-CH_2-)_4$ substituted derivatives – *i.e.* more electron-rich pyridine systems – the pK_i value was much larger (6.09-6.84).

This is in agreement with our hypothesis that π -cation interactions between the guanidinium and aromatic residues in the α_2 -AR active site are important for binding. In Section 3.1 it was discussed how more electron-rich pyridin-2-ylguanidiniums preferred π -cation to π - π stacking interactions, whereas the π -cation interaction was less favoured for electron-poor pyridin-2-ylguanidiniums. Thus, it seems that the more electron rich the aromatic ring is the higher the pK_i is for this type of ligand and this can be applied to the design of future families of compounds. An exception to this trend was that compounds **15f** and **15g** – the nitro- and amino-substituted derivatives, respectively – had identical pK_i values. It was expected that **15g** would have higher binding affinity due to its conjugative donation of electron density to the pyridine ring; however, it was among the lowest affinity members of family **A**. There are several possibilities for this poor binding affinity. The amine could be involved in new HB interactions with the

residues of the active site which disrupt other important contacts between the ligand and receptor. It is also possible that the high negative electrostatic potential of the amine is unfavoured in this area; this would agree with the poor affinities observed for compounds **15a** and **15b**, though it would be interesting to investigate a 5-hydroxy-substituted derivative to gain more information about this.

It must also be noted, however, that compounds from family **A** generally have slightly lower pK_i values than their phenyl analogues. Data is available for the unsubstituted, *p*-chloro, *p*-methyl and *m,p*-(-CH₂)₄ derivatives, showing the average difference in pK_i is only 0.27. Despite this, members of Family **A** have sufficiently large pK_i values and this could hopefully be improved in subsequent analogues. In any case, more attractive than their affinity was their activity at the α_2 -AR.

Functional Assay Results

The activities of all compounds with $pK_i \geq 6.00$, and a representative set of molecules with lower pK_i values were tested using [³⁵S]GTP γ S binding experiments. All members of families **A** and **B** tested displayed either antagonistic or inverse-agonistic activity at the α_2 -AR (Fig. 3.2.10). This was extremely encouraging given the unpredictable activities obtained across families of structurally similar compounds in the past.

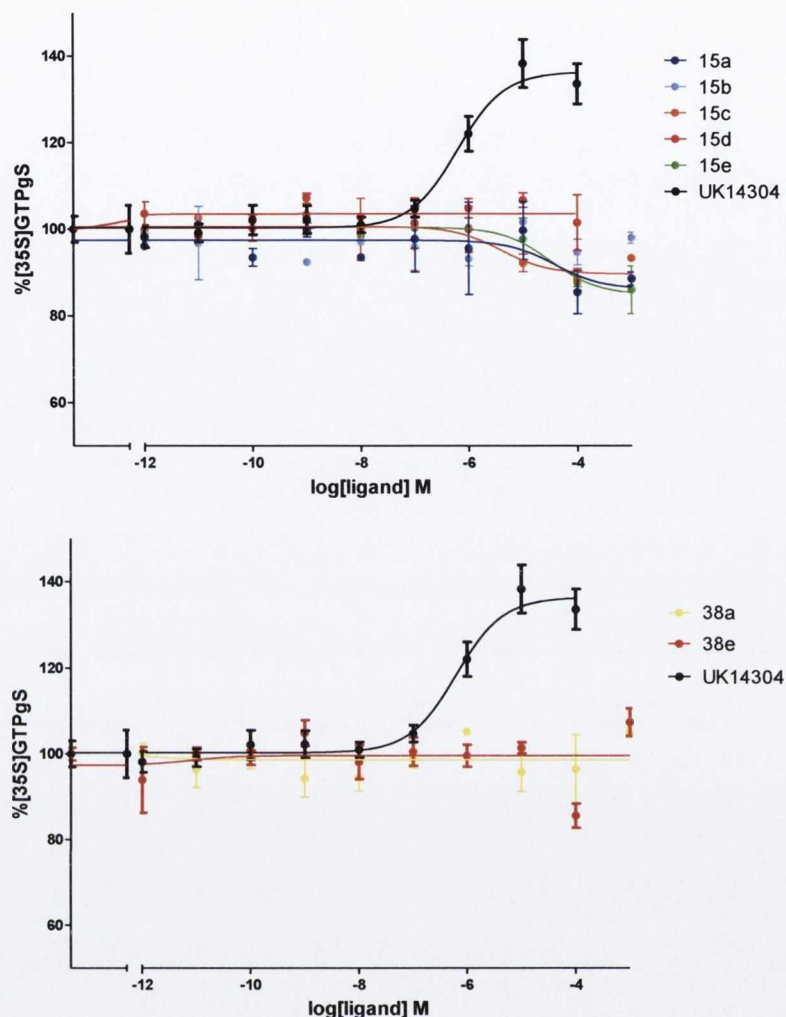


Fig. 3.2.10. Dose-response curves for percentage [^{35}S]GTP γ S binding vs. ligand concentration of families **A** (*top*) and **B** (*bottom*). The standard agonist UK14304 is shown in black.

Thus, two molecules from family **B** – **38a** and **38e** – induced no stimulation of [^{35}S]GTP γ S binding, indicating that they do not activate the α_2 -AR. Several factors need to be considered however. As antagonists display a flat line no further information can be gained from this assay. It is known that low affinity compounds show only small variations in receptor activation if they are acting as agonists. Thus, it cannot be concluded for certain from this assay if **38a** and **38e** are antagonists due to their poor binding affinity, though it is likely that they are. To account for this, the effect of the compounds on levels of bound [^{35}S]GTP γ S can be measured in the presence of an agonist of the receptor. Decreases in bound [^{35}S]GTP γ S in this test indicate competitive

antagonism, with larger decreases meaning greater potency. However, due to their poor pK_i it was not deemed worthy to carry out this experiment.

There was less uncertainty for members of family **A** as these compounds displayed curves characteristic of inverse agonism at high concentrations. This indicates that the compounds are competitively binding to the active site of the receptor and that they are preventing its activation.

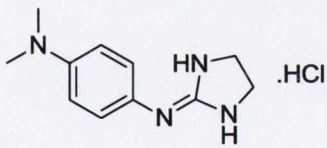
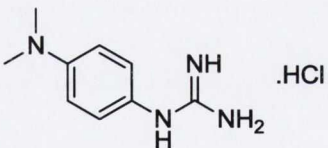
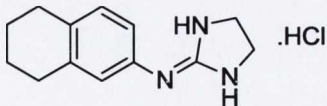
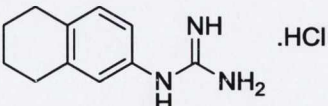
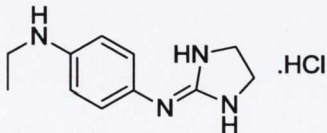
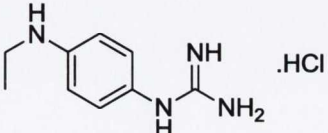
From these data we can conclude that compounds from family **A** are acting as either antagonists or inverse agonists of the α_2 -AR. To prove that this activity is being inferred by the IMHB which gives rigidity to these compounds it would have to be replicated in similar molecules. For this purpose, pyridin-2'-yl-2-iminoimidazolidine hydrochloride analogues were chosen as they incorporate the same IMHB and, thus, should antagonise the α_2 -AR if this is a necessary feature for blocking the receptor. Therefore, it was decided to synthesise and test this family of compounds for pyridin-2-yl and pyridin-3-yl derivatives to investigate preservation of the differences in pK_i between families **A** and **B**.

3.3. Synthesis and Pharmacological Evaluation of Families C1 and C2

Previously in Rozas' group, aryl 2-iminoimidazolidine hydrochlorides have shown higher affinity for the α_2 -AR than analogous guanidine hydrochlorides, an effect that has been reproduced across varied aromatic systems (Table 3.3.1). Considering that a *para* relationship of cationic moiety and substituents on the aromatic ring has been most successful, alkyl, heteroatomic and cyclic groups in this position have all conserved this effect.^{156,157,158}

What has not been consistent is the activity profile of these compounds; agonist (Ag.) or antagonist (Ant.). Table 3.3.1 demonstrates the inconsistent results that have been obtained for the activity of guanidiniums and 2-iminoimidazolidiniums at the α_2 -AR; changes to either the cationic moiety or the *para* substituent on the ring have led to unpredictable activity at the α_2 -AR.

Table 3.3.1. Brief comparison of binding affinity (pK_i) and activity (Ag./Ant.) at the α_2 -AR of 2-iminoimidazolidine (*l*) and guanidine (*r*) hydrochlorides.

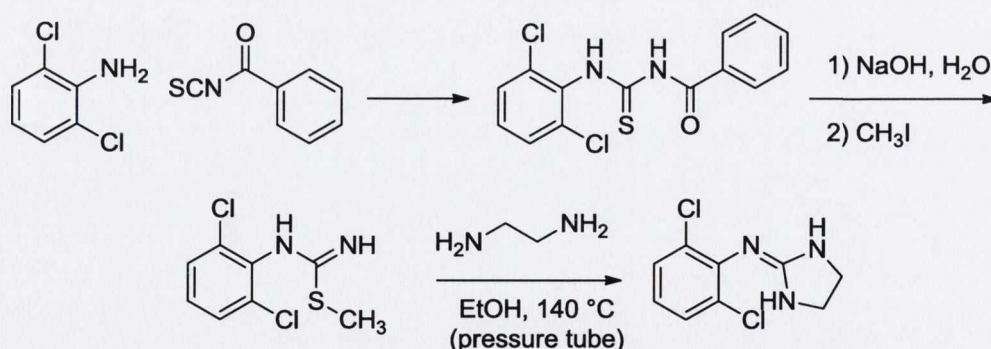
Compound	pK_i Ag./Ant.	Compound	pK_i Ag./Ant.
 3	6.48 Ant.	 7	6.19 Ag.
 6	8.26 Ag.	 2	7.11 Ant.
 40	6.75 Ag.	 1	6.58 Ant.

Thus, it was desirable to synthesise and test a series of pyridin-2'-yl and pyridin-3'-yl 2-iminoimidazolidine hydrochlorides – **C1** and **C2** respectively – for two reasons; firstly, to introduce the 2-iminoimidazolidinium functionality to increase the binding affinity of this type of molecules and, secondly, to learn if the antagonistic activity obtained for pyridin-2-ylguanidiniums could be transferred to pyridin-2-yl-(2-iminoimidazolidiniums), and in the process give further evidence to our hypothesis that this is being conferred by conformational effects.

3.3.1. Methods for the Synthesis of 2-iminoimidazolidines

Intramolecular ring closure by condensation of thioureas with ethylenediamine derivatives has been a popular method for the synthesis of 2-iminoimidazolidines. This approach was applied to the synthesis of clonidine (Scheme 3.3.1), which involves initial condensation of an amine with benzoyl isothiocyanate to form a thiourea after basic hydrolysis of the intermediate carbamate. Treatment with methyl iodide then leads to an isothiurea which undergoes desulfurisation after nucleophilic attack by ethylenediamine under harsh conditions of 140 °C in a pressure tube.¹⁹⁴

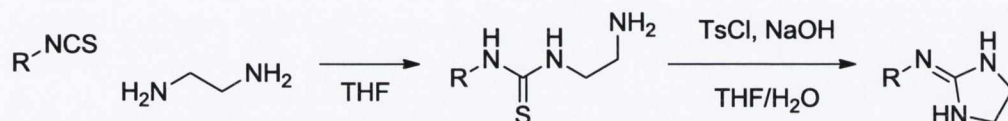
Scheme 3.3.1.



Several similar, yet milder and more atom-efficient, approaches have been developed since. One such example by Heinelt *et al.* again proceeds from the isothiocyanate, which is reacted with ethylenediamine to yield a *N,N'*-di-substituted thiourea (Scheme 3.3.2).¹⁹⁵ Coordination of the thiourea sulfur to *p*-toluenesulfonyl chloride then

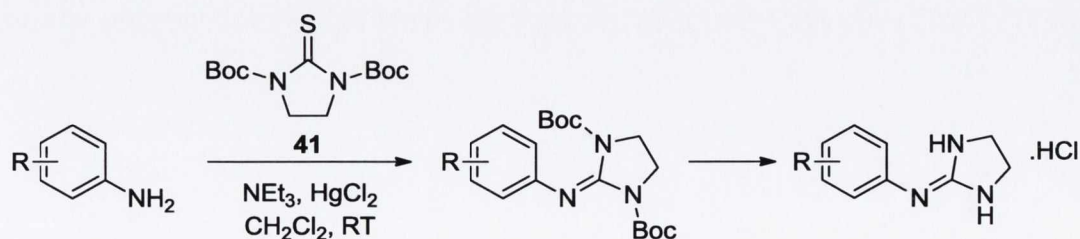
promotes formation of a carbodiimide intermediate, on loss of sulfur, which can undergo a 5-endo trig cyclisation to the 2-iminoimidazolidine product. Yields are quoted at 60% for the formation of these derivatives but the limiting factor is the availability of isothiocyanates and the poor yields for their conversion to thioureas.

Scheme 3.3.2.



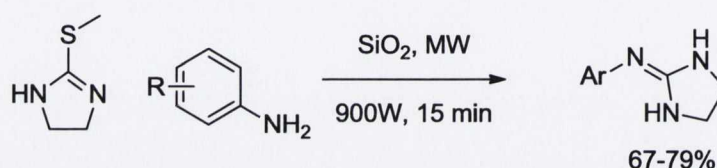
Our group developed a method for the synthesis of 2-iminoimidazolidines which is an extension of Kim and Qian's mercury(II) chloride promoted guanidylation of amines using electron-deficient thioureas (Scheme 3.3.3).¹⁹⁶ The benefits of the procedure are the mild conditions, tolerance of a wide range of functionalities, wide availability of amines and generally high yields. However, the most notable advantage of this methodology is its ability to produce aryl 2-iminoimidazolidines from poorly nucleophilic aryl amines. Coupling of these with *N,N'*-bis-(*tert*-butoxycarbonyl)imidazolidine-2-thione **41** in the presence of mercury(II) chloride yields the *bis*-Boc protected 2-iminoimidazolidine derivatives which can be readily deprotected using a variety of acidic conditions to yield the desired 2-iminoimidazolidine hydrochlorides.

Scheme 3.3.3.



Other current methods favour use of aryl amines over isothiocyanates and substituted thioureas as many are commercially available and their synthesis and manipulation is more easily achieved. One interesting example of such a methodology describes the microwave-assisted coupling of an aryl amine with *S*-methyl imidazolidine-2-thione in the presence of silica gel (Scheme 3.3.4), though the thiourea derivative must first be synthesised from imidazolidine-2-thione using iodomethane.¹⁹⁷

Scheme 3.3.4.



For the synthesis of pyridinyl 2-iminoimidazolidines the method of Rozas and Dardonville was chosen due to the availability of the corresponding pyridine amines and our experience in the generation of 2-iminoimidazolidine hydrochlorides from the *bis*-Boc protected intermediates.

3.3.2. Synthesis of Pyridinyl 2-iminoimidazolidines

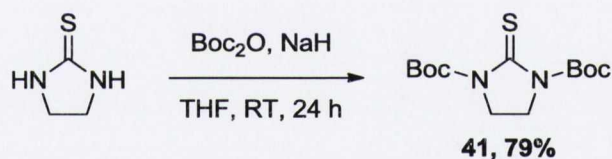
Following the mentioned procedure of Rozas and Dardonville, two families of pyridin-2'-yl (**C1**) and pyridin-3'-yl (**C2**) 2-iminoimidazolidine hydrochlorides were prepared. The objectives were to see if the conformational preferences – and concomitant pharmacological differences – observed in pyridin-2-yl and pyridin-3-yl guanidiniums were maintained in the analogous 2-iminoimidazolidinium families, and to improve upon the binding affinities of families **A** and **B**.

Pyridin-2'-yl-2-iminoimidazolidine Hydrochlorides – C1

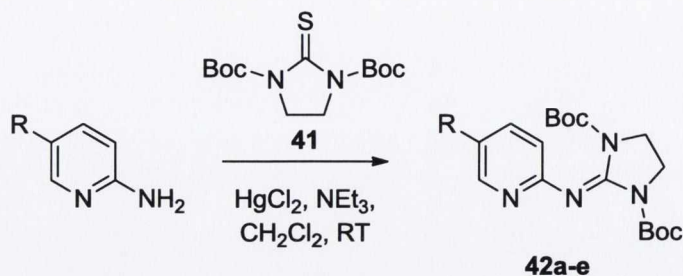
Armed with the binding affinity data obtained for families **A** and **B**, pyridin-2'-yl-2-iminoimidazolidine hydrochlorides were synthesised for the following *para* substituents; Cl, H, CH₃, 5,6-(-CH₂)₄, NHEt (Tables 3.3.2 and 3.3.3), which were expected to have the highest affinity for the α_2 -AR. The chlorine derivative was included for validation of the patterns observed for electron-donating and withdrawing groups at this position in families **A** and **B**.

Firstly, as it is not commercially available, **41** was synthesised in one step from imidazolidine-2-thione and di-*tert*-butyl dicarbonate in the presence of excess sodium hydride (Scheme 3.3.5). This reaction affords pure product on a large scale in high yield (79%) after trituration in cold hexane.

Scheme 3.3.5.



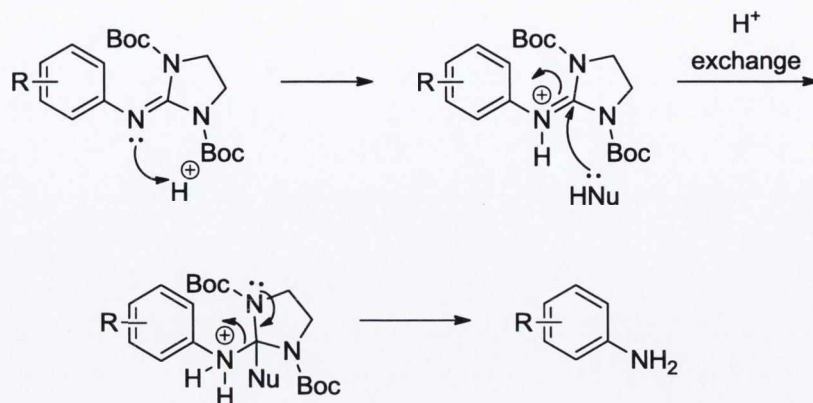
This was then reacted with aromatic amines where addition to the electrophilic carbon was promoted by coordination of sulfur to thiophilic mercury(II) chloride (Table 3.3.2). The mechanism of the reaction is unproven though unlike the guanidylation mechanism described earlier (Scheme 3.2.1) it cannot go through a carbodiimide intermediate as the 5-membered ring is incapable of accommodating such a species with 180° bond angle.

Table 3.3.2. Synthesis of pyridin-2'-yl-2-iminoimidazolidine hydrochlorides, family C1.

Compound	R	Yield (%)
42a	Cl	56
42b	H	56
42c	CH ₃	75
42d	5,6-(-CH ₂ -) ₄	81
42e	NHEt	35

The reaction proceeds in moderate to high yield depending on the reactivity of the aromatic amine, with the more electron-rich pyridine systems giving higher yields. It is also of note that some material is lost during chromatography on compounds **42a-e**, yields of purified compound being much lower than crude yields. Previously in our group, *bis*-Boc protected phenyl 2-iminoimidazolidines have degraded almost completely on silica and high speed alumina chromatography has been necessary to purify them, with poor separation being observed and some product loss still incurring. These molecules are highly acid-sensitive and with the imine being conjugated with the *N*-phenyl moiety, protonation at this position can lead to hydrolysis of the imine, resulting in the aniline (Scheme 3.3.6) which has been isolated and observed by ¹H NMR spectroscopy.

Scheme 3.3.6.



In the case of pyridinyl 2-iminoimidazolidines, degradation on silica was not an issue and purification in this manner proved successful. We attribute this to the presence of the basic pyridine nitrogen acting as a buffer for the system, preventing protonation at the imine nitrogen and subsequent breakdown.

The NAS approach taken for the synthesis of guanidinium **15h** was not an option for the preparation of the analogous **45e** as 2-iminoimidazolidine is not commercially available. Luckily, **45e** was able to be synthesised using the mercury(II) chloride synthetic route from 2-amino-5-*N*-ethylaminopyridine **44**. Remembering that the analogous guanidylation reaction (Section 3.2.1) could not be achieved due to substitution solely occurring at the secondary amine in the 5-position, there are several factors which could be influencing the observed selectivity for substitution at the primary amine in this case.

One such reason is that the electrophilic carbon of **41** is sterically hindered compared to the electrophilic carbon of either **8** or **9** and could prevent access of the bulkier secondary amine nucleophile, directing substitution to the primary amine in the 2-position. This can be rationalised by the fact that the Boc groups in **41** are forced into the space around this carbon by the constrained five-membered ring (Fig. 3.3.1). Free rotation around two C-N bonds in *N,N'*-bis-(*tert*-butoxycarbonyl)thiourea **8** and one in *S*-methyliothiourea **9** – both used as agents for the guanidylation of amines – means that the steric crowding around the electrophilic carbon can be alleviated and bulky nucleophiles such as secondary amines have access.

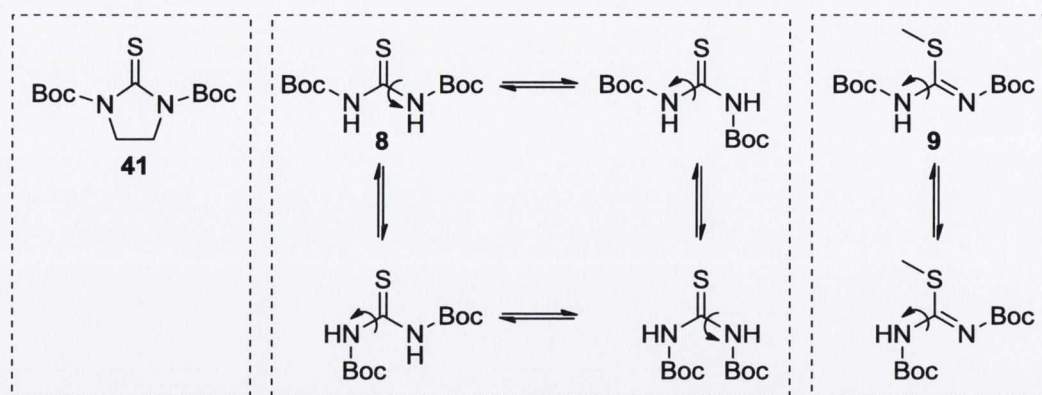
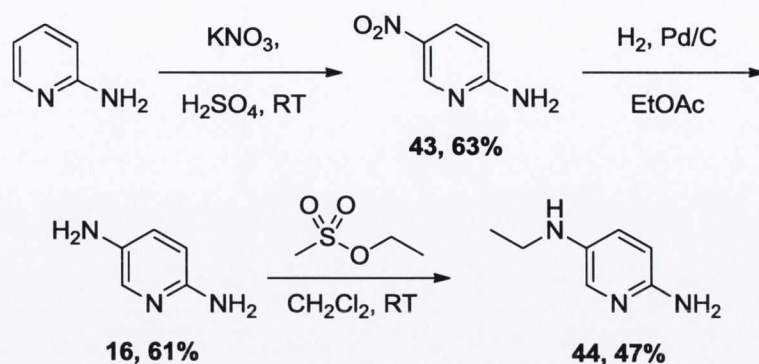


Fig. 3.3.1. Restricted C-N rotation makes **41** a sterically crowded electrophile compared to either **8** or **9**.

Furthermore, reaction occurring at the secondary amine would lead to an unstable cationic product as it must incorporate a C=N to one of the nitrogen atoms and each of these nitrogens is tri-substituted. Thus, if addition of the secondary amine to imidazolidine-2-thione does occur in the presence of mercury(II) chloride, it is assumed to be either reversible or to lead to degradation of material; an event which would explain the poor yield obtained for this particular reaction (Table 3.3.2).

Synthesis of diamine **44** was achieved in three steps from 2-aminopyridine (Scheme 3.3.7). Nitration using potassium nitrate and excess concentrated sulfuric acid was directed principally to the 5-position of the pyridine ring to yield **43**. The 3-nitrated compound was obtained as a minor side product which was removable by silica gel chromatography. The nitro group was then reduced by palladium-catalysed hydrogenation to give 2,5-diaminopyridine **16**; the yield for this step was lower than expected (61%) due to product loss during workup and purification as these molecules are highly soluble in water and stick to silica.

Scheme 3.3.7.



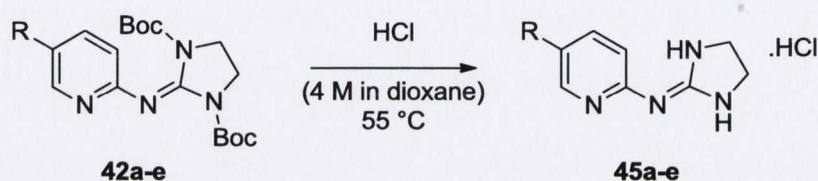
Alkylation of the amine in the 5-position of **16** proved problematic. Several approaches were taken to obtain the ethylated diamine **44** efficiently; however, all methods suffered from low yields and difficult purifications. Reductive alkylation using acetaldehyde and sodium triacetoxyborohydride offered no selection between the two amines, giving a mixture of di-aminopyridines including poly-alkylated products. Acetylation using acetic anhydride was attempted as it was anticipated that the less reactive electrophile might increase selectivity for the more reactive amine in the 5-position; however, no selectivity was obtained and separation of the resultant isomers was difficult. As this route also involved reduction of the amide using lithium aluminium hydride, alkylation of **16** was attempted using ethyl mesylate; this alkylating agent was not predicted to be as reactive as acetaldehyde and if separation of the two resulting *mono*-ethylated di-amines was possible would afford **44** in one step from **16**. This proved successful, separation of the di-amines was possible by silica gel chromatography, eluting with a mixture of dichloromethane and methanol (4:1). Compound **44** had not previously been reported in the literature, an indication of the difficulties in handling these di-aminopyridines.

Deprotection of bis-Boc 2-Iminoimidazolidines

Having isolated the purified *bis*-Boc protected pyridin-2'-yl-2-iminoimidazolidines **42a-e**, deprotection was achieved in high yield using a 4 M solution of hydrochloric acid in dioxane (Table 3.3.3). Due to problems with Amberlite introducing impurities to

products elsewhere in our group, several alternative methods of deprotection were investigated, as described in Section 3.2.1. Hydrochloric acid solutions in either diethylether, methanol or 1,4-dioxane were examined; the highly polar nature of *bis*-Boc protected pyridin-2'-yl-2-iminoimidazolidines **42** meant that they were insoluble in ether, while the nucleophilicity of methanol led to problems of hydrolysis. The most successful solvent was 1,4-dioxane and the optimum reaction concentration of 0.2 M with respect to starting material was attained by adding further dry 1,4-dioxane. Where solubility was an issue this concentration was reached by adding a 1:1 solution of dichloromethane and isopropanol, which maintained the necessary dry conditions.

Table 3.3.3. Generation of 2-iminoimidazolidine hydrochlorides **45a-e**.



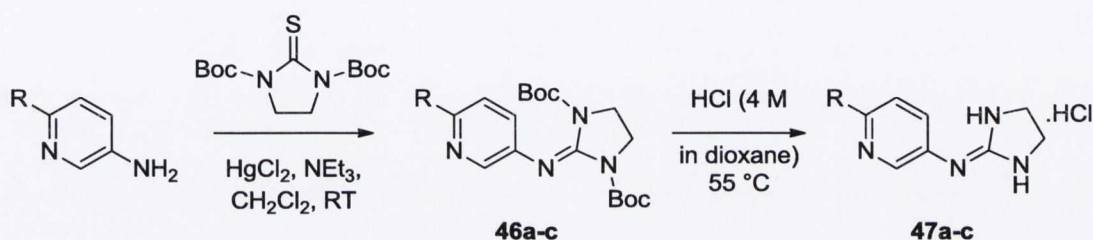
Compound	<i>Bis</i> -Boc/HCl	R	Yield (%)
45a	HCl	Cl	96
45b	HCl	H	92
45c	HCl	CH ₃	93
45d	HCl	5,6-(-CH ₂ -) ₄	93
45e	HCl	NHEt	93

As per guanidine hydrochloride families **A** and **B**, the crude 2-iminoimidazolidine hydrochlorides were dissolved in H₂O and washed twice with dichloromethane to remove any unreacted starting material. They were then purified using reverse phase silica chromatography (C-8 silica) with 100% H₂O as mobile phase, which was sufficient to elute the compounds due to their high polarity and H₂O solubility. Conversion for this deprotection step was almost quantitative and yields for purified compound were all above 92%. All members of family **C1** were obtained as crystalline solids.

Pyridin-3'-yl-2-iminoimidazolidine Hydrochlorides – C2

Family **C2** was synthesised in a similar fashion to family **C1** (Table 3.3.4). Given that pyridin-3-ylguanidiniums showed poor affinity for the α_2 -AR compared to their pyridin-2-ylguanidinium analogues, it was decided to only synthesise three molecules of this type, for the three *para* substituents which were of most pharmacological interest (H, 5,6-(-CH₂)₄, NHEt).

Table 3.3.4. Synthesis of pyridin-3'-yl-2-iminoimidazolidine hydrochlorides, family **C2**.



Compound	<i>Bis</i> -Boc/HCl	R	Yield (%)
46a	<i>bis</i> -Boc	H	87
46b	<i>bis</i> -Boc	5,6-(-CH ₂) ₄	85
46c	<i>bis</i> -Boc	NHEt	64
47a	HCl	H	98
47b	HCl	5,6-(-CH ₂) ₄	91
47c	HCl	NHEt	95

Yields for the preparation of **46a-c** were generally higher than they had been in the case of pyridin-2'-yl-2-iminoimidazolidines **42a-e** (Table 3.3.2). This parallels what was observed in the synthesis of families **A** and **B**, and can be explained by the higher reactivity of amines in the three position of pyridine rings, as they are not in conjugation with the pyridine nitrogen. The intermediate *bis*-Boc protected 2-iminoimidazolidines **46a-c** were again stable to silica gel chromatography and thus purified in this manner.

Removal of the Boc protecting groups was similarly achieved using a 4M hydrochloric acid solution in dioxane with quantitative conversion being obtained. Washing with dichloromethane and further purification by reverse phase silica chromatography yielded the hydrochloride salts as crystalline solids.

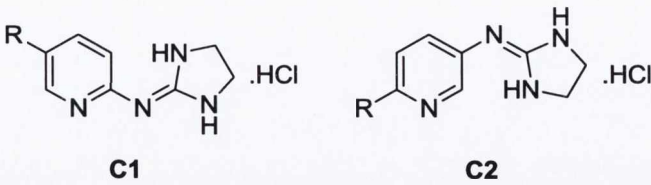
3.3.3. Pharmacological Profile of Families C1 and C2

Eight pyridinyl 2-iminoimidazolidine hydrochlorides were tested for their affinity at the α_2 -AR and those with a $pK_i \geq 6.00$ were tested for their functional activity at the receptor. Based on the results obtained for families **A** and **B**, which indicated that substitution of the cation in the 2-position of the pyridine ring was preferable, five pyridin-2'-yl and three pyridin-3'-yl 2-iminoimidazolidine hydrochlorides (**C1** and **C2**) were chosen.

Binding Affinity (pK_i determination)

Binding affinities were measured in human PFC tissue using a competitive binding assay with the α_2 -AR selective radioligand [3 H]RX821002 at 2 nM concentration. The results of these experiments are presented in Table 3.3.5, where binding affinities are expressed in pK_i values. Interestingly, in a divergence from what was seen for members of families **A** and **B**, the data for the competitive binding assays from several of these compounds fit best to a biphasic curve (Fig. 3.3.2). Where this was the case the pK_i for both the high and low affinity binding curves is provided.

Table 3.3.5. Binding affinities (pK_i) of families **C1** and **C2**. The pK_i values of high (*Hi*) and low (*Lo*) affinity binding sites are indicated.



Compound	Family	R	pK_i
RX821002	-	-	8.72
45a	C1	5-Cl	<i>Hi</i> 11.00 <i>Lo</i> 6.10
45b	C1	5-H	6.43
45c	C1	5-CH ₃	6.39
45d	C1	5,6-(-CH ₂ -) ₄	<i>Hi</i> 9.74 <i>Lo</i> 6.13
45e	C1	5-NHEt	5.64
47a	C2	6-H	5.27
47b	C2	5,6-(-CH ₂ -) ₄	<i>Hi</i> 7.27 <i>Lo</i> 5.84
47c	C2	6-NHEt	<i>Hi</i> 10.03 <i>Lo</i> 6.83

In each case, where a biphasic curve was obtained the goodness of fit was significantly higher than that for a single site binding curve, with R^2 values of 0.987-0.992. There are two main possibilities for the appearance of such a curve in binding experiments which involve displacement of an antagonist ($[^3H]RX821002$). The first possibility is that the compound acting as the displacer is either an agonist or an inverse agonist that binds with different affinity to receptors pre-coupled to their heterotrimeric G-protein complex and uncoupled receptors. This would lead to two changes in the radioactivity counts (Figure 3.3.2), for example one for the binding event to the uncoupled receptors and one for the binding event to the pre-coupled receptors (or *vice versa*).

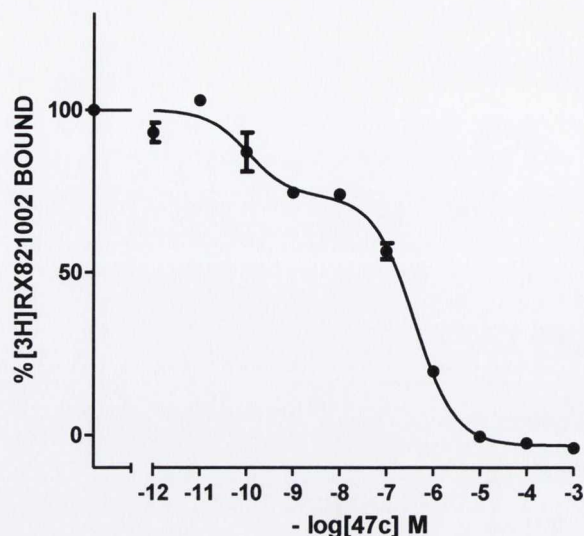


Fig. 3.3.2. Example of a biphasic binding curve for compound **47c** ($R^2 = 0.992$). The extracted pK_i values are 10.03 (Hi) and 6.83 (Lo).

The existence of these two resting states for GPCRs has been demonstrated in living cells using fluorescence studies, including for the case of the α_{2A} -AR.^{198,199} It has also been shown that this pre-assembly of the GPCR complex is only affected by the conformational changes induced when agonists and inverse agonists bind to the receptor, and not when antagonists bind.²⁰⁰ The functional assays of compounds from families **C1** and **C2** (see below) revealed that they are all antagonists, ruling out the possibility of binding with higher affinity to the uncoupled and pre-coupled receptors.

The second possibility for obtaining this type of binding curve is that the compounds are displaying subtype selectivity. The standard antagonist [3 H]RX821002 shows high affinity binding to all α_2 -AR subtypes (α_{2A} -AR, α_{2B} -AR, α_{2C} -AR).²⁰⁰ If the compound being tested has different affinity for different subtypes, then, it will displace the radioligand from these subtypes at different concentrations, resulting in a biphasic curve, and this is postulated to be the case for these compounds.

Comparing the pK_i values of compounds in families **C1** and **C2**, members of **C1** generally have higher affinity for the α_2 -AR; this is true for the tetrahydroquinoline derivatives and the unsubstituted pyridine derivatives while, similarly to their guanidine analogues, the *para* *N*-ethylamino derivatives exhibit the opposite effect. The only analogous derivatives from **C1** and **C2** which both display a biphasic binding curve are

the tetrahydroquinolinyl-2-iminoimidazolidine hydrochlorides **45d** and **47b** and in this case the pattern of pyridin-2-yl compounds having higher affinity than pyridin-3-yl derivatives is maintained for both the high and low affinity binding events.

Functional Assay Results

As mentioned the activities of all compounds with $pK_i \geq 6.00$ were tested using [35 S]GTP γ S binding experiments with the standard agonist UK14304. Thus, three members of **C1** and two members of **C2** were tested and shown to act as antagonists of the α_2 -AR, not modifying the basal [35 S]GTP γ S binding to the receptor (Fig. 3.3.3). No inverse-agonistic features were observed until millimolar concentrations of drug were applied to the assay, which is in line with an antagonistic activity profile.

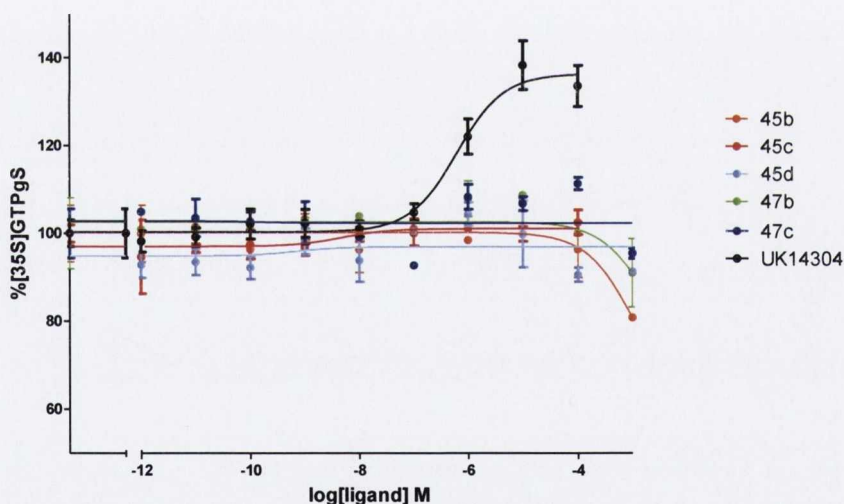


Fig. 3.3.3. Dose-response curves for percentage [35 S]GTP γ S binding vs. ligand concentration of families **C1** and **C2**. The standard agonist UK14304 is shown in black.

3.3.4. Structure Activity Relationships – Families C1 and C2 vs. Families A and B

The first point of comparison between these sets of families (Fig. 3.3.4) was made to investigate if the pharmacological enhancements inferred by placing the cationic moiety in the 2-position as opposed to the 3-position of the pyridine ring would be conserved when the cation was changed from a guanidinium to a 2-iminoimidazolidinium. Encouragingly the same patterns were observed for different *para* substituents in families C1 and C2 as they had been for families A and B. Namely, for all compounds except the *N*-ethylamino substituted derivatives **45e** and **47c** pyridin-2'-yl-2-iminoimidazolidine hydrochlorides C1 have higher affinity for the α_2 -AR. This is the case when either the high or the low affinity pK_i value is considered.

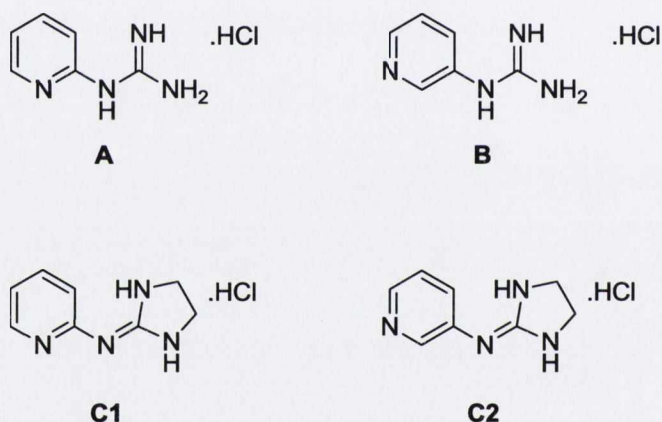


Fig. 3.3.4. Structures of families A, B, C1 and C2.

Having observed this effect across all series to date it seems reasonable to conclude that substitution of the cationic moiety in the 2-position of the pyridine ring is more favourable than substitution in the 3-position. Whether this is due to the conformational restraints induced by the IMHB in families A and C1, or the changes in aromaticity and HB accepting ability introduced by the pyridine ring, is unclear; however, it must be emphasised that the desired antagonistic activity at the α_2 -AR has been attained for all compounds.

Another pattern observed in families A and B was that *para* substituents which increased the electron density of the aromatic ring had higher pK_i values. This is an effect which was investigated computationally (see Section 3.1) and shown to correlate to the preference of ligands to form π -cation complexes with benzene, as a model for

phenylalanine which is thought to be involved in ligand binding at the α_2 -AR. It is difficult to account for this in families **C1** and **C2** as the added variable of biphasic binding curves exists. Furthermore, based on the results obtained in Families **A** and **B** only the 2-iminoimidazolidine hydrochlorides with more electron-donating substituents were synthesised. However, the same patterns are observed when the low affinity pK_i values are compared for those compounds tested.

The second major reason for incorporating the 2-iminoimidazolidinium into the molecules of **C1** and **C2** was to improve the binding affinity relative to guanidinium families **A** and **B**, while hopefully maintaining the antagonistic activity. As already mentioned all molecules tested from **C1** and **C2** are antagonists. The expected increases in pK_i were also observed for almost all derivatives. Table 3.3.6 shows a comparison of the pK_i values for direct analogues with the same substituted aromatic system but with different cationic moieties (either guanidinium or 2-iminoimidazolidinium). Where both a high and low affinity pK_i value was obtained the low affinity value is shown, though the fact that the high affinity site was only observed for 2-iminoimidazolidiniums, and not for guanidiniums, serves to reiterate the point that this type of cation infers higher affinity at the α_2 -AR to ligands.

Table 3.3.6. Comparison of the pK_i values of **A** and **B** (*left*) with **C1** and **C2** (*right*).

No.	Family	R	pK_i	No.	Family	R	pK_i
15b	A	5-Cl	5.77	45a	C1	5-Cl	6.10
15c	A	5-H	6.25	45b	C1	5-H	6.43
15d	A	5-CH ₃	6.09	45c	C1	5-CH ₃	6.39
15e	A	5,6-(-CH ₂ -) ₄	6.84	45d	C1	5,6-(-CH ₂ -) ₄	6.13
15h	A	5-NHEt	4.10	45e	C1	5-NHEt	5.64
38b	B	6-H	3.85	47a	C2	6-H	5.27
38d	B	5,6-(-CH ₂ -) ₄	5.55	47c	C2	5,6-(-CH ₂ -) ₄	5.84
38e	B	6-NHEt	6.34	47c	C2	6-NHEt	6.83

This is a clear indication that 2-iminoimidazolidiniums display higher affinity at the receptor than guanidiniums. While it is unclear if the biphasic binding curves are significant, indications of binding to the receptor with pK_i as high as 11.00 are highly positive and it will be interesting to see in future pharmacological assays if subtype specificity is occurring and if it can be exploited in future ligands of this type.

3.4. *N,N'*-Disubstituted-pyridin-2-ylguanidine Hydrochlorides – Enhancing Antagonistic Activity at the α_2 -AR

In an attempt to establish the structural features necessary to provide antagonistic activity at the α_2 -AR, research in Rozas' group was dedicated to producing a comparative molecular field analysis (CoMFA) model to find quantitative structure activity relationships (QSAR). Two features were probed in this study; the α_2 -AR affinities of compounds previously prepared in our group were used to predict the structural features required for high affinity at the receptor, and α_2 -AR antagonists described in the literature were compared with those discovered in our group to construct an antagonist pharmacophore containing the features required for antagonistic activity at the receptor.

The CoMFA method is a QSAR technique which correlates variations in the activity of molecules to the electrostatic and steric force fields surrounding them. It thus provides a three-dimensional picture of the electrostatic and steric features which correlate to favoured and disfavoured activity, allowing for these characteristics to be incorporated into the design of future molecules. The model was constructed from a library of 41 guanidine and 2-iminoimidazolidine hydrochlorides prepared in Rozas group for which binding affinity and activity data had been obtained.

The steric and electrostatic CoMFA fields were visualised using contour plots to highlight which regions of space correspond to favourable and unfavourable interactions (Fig. 3.4.1). It emerged that steric bulk was predicted to be favoured in the space beyond the cationic moiety (green) and that mildly electrostatically negative groups in this region would favour antagonistic activity (red regions).

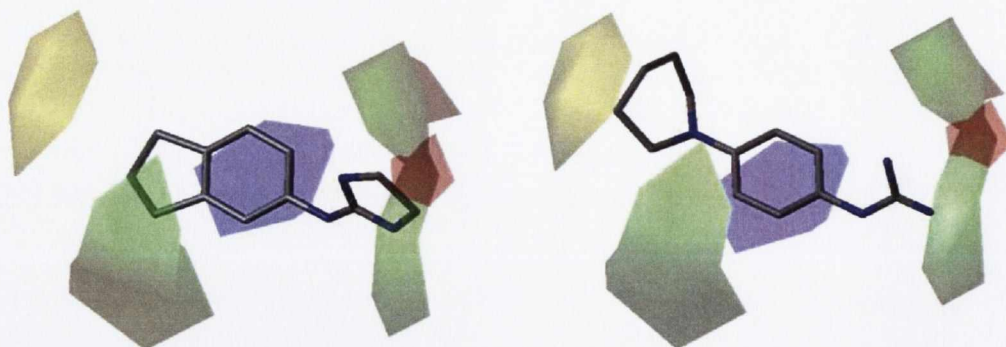


Fig. 3.4.1. CoMFA fields overlaid with a high (*left*) and low (*right*) affinity ligand. Green/yellow regions highlight sterically favoured/disfavoured interactions, red/blue regions represent areas that favour electrostatically negative/positive charges.²⁰¹

The predictions of the model that extension beyond the cationic moiety would infer antagonistic activity to molecules at the α_2 -AR proved successful. Four groups in particular were examined – best fitting the steric requirements – which led to relatively high affinity ligands that, more importantly, were antagonists (Fig. 3.4.2).²⁰¹

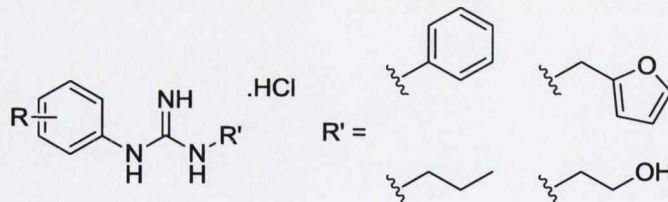


Fig. 3.4.2. Phenyl, 2-furanylmethyl, *n*-propyl and ethoxy substituted aryl guanidine hydrochlorides achieved antagonistic activity at the α_2 -AR.

However, it was notable that the binding affinity of these molecules was significantly reduced compared to their *mono*-substituted guanidine analogues where $R' = H$ (Fig. 3.4.2). The most favourable affinities are associated with the phenyl and 2-furanylmethyl R' substituents, while the *n*-propyl and ethoxy substituents generally provide poorer affinities. It is possible that the lower affinity associated with the latter two substituents is a result of their flexibility. Both groups introduce several additional degrees of rotational freedom which increases the entropic cost of binding and introduces the possibility of other, less favoured, interactions which might alter the

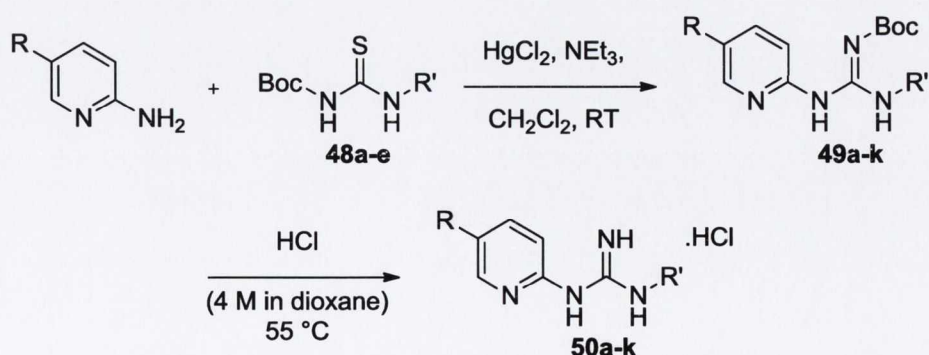
position of either the guanidinium or the aromatic ring, making their contacts with the receptor less effective.

If this is the case, then, reducing this effect would be desirable and could lead to higher affinity ligands, while maintaining the desired antagonistic activity. For this reason, we decided to synthesise a series of *N*-pyridin-2-yl-*N'*-substituted guanidine hydrochlorides, family **D**. These molecules preserve the IMHB described for pyridin-2-ylguanidines **A**, which is predicted to confer rigidity to the pyridinyl guanidinium moiety, allowing for more specific ligand-receptor contacts to be made and decreasing the entropic penalty of binding to the receptor.

3.4.1. Synthesis of *N*-Pyridin-2-yl-*N'*-substituted Guanidine Hydrochlorides

The synthetic approach employed for the synthesis of Family **D** was developed in our lab. It involves the preparation of *N*-(*tert*-butoxycarbonyl)-*N'*-substituted thioureas **48**, followed by their mercury(II) chloride promoted coupling with the suitable 2-aminopyridine to give Boc protected *N*-pyridinyl-*N'*-substituted guanidines **49** and subsequent removal of the Boc group using solutions of hydrochloric acid to generate di-substituted guanidine hydrochlorides **50** for pharmacological testing (Scheme 3.4.1).

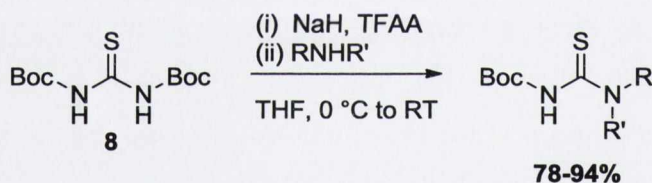
Scheme 3.4.1.



Employing this route has the advantage that the relevant amines are readily available from the synthesis of previous families and that the reaction conditions for the guanidylation and deprotection steps are fully optimised; however, it means that *N*-(*tert*-butoxycarbonyl)-*N'*-substituted thioureas **48** need to be synthesised.

Thus, a method was needed which would give access to derivatives of **48** with a broad range of alkyl, aryl and heteroatomic functionality at the *N'* position. For library preparation, it was also desirable that the conditions for their synthesis would be mild, the reagents involved relatively inexpensive, and the yields high enough to provide the material necessary for the synthesis of families of compounds. Work from Yin *et al.* had recently described the preparation of *N*-Boc-*N'*-substituted thioureas by treatment of *N,N'*-bis-Boc-substituted thiourea **8** with sodium hydride and trifluoroacetic anhydride (TFAA) in the presence of an amine (Scheme 3.4.2).²⁰²

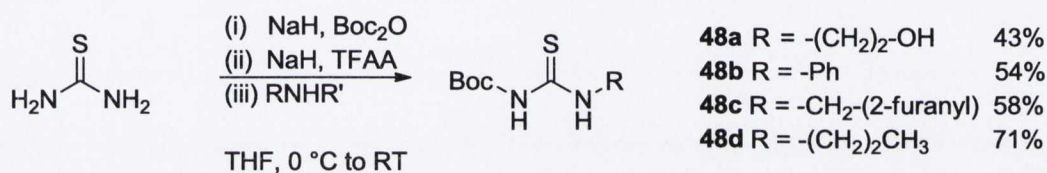
Scheme 3.4.2.



The method is reported to work very well and to be tolerant of primary and secondary alkyl and aryl amines, giving yields of 78-94%. Presumably, the synthesis goes through acylation of one of the thiourea nitrogen atoms to give an intermediate which can be nucleophilically attacked at the thiourea carbon by the amine, with loss of the *N*-Boc-*N*-trifluoroacetyl leaving group, generating the product. The major drawback of this method is the cost of the starting material **8**, which made synthesis of families of thioureas using this route untenable. From our experience using this reagent for the synthesis of *mono*-substituted guanidines, we realised that it was available in one step from unexpensive thiourea and di-*tert*-butyl dicarbonate on treatment with sodium hydride (see Scheme 3.2.2).

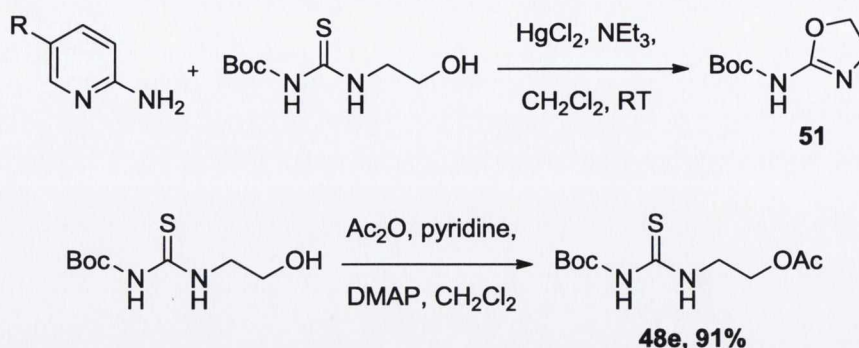
With this in mind, a one-pot procedure was developed in our lab for the synthesis of *N*-Boc-*N'*-substituted thioureas from thiourea (Scheme 3.4.3).²⁰³ Yields obtained were generally in proportion to the reactivity of the amine; alkyl and primary amines gave the highest yields, while aryl and secondary amines gave the lowest yields. This method was applied during the synthesis of family **D**. Firstly, four *N*-Boc-*N'*-di-substituted thioureas **48a-d** were prepared according to Scheme 3.4.3, for the groups which had proved most pharmacologically successful in the analogous family of *N*-phenyl-*N'*-substituted guanidiniums. Yields obtained were similar to the reported values.

Scheme 3.4.3.



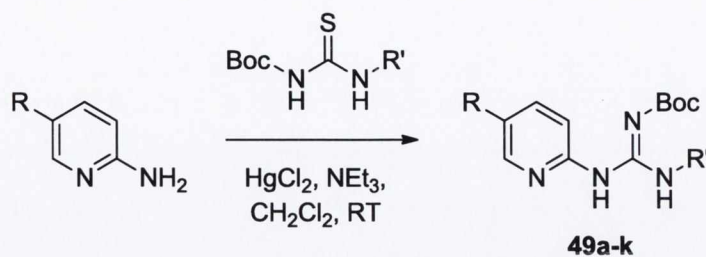
It was necessary to protect the hydroxyl group of *N*-Boc-*N'*-ethoxy thiourea as when this compound was exposed to mercury(II) chloride the available intramolecular ring-closing reaction was faster than guanidylation of the pyridin-2-amine and the dominant product was oxazolidine **51** (Scheme 3.4.4, *top*) and not the desired tri-substituted guanidine. The hydroxyl group was protected with an acetyl group using acetic anhydride, employing pyridine as base and DMAP as nucleophilic catalysts to give **48e** (Scheme 3.4.4, *bottom*). Given that this reaction proceeded in 91% yield and that the acetyl group was readily removed at the same time as the Boc group using a methanolic solution of hydrochloric acid, these steps did not have a detrimental effect on the overall yield.

Scheme 3.4.4.



The family decided on also incorporated the pyridin-2-ylamines which had led to the highest binding affinity in pharmacological tests on families **A**, **B** and **C1**. Thus, these were coupled with thioureas **48b-e** in the presence of mercury(II) chloride, using triethylamine as base. The yields for the guanidylation step (Table 3.4.1) were slightly lower than those for the synthesis of 1-(pyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidines **14a-e** had been (Section 3.2.1). This was expected since the principal reason for introducing the Boc groups to thiourea is for their electron-withdrawing ability which renders the thiourea carbon more electrophilic and more susceptible to attack from nucleophiles. However, in this case, one Boc group is replaced by either a *n*-propyl, ethoxy, phenyl, or 2-furanylmethyl group, none of which are as electron-withdrawing as a Boc group, making the thiourea carbon less electrophilic and lowering the yields for the guanidylation reaction.

Nonetheless all compounds were obtained in reasonable yield (41-89%). These yields do not reflect the fact that some of compounds **49** were obtained as a mixture of isomers (see Section 3.4.2). Where these isomers were separable, the yield is given for the single isomer and where they were inseparable the yield includes both isomers present. Also included in Table 3.4.1 are the two compounds **39a** and **39b** which were used in the study of conformational control in *N*-Boc-*N'*-alkyl pyridin-2-ylguanidines (Section 3.2.2).

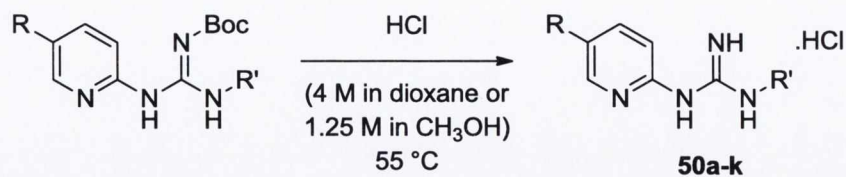
Table 3.4.1. Synthesis of Boc protected *N*-pyridin-2-yl-*N'*-substituted guanidines **49a-i**, **39a-b**).

Compound	R	R'	Yield (%)
49a	H	-(CH ₂) ₂ -OAc	43 ^a
49b	CH ₃	-(CH ₂) ₂ -OAc	54
49c	5,6-(-CH ₂ -) ₄	-(CH ₂) ₂ -OAc	69
49d	H	-C ₆ H ₅	63 ^a
49e	CH ₃	-C ₆ H ₅	89 ^a
49f	5,6-(-CH ₂ -) ₄	-C ₆ H ₅	88 ^a
49g	H	-CH ₂ -(2-furanyl)	61
49h	CH ₃	-CH ₂ -(2-furanyl)	41
49i	5,6-(-CH ₂ -) ₄	-CH ₂ -(2-furanyl)	65
39a	Cl	-(CH ₂) ₂ CH ₃	39
39b	CH ₃	-(CH ₂) ₂ CH ₃	28

^a Denotes that compound was obtained as an inseparable mixture of isomers.

However, the problem of isomerism in intermediates **49** was not serious as removal of the Boc group using hydrochloric acid – to access compounds **50** which are tested pharmacologically – led to a single isomer (Table 3.4.2). Solutions of hydrochloric acid

were used to remove the Boc group and generate the guanidine hydrochloride in one step, similar to the synthesis of 2-iminoimidazolidine hydrochloride families **C1** and **C2**. Where possible, solutions of hydrochloric acid in non-nucleophilic solvents such as 1,4-dioxane were used, but for deprotection of the acetoxy derivatives **49a-c** a 1.25 M solution of hydrochloric acid in methanol was required to allow simultaneous removal of the acetyl protecting group. The yields for the deprotection were generally high and are for purified compound. All compounds were purified using small scale reverse phase (C-8 silica) chromatography and some required recrystallisation further to this. This was carried out using a slow diffusion of diethyl ether in methanol in a closed jar.

Table 3.4.2. Synthesis of *N*-pyridin-2-yl-*N'*-substituted guanidine hydrochlorides, family **D**.

Compound	Method	R	R'	Yield (%)
50a	HCl/CH ₃ OH	H	-(CH ₂) ₂ -OH	83
50b	HCl/CH ₃ OH	CH ₃	-(CH ₂) ₂ -OH	87
50c	HCl/CH ₃ OH	5,6-(-CH ₂ -) ₄	-(CH ₂) ₂ -OH	81
50d	HCl/dioxane	H	-C ₆ H ₅	90
50e	HCl/dioxane	CH ₃	-C ₆ H ₅	86
50f	HCl/dioxane	5,6-(-CH ₂ -) ₄	-C ₆ H ₅	86
50g	HCl/dioxane	H	-CH ₂ -(2-furanyl)	82
50h	HCl/dioxane	CH ₃	-CH ₂ -(2-furanyl)	95
50i	HCl/dioxane	5,6-(-CH ₂ -) ₄	-CH ₂ -(2-furanyl)	86
50j	HCl/dioxane	Cl	-(CH ₂) ₂ -CH ₃	93
50k	HCl/dioxane	CH ₃	-(CH ₂) ₂ -CH ₃	90

3.4.2. Study on Isomerism in Tri-substituted Acyl Guanidines

Acyl guanidines are useful as they act as guanidine analogues with decreased basicity, diminished polarity, and a corresponding increased lipophilicity. For example they have recently been applied to the design of a new class of neuropeptide Y2 receptor antagonists with improved pharmacokinetic profiles.²⁰⁴ Aryl guanidines have long been

of interest, finding use in many pharmaceuticals ranging from clonidine (an α -AR agonist antihypertensive and anaesthetic) to the anticancer treatment imatinib (a Tyrosine kinase inhibitor).

The structure of aryl and acyl guanidines is complex, with the possibility existing for tautomerism, as well as geometrical and conformational isomerism. This has been the subject of several investigations including a ^{15}N NMR spectroscopy study²⁰⁵ of guanidine tautomerism and an analysis of the conformational preferences of *N*-alkyl-acyl guanidines.¹⁹⁰ However, new guanidine derivatives still uncover unpredictable conformational and tautomeric preferences.

On preparation of Boc protected *N*-pyridin-2-yl-*N'*-substituted guanidines **49a-k**, the synthetic intermediates to the guanidines of biological interest, it became evident that the ^1H and ^{13}C NMR spectra of these compounds differed significantly from other classes of acyl guanidines. In contrast with di-acyl guanidines prepared previously in our group, the NMR spectra of *N,N'*-bis-aryl-*N''*-acyl guanidines were characterised by broad, poorly resolved signals. It seemed likely that this was due to interconversion between two or more tautomers induced by the sterically congested environment surrounding the central guanidine moiety.

It was untenable to study each of the compounds synthesised, thus in order to justify the observed mixtures variable temperature (high and low temperature) NMR spectroscopy and B3LYP DFT calculations were carried out on the most instructive compound **49d**, 1-(pyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-phenylguanidine. This compound was part of a larger study²⁰⁶ involving compounds from elsewhere in our research group – *N,N'*-bis-phenyl-*N''*-(*tert*-butoxycarbonyl)guanidine **51** and the structurally complex **52** – which exhibited the same behaviour (Fig. 3.4.3). Only compound **49d** will be discussed in this section, though strong agreement in both the theoretical and NMR studies was observed for each compound.

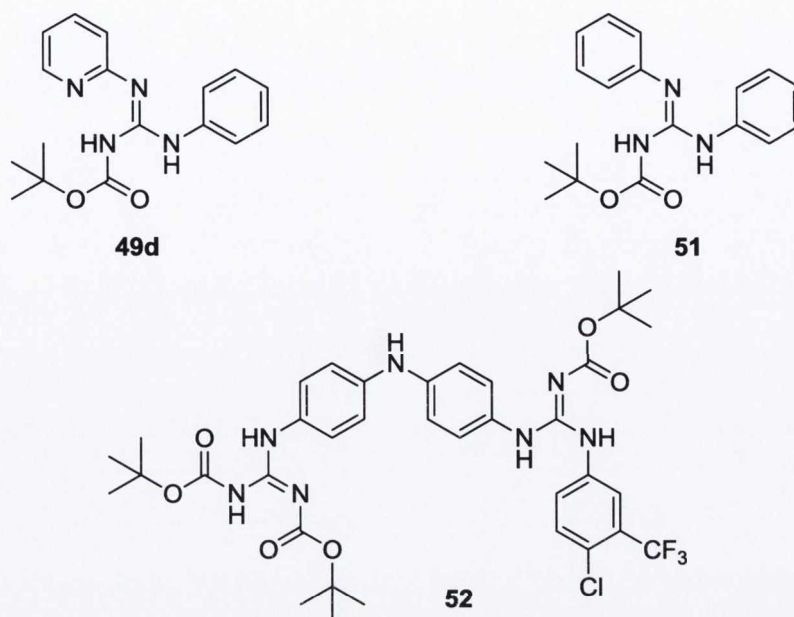


Fig. 3.4.3. Di-aryl acyl guanidines exhibiting tautomeric and conformational isomerism. Only **49d**, arising from this work, will be discussed here.

Variable temperature NMR spectroscopy is commonly used to investigate tautomerism and conformational isomerism. Where X-ray crystallographic data is unavailable – as is the case in this study, all compounds being obtained as sticky gum – DFT calculations are also used in conjunction with spectroscopic techniques to provide a picture of the three-dimensional shape of the molecules. The Gauge-Independent Atomic Orbital (GIAO) method for calculating NMR shifts also proved useful in supporting our experimental NMR data.

In a structural study of non-acylated *N,N'*-bis-phenylguanidines by Tanatani *et al.*,²⁰⁷ extensive use of X-ray crystallography was made. This highlights the fact that small modifications to the guanidine subunit can have an enormous impact on the structural behaviour of the resulting compounds; attempts at recrystallisation of our systems never yielded solid material.

Compound **49d** is obtained in neutral, unprotonated form. Thus, the double bond is localised and can, in theory, conjugate with any of the three substituents on the guanidine. Considering *E* and *Z* isomerism for each of these tautomers a total of six isomers are possible (Fig. 3.4.4). The notation used indicates the group with which the

double bond is in conjugation, followed by the configuration of the double bond (group order of priority: $\text{ac} > \text{py} > \text{ph}$). Two of these isomers can be discounted (py.E , ph.E) due to either steric clashes of the aryl rings or lone-pair:lone-pair repulsion. This was verified by the failure to optimise any such structures in the DFT study. Each of the remaining four tautomers (py.Z , ac.Z , ph.Z , ac.E) incorporates a stabilising IMHB which we have shown previously to be decisive in determining the conformation of acyl-substituted guanidines (see Section 3.2.2).¹⁹¹

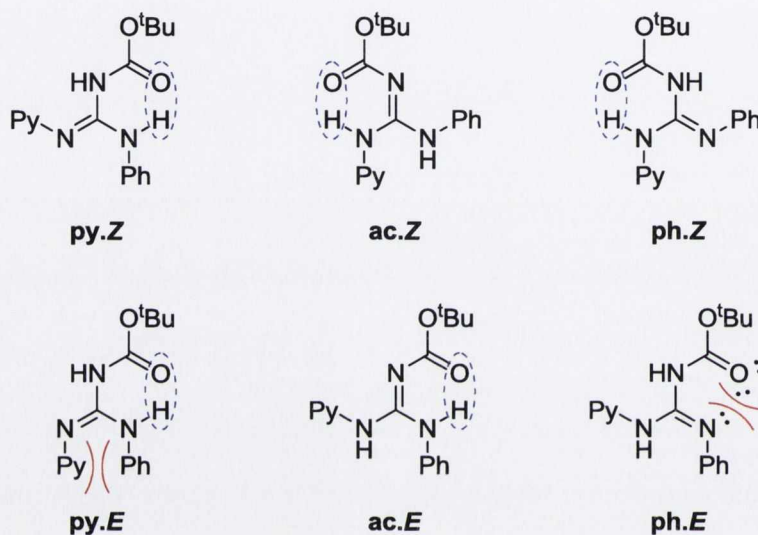


Fig. 3.4.4. Six possible isomers of **49d**. Blue ellipses indicate IMHBs, red arcs indicate steric or electronic repulsion respectively.

Considering these four most energetically favourable isomers in more detail, all are conformationally restrained as rotation about the rotatable C-N bonds leads to loss of IMHB interactions and forces the nitrogen lone-pairs out of conjugation with the imine double bond and the carbonyl moiety. An additional factor to consider for compound **49d** is that a second IMHB is available between the pyridine nitrogen and a second guanidine NH (Fig. 3.4.5). This interaction is only possible for isomers **py.Z** and **ac.Z**. For this reason the expected isomers of **49d** to exist on its formation are **py.Z** and **ac.Z** (Fig. 3.4.5)



Fig. 3.4.5. Predicted isomers of **49d** incorporate two IMHBs (indicated by dashed ellipses).

Accordingly, each of these isomers was optimised using the B3LYP functional with the 6-31+G(d,p) basis set. The calculations predicted that **49d py.Z** would be slightly more stable than **49d ac.Z** (by 5.0 kJ mol⁻¹). While not a substantial difference in energy this predicts that an excess of the **49d py.Z** should be observed in the NMR spectra of the mixture.

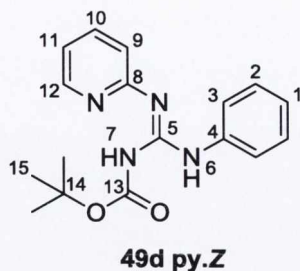
NMR Spectroscopic Evidence for the Existence of 49d py.Z and 49d ac.Z in solution

It was clear from the NMR spectrum of **49d** that two tautomers were being observed. The spectra at room temperature were poorly resolved; in particular, the ¹³C NMR spectrum displayed broad, amorphous peaks. It was decided to perform low temperature NMR experiments on the sample to hamper interconversion of isomers occurring. Thus, **49d** was cooled in 20 °C increments to a temperature of -40 °C, at which point the spectra became sharp enough to observe the two distinct isomers well resolved from each other, with minimal overlap of signals.²⁰⁸

Integration of the Boc CH₃ signals in the ¹H NMR spectrum suggested that the two isomers exist in an approximate ratio of 10:3, the major tautomer being **49d ac.Z**, which places the C=N double bond in conjugation with the Boc group rather than with the pyridine ring. Relative integration of signals in the ¹H NMR spectrum, as well as selective 1D-TOCSY experiments allowed assignment of each signal to a particular isomer. Subsequently, HSQC and HMBC correlation experiments allowed assignment of the ¹³C NMR spectral signals to their respective isomers.

These assignments are supported by the low temperature ^{13}C NMR spectrum of **49d** (-40 °C, 400 MHz, CDCl_3), in which the C5 signal of the major tautomer **2.ac.Z** appears at 152.4 ppm due to conjugation between the C=N double bond and the Boc carbonyl, while in the minor tautomer **2.ar1.Z** the C5 signal is found at 146.4 ppm (Table 3.4.3). Notably, this is significantly downfield from the corresponding signal in the analogous tautomer of the *bis*-phenyl compound **51** (140.2 ppm), suggesting that the double bond in this isomer is conjugated with the more electron-withdrawing pyridine ring, rather than the phenyl ring. Other instructive signals in the ^{13}C NMR spectrum are the C=O carbon signals (C13). Isomer **49d ac.Z** is similar to the dominant isomer obtained for 1-pyridin-2-yl-2,3-*bis*-Boc guanidines **14a-e** for which we showed the dominant isomer to be one with the C=N double bond in conjugation with a Boc group. This Boc C=O signal always appeared from 162.8-163.3 ppm for **14a-e** while the C=O peak of **49d ac.Z** occurs at 164.4 ppm. In contrast, the second Boc C=O peak appears at 154.0, indicating that it is not in conjugation with the guanidine C=N and belongs to **49d py.Z**.

Table 3.4.3. Low temperature ^1H (400 MHz, $-40\text{ }^\circ\text{C}$) and ^{13}C NMR (100 MHz, $-40\text{ }^\circ\text{C}$) shifts of tautomers **49d py.Z** and **49d ac.Z** in CDCl_3 .



Atom No.	δH (49d py.Z)	δC (49d py.Z)	δH (49d ac.Z)	δC (49d ac.Z)
1	7.11	123.3	7.11	124.3
2	7.36	129.0	7.36	129.1
3	7.78	120.8	7.67	122.4
4	-	138.7	-	138.3
5	-	146.4	-	152.4
6	10.11	-	12.27	-
7	13.57	-	12.63	-
8	-	160.6	-	155.2
9	6.88	117.3	6.93	113.7
10	7.62	137.5	7.72	139.1
11	7.03	121.8	7.03	118.4
12	8.27	145.6	8.27	145.9
13	-	154.0	-	164.4
14	-	82.8	-	79.3
15	1.55	28.3	1.52	28.3

Evidence in the ^1H NMR spectrum comes mainly from the guanidine NH peaks which are all in different environments. While not diagnostic in their own right, when viewed in the context of the GIAO results (see below) they support both the accuracy of the GIAO calculation and the assignments of the isomers. In qualitative terms, the NHs of **49d py.Z** appeared at 10.11 and 13.57 ppm. A HMBC connection of the peak at 13.57 ppm with C-13 identified it as H-7, making the peak at 10.11 ppm the proton H-6.

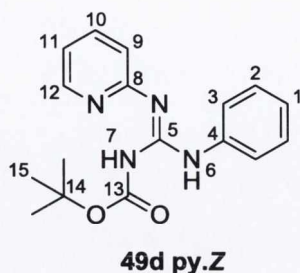
Similarly, a HMBC connection from the proton at 12.63 ppm to C-9 identified this peak as H-7 in **49d ac.z**, making the peak at 12.27 ppm that of H-6 in the same isomer.

The inconsistency between the DFT prediction and the observed ratio of isomers in NMR spectrum was undesired. Factors such as the relative stability of each isomer, or loss of material during silica gel chromatography could tentatively be blamed for the discrepancy. However, the accuracy of the GIAO calculations and their support in assigning the NMR spectrum was undoubted.

*GIAO Calculations of **49d py.Z** and **49d ac.Z***

The GIAO calculations were carried out at the B3LYP theoretical level using the 6-31+G(d,p) basis set. Both ^1H and ^{13}C NMR shifts (given relative to those of tetramethylsilane calculated using the same level of theory) strongly agreed with experimental values and proved useful in confirming the assignments made from the NMR spectra. In particular, the NH protons of the guanidine system, and the guanidine and Boc group carbonyl signals of the ^{13}C NMR spectrum, reinforced our assignments of isomers **49d py.Z** and **49d ac.Z**, despite the tautomeric preference being incorrectly predicted during optimisation (**Table 3.4.4**).

Table 3.4.4. Theoretical ^1H and ^{13}C NMR chemical shifts of **49d py.Z** and **49d ac.Z** at the B3LYP/6-31+G(d,p) using the GIAO method.



Atom No.	δH (49d py.Z)	δC (49d py.Z)	δH (49d ac.Z)	δC (49d ac.Z)
1	7.83	121.0	7.85	121.7
2	8.07/8.21	112.0	8.09/8.21	126.7/128.9
3	7.71/10.24	127.0	7.78/10.23	117.8/122.0
4	-	128.5	-	140.4
5	-	145.1	-	151.9
6	11.16	-	12.71	-
7	14.49	-	13.71	-
8	-	161.9	-	153.9
9	8.00	122.7	7.52	112.0
10	8.34	136.1	8.37	136.9
11	7.49	115.2	7.60	115.3
12	9.04	145.1	9.14	146.2
13	-	154.9	-	165.2
14	-	87.8	-	84.5
15	2.03/2.40/2.40	28.4/28.4/34.7	2.03/2.37/2.37	28.3/28.3/35.2

Shifts are given relative to the isotropic values of TMS calculated at the same level of theory. Multiple values for chemically equivalent protons arise as GIAO is a single point calculation.

Importantly, the theoretical NMR shifts for the most instructive signals of each isomer agree strongly with the experimental values. The computed NH signals of tautomer **49d py.Z** appeared at 11.16 and 14.49 ppm (experimental shift 10.11 and 13.57 ppm), while

the NH signals of **49d ac.Z** appeared at 12.71 and 13.71 ppm (experimental shift 12.27 and 12.63 ppm), maintaining the same patterns observed experimentally.

The ^{13}C NMR shift for guanidine carbon C5 in tautomer **49d py.Z** is unusually low for carbons of this type, being observed at 146.4 ppm. However, its shift is replicated almost exactly by the GIAO calculation, appearing at 145.1 ppm. The theoretical (154.9 ppm) and experimental (154.0 ppm) shifts for the Boc C=O signals for this isomer were also consistent, further supporting our assignments of the NMR spectra. Experimental and theoretical shifts also showed good agreement for isomer **49d ac.Z**. The experimental signal of the guanidine (C5) and Boc carbonyl (C13) carbons appeared at 152.4 and 164.4 ppm respectively, while their GIAO shifts correlated strongly to these values, appearing at 151.9 and 165.2 ppm, respectively. These values are more similar to the values reported for 1-pyridin-yl-2,3-*bis*-Boc guanidines **14a-e**, which also have the C=N double bond conjugated with the Boc group.

It is worth noting that, in our study, the ^{13}C NMR spectrum was only fully assignable for compound **49d**. Its two IMHBs reduced the conformational freedom relative to compound **51** whose ^{13}C NMR spectrum was too amorphous in the aromatic region for assignments to be made, even at -40 °C.

Isomerism in Compounds 49a-k

The phenomenon of isomeric mixtures was prevalent throughout compounds **49a-k**. The two isomers obtained always corresponded to those described above for compound **49d**; however, the ratio of isomers differed based on the relative stability of the isomers involved. In general, the more similar the two substituents on the acyl guanidine were the more degenerate the two isomers were and the closer to a 50:50 mixture was obtained experimentally. Where possible, the NMR spectra of both isomers were assigned; however, in some cases only trace amounts of the minor isomer were observed by ^1H NMR spectroscopy (being unobservable by ^{13}C NMR spectroscopy) and so the minor isomer was not characterised. Furthermore, in some cases the isomers had sufficiently different physical properties that the major isomer could be isolated by silica gel chromatography. In these cases only the major isomer was characterised fully. The specifics for each compound are indicated in Experimental Section 5.

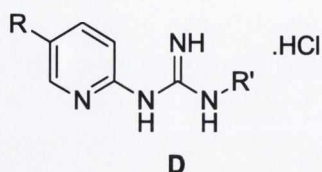
3.4.3. Pharmacological Results for Family D – *N*-Pyridin-2-yl-*N'*-substituted Guanidine Hydrochlorides

All eleven *N*-pyridin-2-yl-*N'*-substituted guanidine hydrochlorides were tested for their affinity at the α_2 -AR and those with a $pK_i \geq 6.00$ were tested for their functional activity at the receptor. These molecules represented incorporation of the most successful pyridin-2-ylamines according to the pharmacological testing of families **A** and **C1**, as well as the groups in the *N'*-position which gave best results in the analogous family of *N*-phenyl-*N'*-substituted guanidine hydrochlorides previously prepared and tested in Rozas group.²⁰¹

Binding Affinity (pK_i determination)

Binding affinities were measured in human PFC tissue using a competitive binding assay with the α_2 -AR selective radioligand [³H]RX821002 at 2 nM concentration. The results of these experiments are presented in Table 3.4.5, where binding affinities are expressed in pK_i values. As was also observed for some members of families **C1** and **C2**, the data from these binding experiments led to biphasic binding curves, indicative of two binding events; one with high affinity and one with lower binding affinity, suggested to correspond to a degree of α_2 -AR subtype selectivity.

Table 3.4.5. Binding affinities (pK_i) of family **D**. The pK_i values of high (*Hi*) and low (*Lo*) affinity binding sites are indicated.



Compound	R	R'	pK_i
RX821002	-	-	8.72
50a	H	-(CH ₂) ₂ -OH	<i>Hi</i> 8.85
			<i>Lo</i> 5.31
50b	CH ₃	-(CH ₂) ₂ -OH	5.20
50c	5,6-(CH ₂) ₄	-(CH ₂) ₂ -OH	<i>Hi</i> 9.55
			<i>Lo</i> 5.65
50d	H	-C ₆ H ₅	<i>Hi</i> 8.92
			<i>Lo</i> 6.06
50e	CH ₃	-C ₆ H ₅	<i>Hi</i> 8.55
			<i>Lo</i> 5.87
50f	5,6-(CH ₂) ₄	-C ₆ H ₅	6.74
50g	H	-CH ₂ -(2-furanyl)-	6.29
50h	CH ₃	-CH ₂ -(2-furanyl)-	6.25
50i	5,6-(CH ₂) ₄	-CH ₂ -(2-furanyl)-	<i>Hi</i> 10.15
			<i>Lo</i> 6.45
50j	Cl	-(CH ₂) ₂ -CH ₃	5.78
50k	CH ₃	-(CH ₂) ₂ -CH ₃	<i>Hi</i> 7.51
			<i>Lo</i> 6.01

Comparison of the various sub-families of **D** allows some SAR conclusions to be made. Again, to allow evaluation of all groups, the low affinity binding site is compared when both high and low affinity sites were indicated. As in all previous families, the order of the R groups on the pyridine ring which give the best pK_i from best to worst is; -(CH₂)₄-

, H, CH₃. The only chlorine-substituted derivative, **50j**, also continued the trend observed for electron-withdrawing substituents, resulting in a lower pK_i than the corresponding methyl-substituted compound **50k**.

It is more difficult to see a general trend when the R' groups are compared. With the exception of tetrahydroquinoline derivatives **50f** and **50i** it can be said that the 2-furanylmethyl derivatives result in the highest pK_i values, though **50i** displays a biphasic binding curve indicative of binding to both a high and low affinity binding site. One consequence of this is that, if some compound is bound to a high affinity site, there is less available for binding to the low affinity site and the pK_i value for the low affinity site becomes distorted, seeming lower than it should be.

It is clear that the ethoxy-substituted guanidiniums have lower pK_i values than both the phenyl and the 2-furanylmethyl derivatives. This was shown to be the case for *N*-phenyl-*N*'-substituted guanidines as well; it seems that the polarity and HB profile of the hydroxyl group are unfavourable for binding to this pocket of the active site. Not a lot of information can be gleaned from the *n*-propyl-substituted guanidines; **50k** binds to two sites with similar affinity, while **50j** has no direct analogues in **D**.

Functional Assay Results

The activities of all compounds with pK_i ≥ 6.00 were tested using [³⁵S]GTPγS binding experiments with the standard agonist UK14304. Thus, all three 2-furanylmethyl compounds, the tetrahydroquinoline derivative of the phenyl guanidiniums and both *n*-propyl guanidiniums were tested using this assay. None of the ethoxy-substituted guanidiniums were tested for activity at the α₂-AR as their affinity for the receptor was too low to provide a useful antagonist. The dose-response curves for all of these compounds (Fig. 3.4.6) are suggestive of an inverse-agonistic effect at the α₂-AR, switching down the basal binding of [³⁵S]GTPγS at the receptor.

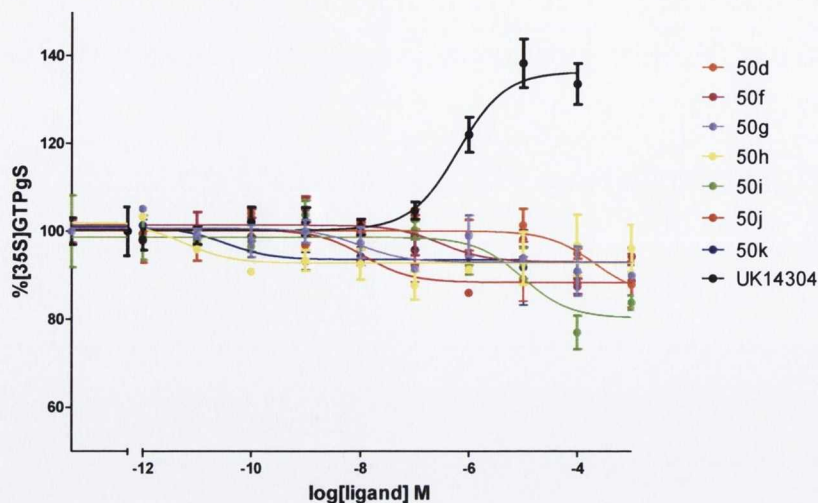


Fig. 3.4.6. Dose-response curves for percentage [^{35}S]GTP γ S binding vs. ligand concentration of family **D**. The standard agonist UK14304 is shown in black.

While it is clear from these curves that the concentration at which the inverse agonistic effects begin to appear is varied, reliable EC_{50} values cannot be obtained from the functional [^{35}S]GTP γ S assay either for agonists or inverse agonists and so nothing can be drawn from these differences without further assays.

3.4.4. Structure-Activity Relationships – Family **D** in Comparison with Families **A**, **B** and *N*-Phenyl-*N'*-substituted Guanidine Hydrochlorides

Binding Affinity (pK_i)

The pK_i values for compounds from family **D** compare favourably to those of families **A**, **B**, **C1** and **C2** (Fig. 3.4.7). For the purpose of this discussion, the members of these families with unsubstituted, *p*- CH_3 and 5,6- $(-\text{CH}_2)_4$ pyridine systems will be considered.

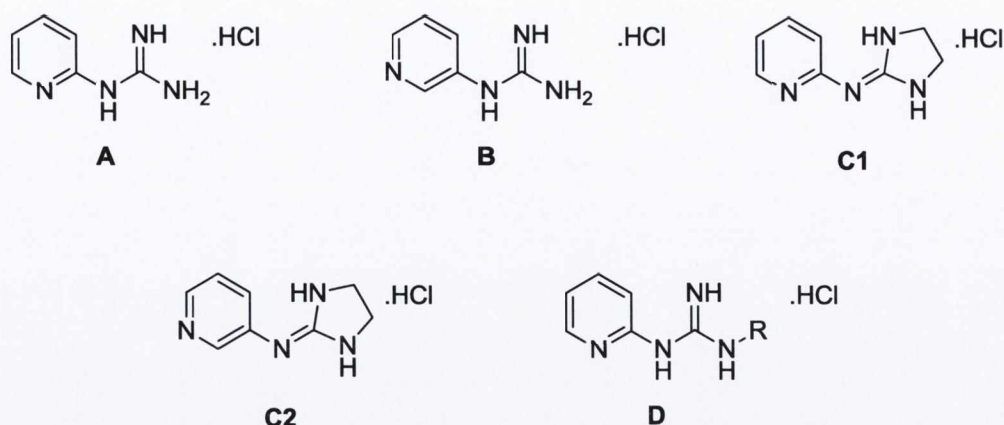
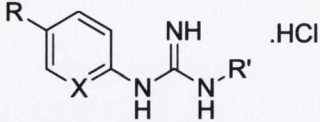
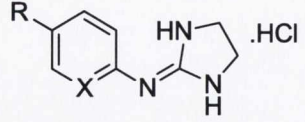


Fig. 3.4.7. Structures of families **A**, **B**, **C1**, **C2** and **D**.

As was expected, compounds from family **D** have significantly higher pK_i values than any of their analogous pyridin-3-yl cationic compounds from families **B** and **C2**. This can be said for all compounds even when the low affinity pK_i of compounds that bind to two sites with different affinity is compared, further reinforcing the finding that introduction of the cationic moiety in the 2-position is preferable to in the 3-position.

Of more relevance is a comparison of family **D** with families **A** and **C1** (Table 3.4.6). The ethoxy-substituted guanidiniums of **D** have the lowest pK_i values of all these groups; those for the low affinity site and those for the high affinity site are lower than for compounds from the other families. The phenyl-substituted guanidiniums of **D** have improved pK_i relative to this; however, the values fall short of both family **A** and family **C1**, indicating that substitution in this region with a bulky phenyl group is unfavoured in terms of binding affinity. The 2-furanylmethyl guanidiniums gave the highest pK_i values from family **D**. When considering the low affinity binding site, values of pK_i fall between those of families **A** and **C1**, being better than *mono*-substituted guanidiniums and only slightly worse than 2-iminoimidazolidiniums.

Table 3.4.6. Binding affinities (pK_i) of **D** and subsets of **A** and **C1**.

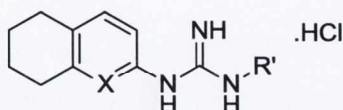
Family	R'	R	R	R
		5-CH ₃	5-H	5,6-(-CH ₂ -) ₄
A	H	6.09	6.25	6.84
C1	-	6.39	6.43	Hi 9.74 Lo 6.13
D	-(CH ₂) ₂ -OH	5.20	Hi 8.85 Lo 5.31	Hi 9.55 Lo 5.65
D	-CH ₂ -(2-furanyl)-	6.25	6.29	Hi 10.15 Lo 6.45
D	-C ₆ H ₅	Hi 8.85 Lo 5.87	Hi 8.92 Lo 6.06	6.74

This was a positive result, as extension beyond the guanidinium moiety in the analogous *N*-phenyl-*N'*-substituted guanidiniums had led to decreased binding affinity relative to the *mono*-substituted phenyl guanidiniums for all groups. It is difficult to obtain direct comparisons between family **D** and *N*-phenyl-*N'*-substituted guanidiniums as the phenyl systems investigated in the work that led to this series of compounds are generally different to those in family **D**. However, it can be said that within both families similar trends in the pK_i values were observed; 2-furanylmethyl derivatives gave the highest pK_i values, being better than phenyl and ethoxy, respectively.

Data is available for the phenyl analogues of the tetrahydroquinoline derivatives however (Table 3.4.7). Encouragingly, the data suggests that improvements to pK_i have been obtained in family **D** relative to the phenyl derivatives. Even when the low affinity pK_i values for family **D** are considered they are either very similar or improved

compared to the phenyl derivatives. Also, none of the phenyl compounds showed any high affinity binding event.

Table 3.4.7. Comparison of family **D** with *N*-phenyl-*N'*-substituted guanidine hydrochlorides.



X	R'	pK _i	X	R'	pK _i
CH	-(CH ₂) ₂ -OH	5.76	N	-(CH ₂) ₂ -OH	Hi 9.55 Lo 5.65
CH	-C ₆ H ₅	6.58	N	-C ₆ H ₅	6.74
CH	-CH ₂ -(2-furanyl)	6.29	N	-CH ₂ -(2-furanyl)	Hi 10.15 Lo 6.45

Functional Activity

In terms of the activity profile obtained using [³⁵S]GTPγS assays for compounds from these families, some differences occur. All members of families **B**, **C1** and **C2**, as well as *N*-phenyl-*N'*-substituted guanidiniums exhibit antagonist binding curves in these experiments, with neither partial agonism nor inverse agonism being observed. Members of families **A** and **D** mainly displayed dose-response curves characteristic of inverse agonists. There are several factors which could be decisive in this discrepancy. The 2-iminoimidazolidiniums have increased hydrophobicity due to their ethylene bridge which could be altering their interactions with the active site of the receptor. They will also have reduced pK_{aH} relative to guanidines due to disruptions to the delocalisation of the positive charge of the conjugate acid caused by the ethylene bridge. No crystal structure data was available for this type of molecule however the ¹H NMR spectra of pyridin-2'-yl-2-iminoimidazolidine hydrochlorides **C1** strongly suggests that the molecules adopt the planar conformation seen in pyridin-2-ylguanidine hydrochlorides **A**. Members of families **A** and **D** share the planar geometry induced by

an IMHB between their pyridine nitrogen and a guanidinium NH and this could be a decisive factor in their activity profiles.

However, it is difficult to make conclusions on these differences and, in order to do so, further examination of the significance of inverse agonism at the α_2 -AR, as well as measurements of EC_{50} for these events, is needed. What is clear is that agonistic activity at the receptor has not been observed for any of these compounds where it had been a recurring, yet unpredictable, theme for the previously prepared phenylguanidiniums and phenyl-2-iminoimidazolidiniums.

3.5. *N*-Substituted-1,4-dihydroquinazolin-2-amines — Conformationally Restricted Guanidines

Having seen that family **A** gave exclusively antagonistic activity at the α_2 -AR, whereas phenyl guanidiniums had given mixed activity profiles, and given that conformationally restricting the rotatable bonds of ligands has long been a strategy in amplifying antagonistic effects,²⁰⁹ we decided to add a CH₂ bridge between the guanidinium moiety and the aryl ring, resulting in fused bicyclic aryl guanidinium conformationally restricted analogues, family **E** (Fig. 3.5.1).

The first point of note is that in these derivatives the pyridine nitrogen cannot be accommodated at this position any more. Instead of incorporating it elsewhere in the aryl ring it was decided to remove it, opting instead for a phenyl ring. The reason for this was that the pK_i values of family **A** compared unfavourably to those of their analogous phenyl guanidine hydrochlorides. It was postulated that this reduction was due to the pyridine ring and thus the benzene core was re-introduced to family **E** in an attempt to increase binding affinity. Our hypothesis was that in this manner we could obtain high affinity binding at the α_2 -AR, while at the same time maintaining antagonistic activity.

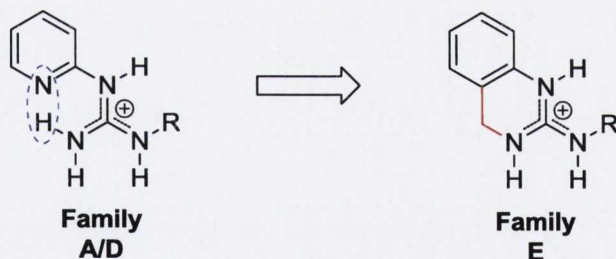


Fig. 3.5.1. Family **E** arose from the positive results of families **A** (R = H) and **D** (R = -(CH₂)₂-OH, -C₆H₅, -CH₂-(2-furanyl), -(CH₂)₂-CH₃), the IMHB being replaced by a CH₂ bridge.

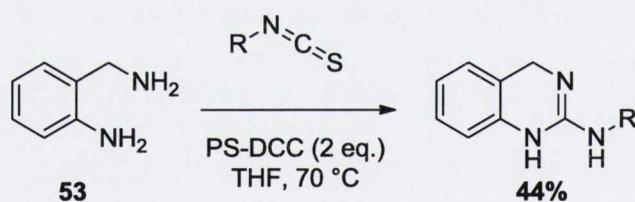
Having also learned that substitution of the benzene ring was not necessarily beneficial to the binding properties of the molecules (this is also suggested by the CoMFA model), it was left unsubstituted; however, based on the results of family **D**, and the analogous disubstituted phenyl guanidinium derivatives, di-substitution of the guanidinium moiety

was employed. Thus, a small family of four ligands (Fig. 3.5.1) was prepared for biological testing to obtain proof of concept on this new type of ligand for the α_2 -AR.

Literature Methods for the Synthesis of N-Substituted-1,4-dihydroquinazolin-2-amines

The synthesis of *N*-substituted-1,4-dihydroquinazolin-2-amines is not widely reported in the literature and, of the routes that exist, many are long and laborious. One reported method utilises *N,N'*-dicyclohexylcarbodiimide (DCC) supported on a polymer (PS-DCC) to couple 2-aminobenzylamine **53** to isothiocyanates (Scheme 3.5.1).²¹⁰ This method was reported to work in very high yield for the formation of 2-aminobenzimidazoles when 2-aminoaniline was used in place of 2-aminobenzylamine; however, the yield for formation of *N*-substituted-1,4-dihydroquinazolin-2-amines was significantly lower (44%), when phenyl-isothiocyanate was used.

Scheme 3.5.1.

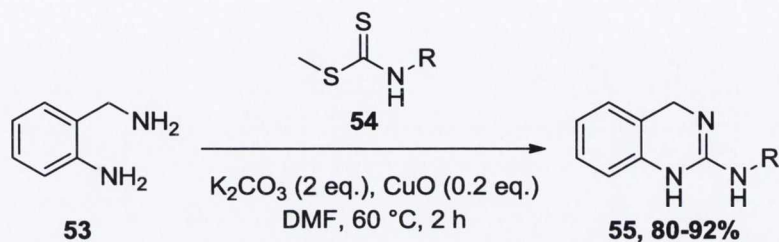


Another limiting factor for this route is the poor availability of isothiocyanates and their relative difficulty of preparation. The most common route to their synthesis involves coupling of an amine with carbon disulfide in the presence of ammonium hydroxide to yield an ammonium dithiocarbamate, which is then reacted with lead(II) nitrate to form the isothiocyanate.²¹¹

By far a more efficient and high yielding synthesis was reported by Das *et al.*, which also utilises **53** described above; however, in this case it is catalytically coupled with the easily accessible *S*-methyl dithiocarbamates **54** using copper(II) oxide (Scheme 3.5.2).²¹² This method was also reported for the preparation of 2-aminobenzimidazoles along with *N*-substituted-1,4-dihydroquinazolin-2-amines **55**; yet, in this case the yields

were high for both reaction types, with yields of 80-92% for the coupling of varied dithiocarbamates with 2-aminobenzylamine being reported.

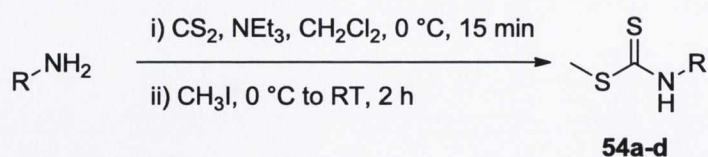
Scheme 3.5.2.



The *S*-methyl dithiocarbamates themselves can be synthesised from the desired amine, carbon disulfide and iodomethane in the presence of triethylamine in a two hour reaction (Table 3.5.1) and for these reasons this method was chosen for the preparation of family **E**.

3.5.1. Synthesis of *S*-Methyl Dithiocarbamates

Accordingly, four *S*-methyl dithiocarbamates **54a-d** were prepared using a literature procedure (Table 3.5.1).²¹³ The method reported involves the solvent-free reaction of an amine with carbon disulfide in triethylamine to form an intermediate dithiocarbamate, which is then trapped by iodomethane to make the isolable *S*-methyl dithiocarbamate with the leaving-group ability installed in the shape of the *S*-methyl group.

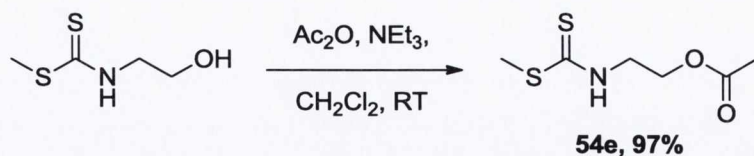
Table 3.5.1. Preparation of *S*-methyl dithiocarbamates.

R	Product	Yield (%)
-(CH ₂) ₂ -OH	54a	68
-C ₆ H ₅	54b	68
-CH ₂ -(2-furanyl)	54c	80
-(CH ₂) ₂ -CH ₃	54d	89

In our experience, the reaction was difficult to control in the absence of solvent, particularly for the more reactive amines *n*-propylamine and ethanolamine, and the rate of reaction was not diminished when dichloromethane was added. Thus, the reactions were performed at 1 M concentration in dichloromethane with respect to the amine starting material. The possibility of conformational isomerism at the *S*-methyl group led to two systems being observed by NMR spectroscopy, which could clearly be identified as the two isomers of **54a-d** resulting from this. However, this did not cause any problems as both isomers coupled effectively with 2-aminobenzylamine in the next step of the synthesis (see below).

The ethoxy substituted *S*-methyl dithiocarbamate **54a** was *O*-protected with an acetyl group as it was expected that exposure to the desulfurizing agent (CuO) would lead to ring-closing and formation of oxazolidine-2-thione, even in the presence of 2-aminobenzylamine, in a similar side-reaction to the one observed during the synthesis of the analogous derivatives from family **D** (Section 3.4.1). It was, thus, reacted with acetic anhydride in the presence of triethylamine and almost quantitative formation of the acetyl-protected *S*-methyl dithiocarbamate **54e** was obtained (Scheme 3.5.3).

Scheme 3.5.3.

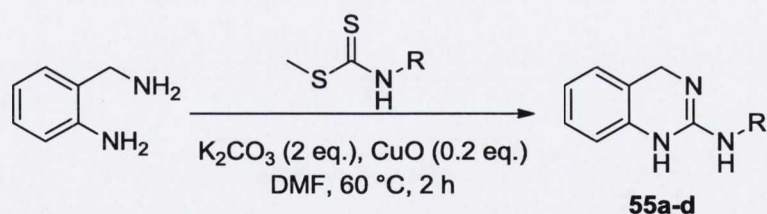


With the *S*-methyl dithiocarbamates in hand, the conditions of Das *et al.* were applied to form the neutral *N*-substituted-1,4-dihydroquinazolin-2-amines **55a-d**.

Formation of N-Substituted-1,4-Dihydroquinazolin-2-amines

Coupling of 2-aminobenzylamine with *S*-methyl dithiocarbamates **54** went in relatively high yield (60-81%). Removal of dimethylformamide proved difficult, additional aqueous workup often being required even after silica gel chromatography, and it is anticipated that this had a detrimental effect on yield. Nevertheless, the neutral *N*-substituted-1,4-dihydroquinazolines **55a-d** were obtained in high purity and without major problems, as low-melting solids (Table 3.5.2).

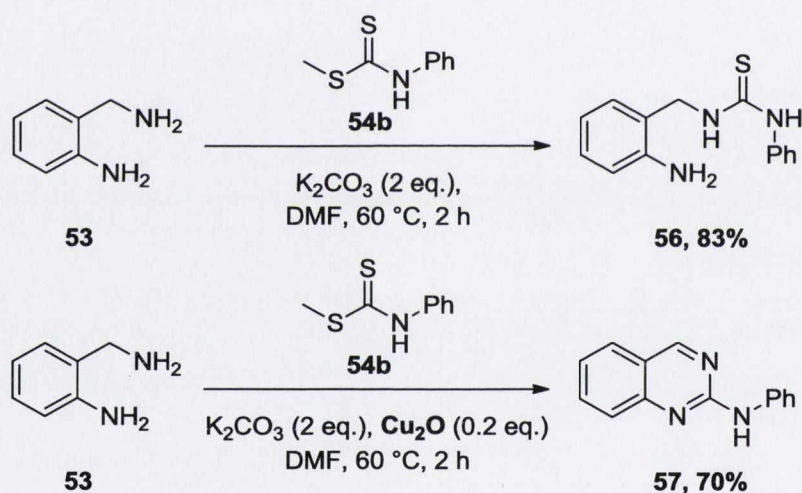
Table 3.5.2. Synthesis of *N*-substituted-1,4-dihydroquinazolin-2-amine hydrochlorides.



Compound	R	Yield (%)
55a	-(CH ₂) ₂ -OAc	60
55b	-C ₆ H ₅	76
55c	-CH ₂ -(2-furanyl)	63
55d	-(CH ₂) ₂ -CH ₃	81

The Mechanism of Ring Closure

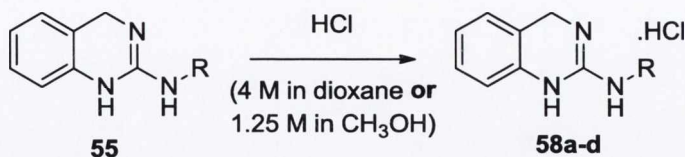
No discussion on the mechanism of ring-closure is provided by the authors; however, it is likely to go through a thiourea intermediate, which then undergoes intramolecular nucleophilic attack from the benzyl amino group and loss of sulfur to yield the cyclic product. During a brief study on the reactivity of these dithiocarbamates, we found that in the absence of copper(II) oxide the product formed is the thiourea **56** (Scheme 3.5.4). Interestingly, when copper(I) oxide was used instead of copper(II) oxide, the product was the aromatic quinazoline species **57**. This proved to be a general pattern; however, attempts at optimising the modest yields never surpassed 70% on our test reaction.

Scheme 3.5.4.

As the neutral dihydroquinazoline molecules **55a-d** are not water-soluble, and this is preferred for pharmacological testing, their hydrochloride salts were prepared by stirring the neutral compounds in solutions of hydrochloric acid (Table 3.5.3). The reaction went to full completion, as monitored by the disappearance of starting material in TLC. A 4 M solution of hydrochloric acid in 1,4-dioxane was favoured; however, due to the high polarity of the starting materials, additional methanol was added as necessary to aid solubility during the formation of **58b** and **58d**. A solution of

hydrochloric acid in methanol (1.25 M) was required to remove the acetyl group of **55a** and simultaneously generate the hydrochloride salt **58a**.

Table 3.5.3. Protonation of **55a-d** to yield **58a-d**.

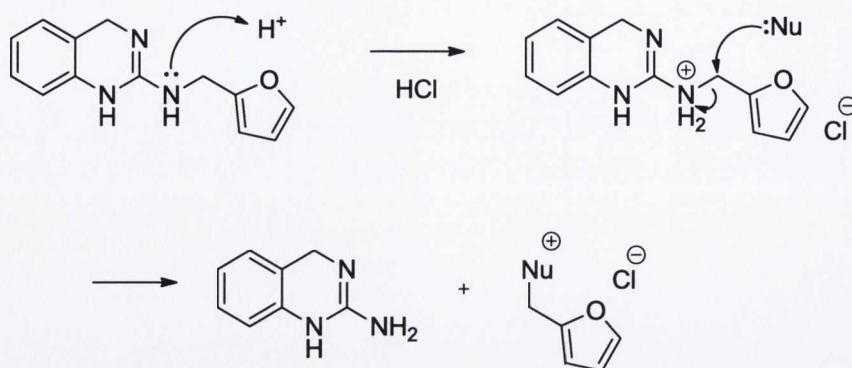


Compound	Conditions	R	Yield (%)
58a	a	-(CH ₂) ₂ -OH	95
58b	b	-C ₆ H ₅	93
58c	c	-CH ₂ -(2-furanyl)	30
58d	b	-(CH ₂) ₂ -CH ₃	90

^a 1.25 M HCl in methanol, ^b 4 M HCl in 1,4-dioxane with CH₃OH to aid solubility, ^c 4 M HCl in 1,4-dioxane diluted with CH₂Cl₂, 0 °C, argon atmosphere.

The addition of methanol did not introduce any problems of hydrolysis as had been seen before for guanidine derivatives; however, in the case of the 2-furanylmethyl derivative **58c** degradation was observed. In fact, this molecule proved highly acid sensitive, even in dry conditions. Protonation of the exocyclic nitrogen atom leads to a molecule which not only has a highly electrophilic furanylic carbon, but also has a neutral leaving group attached. It is thought that degradation was occurring by this pathway (Scheme 3.5.5), though no spectroscopic proof was observed by NMR generally gave a spectrum indicative of a polymeric product. Eventually, diluting the 4 M solution of hydrochloric acid in 1,4-dioxane with dichloromethane, and using an inert atmosphere of argon at 0 °C, allowed isolation of **58c** in low yield (30%).

Scheme 3.5.5.



It is worth noting that all of the four molecules were protonated without difficulty indicating that the guanidine-like moiety maintains its basicity despite the constraints imposed by the CH₂ bridge to the aromatic ring. Protonation was nearly instantaneous, as judged by the disappearance of the neutral molecule in TLC and this gives a good indication that the salts will behave similarly to guanidines at physiological pH. Furthermore, a study on the pK_{aH} of highly similar molecules carried out by Peters *et al.* – studying 4-substituted-1,4-dihydroquinazolin-2-amines as 5-HT receptor ligands – indicates that for alkyl substituted amine groups in the 2-position the pK_{aH} is in the region of 10-11, while for those amines with slightly more electron-withdrawing substituents the pK_{aH} drops to 9-10, meaning that these molecules will most likely be protonated at physiological pH.

Encouragingly, the same study indicates that such molecules have ideal physicochemical features for CNS drugs, having pK_{aH} values of less than 11 and improved log D values (< 2).²¹⁴

3.5.2. Pharmacological Profile of *N*-Substituted-1,4-dihydroquinazolin-2-amine Hydrochlorides **E**

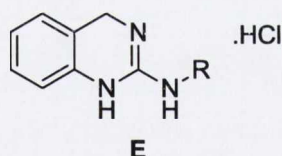
All four *N*-substituted-1,4-dihydroquinazolin-2-amine hydrochlorides **E** were tested for their affinity at the α₂-AR and any with a pK_i ≥ 6.00 were tested for functional activity at the receptor. These molecules incorporated the groups in the *N*-position which had

previously given best results in the analogous family of *N*-phenyl-*N'*-substituted guanidine hydrochlorides and family **D** from this work.

Binding Affinity (pK_i determination)

Binding affinities were measured in human PFC tissue using a competitive binding assay with the α_2 -AR selective radioligand [³H]RX821002 at 2 nM concentration. The results of these experiments are presented in Table 3.5.4, where binding affinities are expressed as pK_i values. Three of the four compounds resulted in data which fit best to a monophasic curve, indicative of a single binding event occurring, while one set of data fit best to a biphasic curve. As seen with families **C1**, **C2** and **D** this is thought to arise from the molecule having a higher affinity for one subtype of the α_2 -AR (α_{2A} -AR, α_{2B} -AR, α_{2C} -AR) than for others.

Table 3.5.4. Binding affinities (pK_i) of family **E** to the α_2 -AR. The pK_i values of high (*Hi*) and low (*Lo*) affinity binding sites are indicated, as is that of the standard ligand RX821002.



Compound	R	pK _i
RX821002	-	8.72
58a	-(CH ₂) ₂ -OH	6.25
58b	-C ₆ H ₅	<i>Hi</i> 7.80 <i>Lo</i> 4.24
58c	-CH ₂ -(2-furanyl)	5.34
58d	-(CH ₂) ₂ -CH ₃	4.32

Disappointingly, compounds from family **E** did not show the expected improvements to pK_i relative to the pyridine-containing families **A-D**. It is difficult to rationally explain the poor binding affinity of these compounds at the α_2 -AR; due to the reduced pK_i of pyridinyl guanidiniums compared to their phenyl analogues, it had been anticipated that reintroduction of the phenyl ring would lead to increased binding affinity at the receptor. Further reason to believe that these molecules would have high pK_i values came from the relationship between the aromatic ring and the cationic moiety, which is similar in terms of geometry to that in families **A**, **C1** and **D**, the most successful families in this project in terms of pK_i . A third factor to consider was substitution of the phenyl ring *para* to the nitrogen of the cation, which had been investigated in previous families. The unsubstituted derivatives had always been among the highest affinity members of these families and so lack of a substituent cannot be blamed for the poor pK_i either.

*π -Cation and π - π Complexes of Family **E***

One possibility to explain this poor affinity is that the interactions of the guanidinium-like moiety with the active site are disrupted by the introduction of the methylene bridge, and corresponding conformational restriction, to the system. We have suggested that π -cation interactions between an aromatic amino acid residue in the active site and guanidinium are important for the binding of these compounds (see Section 3.1) and disruption of this could be a reason for reduced pK_i . When the same calculations carried out for family **A** (M06-2X/6-311++G(d,p) optimisations in PCM-water solvation) were applied to a test compound representing family **E**, several features were revealed which might explain these low pK_i values.

First of all, the lowest energy π - π complex was more stable (had higher interaction energy) than the lowest energy π -cation complex, a property which correlated with lower binding affinity within family **A**; the π -cation complex of **E** was 2.2 kJ mol⁻¹ less stable than the π - π complex. Secondly, the geometry of the π -cation complex (and of the optimised monomer **E**) revealed that the cationic moiety has no freedom to move from its position due to the boat-like conformation of the dihydroquinazoline ring enforced by the CH₂ bridge (Fig. 3.5.2); in pyridin-2-ylguanidiniums (like **A**, **C1** and

D) there is more flexibility to adopt different geometries which maintain the IMHB between the pyridine nitrogen and the guanidinium N-H.

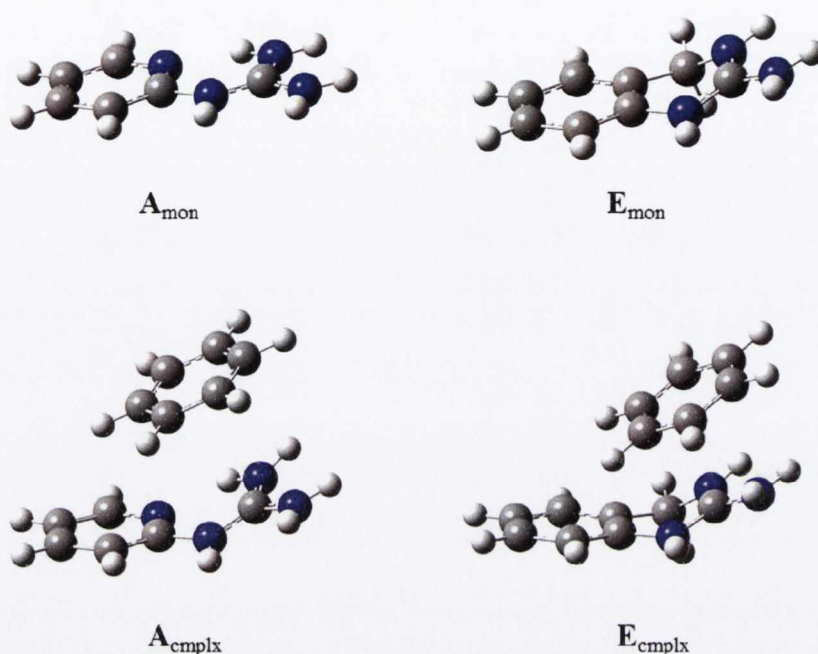


Fig. 3.5.2. Comparison of the optimised geometries of monomers **A** and **E** (A_{mon} , E_{mon}) and their π -cation complexes with benzene (A_{cplx} , E_{cplx}).

This freedom is illustrated when the interatomic distances and dihedral angles of monomers **A** and **E** and those of their complexes are compared. For **A**, the IMHB distance is 1.87 Å in the monomer and 1.93 Å in the complex. For **B**, the equivalent $C^{\text{CH}_2}-C^{\text{Ar}}$ distance is exactly the same in the monomer and the complex (1.50 Å). The dihedral angle $N^{\text{py}}-C^{\text{py}}-N^{\text{gua}}-C^{\text{gua}}$ represents the angle between the aromatic plane and the plane of the cationic group in **A**. This angle is 0.23° in A_{mon} and moves to 29.17° in A_{cplx} , indicating a certain amount of flexibility – it must be remembered that our AIM and NBO calculations showed that the IMHB is maintained on complexation despite this flexibility. There is again little change between the monomer and complex of **E**; the equivalent angle is 20.07° in E_{mon} and 27.40° in E_{cplx} .

It seems likely that flexibility in this region is required to undergo optimum interactions with the residues of the active site and that the rigidity of compounds **E** is preventing some of these interactions from occurring, resulting in low pK_i values.

NICS Indices of Aryl Guanidinium Systems

Another property which can influence the interactions of these ligands with the active site is the aromaticity of the aryl ring. This ring is thought to undergo π - π interactions with aromatic amino acid residues in the α_2 -AR active site. Given that phenyl guanidiniums/2-iminoimidazolidiniums had higher affinity than pyridin-yl guanidiniums/2-iminoimidazolidiniums (**A**, **B**, **C1** and **C2**), it is possible that the altered aromaticity of these aryl systems is influencing their binding to the α_2 -AR.

With this in mind NICS calculations were carried out on the optimised geometries of phenylguanidinium, pyridin-2-ylguanidinium and dihydroquinazoline-2-ammonium (Fig. 3.5.3) to evaluate their relative aromaticities using the M06-2X functional at the 6-311++G(d,p) level in PCM-water solvent.

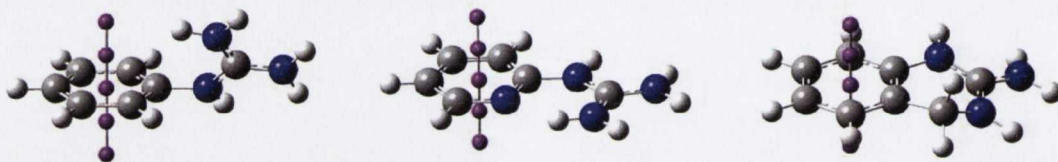


Fig. 3.5.3. NICS(0), NICS(1) and NICS(2) (indicated by pink dummy atoms) were calculated for phenyl guanidinium, pyridin-2-ylguanidinium and dihydroquinazoline-2-ammonium.

As expected, the benzene ring of phenylguanidinium had the highest aromaticity value for NICS(0), NICS(1) and NICS(2), being larger than that of the pyridine ring of pyridin-2-ylguanidinium and the benzene ring of dihydroquinazoline-2-ammonium, respectively. As NICS(1) values are the most often discussed of the three indices – including in the discussion presented in Section 3.1.2.4 – these particular values will be the ones chosen to compare the systems.

The average of the NICS 1 values for above and below the plane of phenylguanidinium is -10.32, while this value is -10.27 for pyridin-2-ylguanidinium and -10.16 for dihydroquinazoline-2-ammonium. It was expected that the pyridine ring would have lower aromaticity index than the benzene ring of phenylguanidinium; however, the low aromaticity of the benzene ring in dihydroquinazoline-2-ammonium was unexpected and indicates that the CH₂ bridge to the guanidinium system is disrupting the π -system

of the ring. If interactions between the active site and this aromatic moiety are important for ligand binding then this could be a reason for the low affinity of compounds of this type and this will have to be addressed in future ligands.

Functional Assay Results

Only compound **58a** had $pK_i \geq 6.00$ and its activity was tested using [35 S]GTP γ S binding experiments with the standard agonist UK14304 (Fig. 3.5.4). It was shown to be an antagonist, with inverse agonistic features only at high concentrations (almost millimolar). This result indicates that the geometry of these molecules is more suited for antagonistic activity at the receptor; however, improvements to binding affinities will have to be made through chemical modifications for this type of molecule to have any utility as α_2 -AR antagonists.

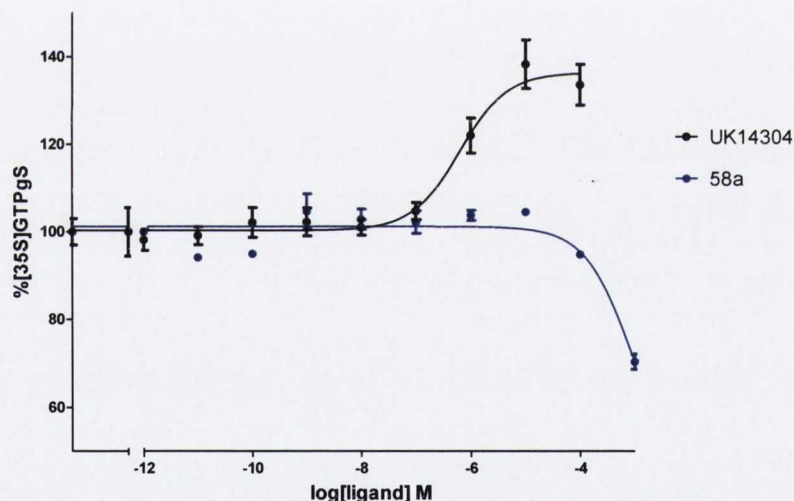
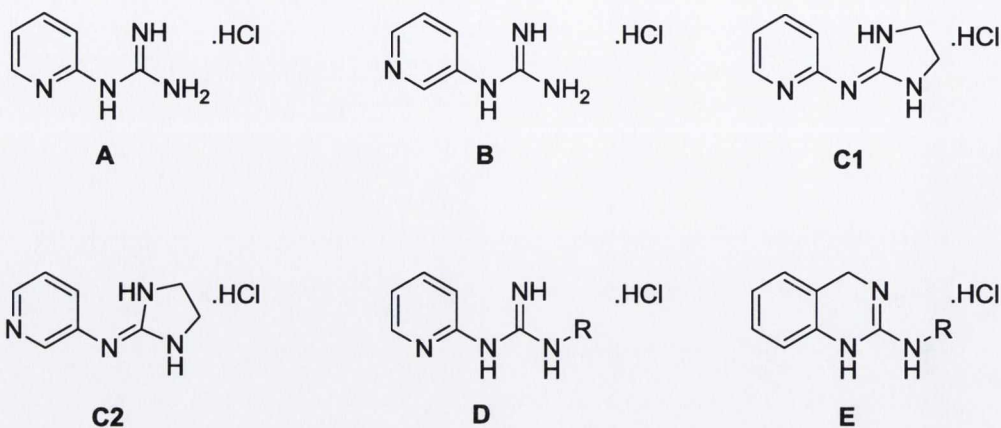


Fig. 3.5.4. Dose-response curve for percentage [35 S]GTP γ S binding vs. ligand concentration of **58a**. The standard agonist UK14304 is shown in black.

3.5.3. Structure-Activity Relationships from Family E

A comparison of the molecules from family E can be made with the 5-unsubstituted derivatives of family A (**15c**), B (**38b**), C1 (**45b**), C2 (**47a**) and D (**50a**, **50d**, **50g**), as shown in Table 3.5.5.

Table 3.5.5. Comparison of pK_i across families A-E. The pK_i values of high (*Hi*) and low (*Lo*) affinity binding sites are indicated.



Compound	Family	R	pK_i
15c	A	-	6.25
38b	B	-	3.85
45b	C1	-	6.43
47a	C2	-	5.27
50a	D	-(CH ₂) ₂ -OH	<i>Hi</i> 8.85 <i>Lo</i> 5.31
50d	D	-C ₆ H ₅	<i>Hi</i> 8.92 <i>Lo</i> 6.06
50g	D	-CH ₂ -(2-furanyl)	6.29
58a	E	-(CH ₂) ₂ -OH	6.25
58b	E	-C ₆ H ₅	<i>Hi</i> 7.80 <i>Lo</i> 4.24
58c	E	-CH ₂ -(2-furanyl)	5.34
58d	E	-(CH ₂) ₂ -CH ₃	4.32

These results demonstrate that compounds from family **E** are among the poorest affinity ones from this project. In particular, the phenyl, 2-furanylmethyl and *n*-propyl substituted compounds are on a par with pyridin-3-ylguanidiniums **B** and are worse than all other families, including pyridin-3-yl-(2-iminoimidazolidiniums) **C2**. The high-affinity pK_i of **58b** is also lower than corresponding values in all other families. The

ethoxy-substituted derivative **58a** shows more favourable binding affinity, being exactly the same as pyridin-2-ylguanidinium **15c**, and having a higher binding affinity than corresponding compounds from families **B** and **C2**. While it is difficult to draw comparisons between **58a** and family **D**, it has a pK_i value in the same range as all of these compounds, when the low affinity pK_i is considered for molecules from family **D**.

The pharmacological results obtained for family **E** suggest that the constrained geometry of the cationic moiety is unfavourable for optimum binding affinity at the α_2 -AR, but that the antagonistic activity is maintained. This gives the hope that related molecules could improve on this profile and this type of molecule will not be abandoned in the search for new α_2 -AR antagonists.

3.6. New Synthetic Methodologies for the Guanidylation of Amines

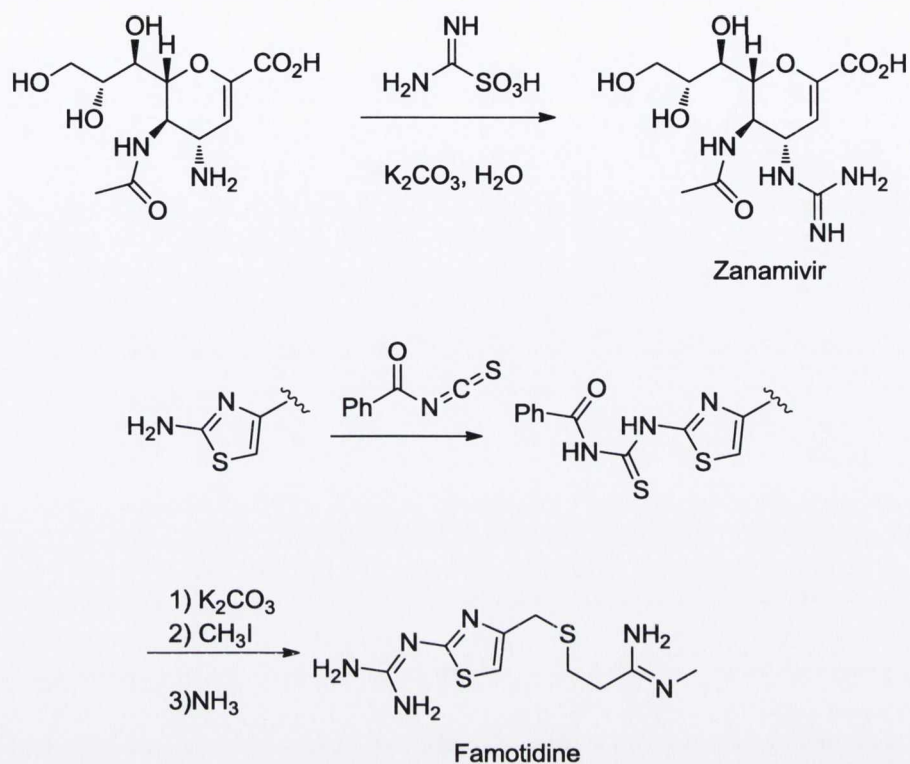
3.6.1. Mercury(II) Chloride and the Need for New Methods of Guanidine Preparation

Rozas group has been interested in the preparation of aryl guanidine and 2-iminoimidazolidine derivatives with varying pharmacological applications over the last fifteen years.^{156,157,158,196} Throughout this time, the synthetic approach involved the mercury(II) chloride promoted coupling of a primary aryl amine with either *N,N'*-di-(*tert*-butoxycarbonyl)thiourea **8** to form guanidines or *N,N'*-di-(*tert*-butoxycarbonyl)imidazolidin-2-thione **41** to form 2-iminoimidazolidines. Generally, thiourea bearing one or more electron-withdrawing groups is required to increase the electrophilicity of the central thiourea carbon. Mercury(II) chloride is also necessary to facilitate nucleophilic attack of the amine at this carbon, by complexation to the thiourea sulfur.

The method works extremely well and despite the undesirable use of mercury salts – and the mercury-containing waste that ensues – it is the method of choice in late stage synthesis of guanidines, and is particularly useful where unreactive aryl amines are involved. For example, in Du Bois' famous synthesis of (-)-Tetrodotoxin the guanidine is introduced in the penultimate step using mercury(II)chloride promoted coupling of an amine and **8**, followed by Boc deprotection using trifluoroacetic acid.²¹⁵

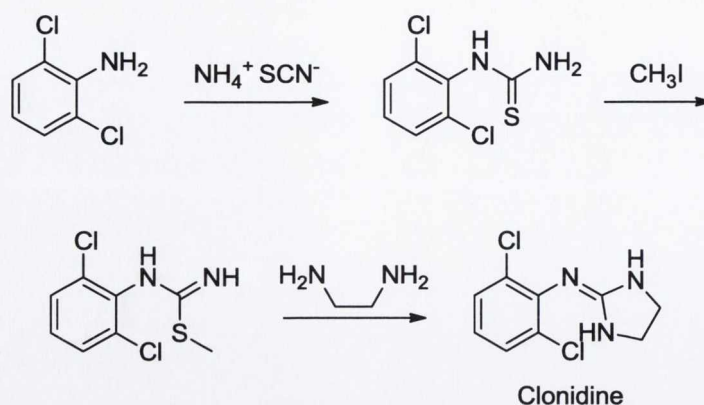
It is worth mentioning that none of the commercially available guanidine-containing drugs use mercury(II) chloride in their synthesis as it is not feasible for use on a large scale (Scheme 3.6.1). The synthesis of the neuraminidase inhibitor zanamivir employs amidine sulfonic acid²¹⁶ which is not successful with deactivated amines. The guanidine of the histamine H₂-receptor antagonist famotidine, used in the treatment of peptic ulcer disease, is introduced using benzoyl isothiocyanate. This is reacted with an aryl amine to give a benzoylthiourea, which is subsequently *S*-methylated by iodomethane and cleaved in two steps by ammonia to yield the guanidine.

Scheme 3.6.1.



The 2-iminoimidazolidine moiety of clonidine (Scheme 3.6.2), an α_2 -AR agonist used in the treatment of ADHD and panic disorder, is introduced from the corresponding aryl amine by first converting it to the thiourea using ammonium thiocyanate. This is then *S*-methylated by iodomethane and attacked by the powerfully nucleophilic ethylenediamine. This is a clean synthesis but only works for preparing 2-iminoimidazolidines and, like the synthesis of famotidine, still requires the use of the light-sensitive and highly toxic iodomethane. Clearly, there is a need for safe and efficient syntheses of guanidines considering their potential as therapeutics.

Scheme 3.6.2.

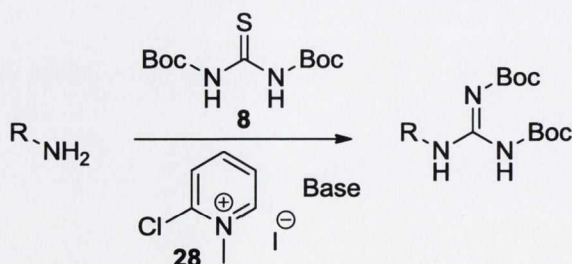


3.6.2. Alternative Guanidylating Agents to Mercury(II) Chloride

There is a plethora of methods for the formation of guanidine containing compounds; however, most of them work only for highly nucleophilic amines. The majority involve a starting amine and the subsequent introduction of a masked electrophilic carbodiimide, with the amine nucleophilically attacking the carbodiimide to form a new C-N bond.²¹⁷ By far, the most used is Kim and Qian's mercury(II) chloride promoted guanidylation reaction described earlier (Section 3.2.1).

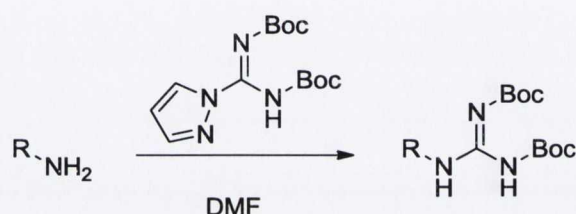
A feasible alternative is the use of Mukaiyama's reagent **28**,²¹⁸ instead of mercury(II) chloride, with a base to couple **8** with amines (Scheme 3.6.3). The method is effective for aliphatic amines; however, it does not work well, if at all, for aryl amines.

Scheme 3.6.3.



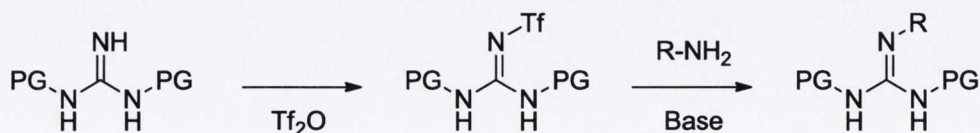
Another approach for the guanidylation of amines is to turn one of the nitrogens of a guanidine derivative into a leaving group. An example employs pyrazole-1-carboximidamides (Scheme 3.6.4); their reaction with an amine leads to pyrazole (pK_a 19.8) acting as a leaving group. Again, however, yields for the guanidylation with aryl amines are low.²¹⁶ This is improved upon with the introduction of electron-withdrawing groups to the pyrazole ring, which lower the pK_a of the conjugate-base leaving group; yet, synthesis of these derivatives is not trivial.

Scheme 3.6.4.



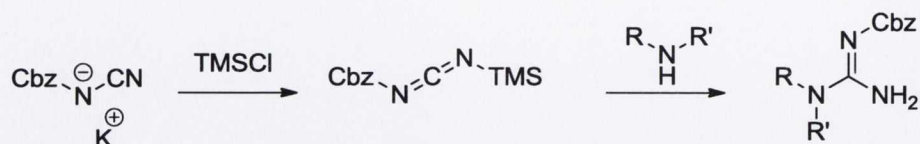
Another example of this is triflylation of *N,N'*-bis-protected guanidines (Scheme 3.6.5). Trifluoromethanesulfonic anhydride is highly electrophilic and can add to the imine nitrogen, converting this into a good leaving group. Subsequent nucleophilic attack at the guanidine carbon with an amine yields *N,N'*-bis-protected guanidines on loss of trifluoromethanesulfonamide (pK_a 6.39). Even still, this method is not as successful for guanidylation of aryl amines as mercury(II) chloride. Furthermore, it is recommended that the triflate be made in-situ prior to addition of the amine, and it is made in two steps from guanidine, while use of the highly reactive trifluoromethanesulfonic anhydride is not suitable on a large scale.

Scheme 3.6.5.



A recent synthesis uses the potassium salt of Cbz-protected cyanamide (Scheme 3.6.6). Addition of trimethylsilyl chloride leads to a reactive carbodiimide intermediate which affords *mono*-protected guanidines on addition of an amine.²¹⁹

Scheme 3.6.6.



High yields are obtained for guanidylation of aliphatic and benzylic amines, with moderate yields being reported for aryl amines as well. It is worth noting, however, that some substrates gave no reaction, namely *para*-nitroaniline. This paper also illustrates that the synthesis of guanidines from amines is still an area of active research due to the lack of non-toxic, efficient synthetic routes to guanidines from amines.

3.6.3. Copper(II) Chloride as a Guanidylating Agent

Thus, we sought to find a method which would allow the guanidylation of unreactive aryl amines without the need for mercury salts. During the synthesis of the related family of 1,4-dihydroquinazoline compounds from family **D**, copper(II) oxide was used in catalytic amounts as a desulfurizing agent to promote the intramolecular coupling of an aryl amine and a thiourea.

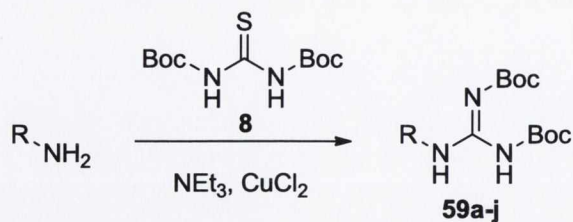
With this in mind an investigation into the possibility of extending this methodology to intermolecular guanidine formation was undertaken. Under the same conditions only trace amounts of product were isolated (< 5%). Similarly, using a stoichiometric amount of copper(II) oxide gave very low yields (< 5%). Use of a stoichiometric re-oxidant (*N*-methylmorpholine-*N*-oxide) was attempted to examine if the catalytic species was being used up in the reaction but no improvements in yield were observed.

It seemed that a leaving group was required on the copper(II) species for reaction to occur. Revisiting copper(II) chloride – which had been mentioned in Kim and Qian's

original paper¹⁸²Error! Bookmark not defined. but concluded to be less effective than mercury(II) chloride – the guanidylation was affected in high yield (73%) for the test compound aniline. Notably, the yield matched published values for the reaction using mercury(II) chloride (73%) exactly.¹⁵⁷

It should be noted that use of the commercially available *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiourea **9**, which can often be used in place of **8** in mercury(II) chloride promoted guanidylations, was not successful when copper(II) chloride was employed. Use of **8**, however, proved successful and it can be synthesised in high yield and on large scale from thiourea and di-*tert*-butyl dicarbonate (see Scheme 3.2.2).²²⁰

To investigate the general applicability of this method a series of amines (Table 3.6.1) of varied reactivity was subjected to the conditions. When available, yields were compared to published values for the corresponding mercury(II) chloride promoted guanidylation. Reactions were carried out at room temperature in dichloromethane, but for more deactivated amines slightly more forceful conditions using dimethylformamide as solvent and heating at 60 °C were employed, also yielding products in acceptable yields. It is significant that 4-aminopyridine was successfully guanidylated (26% yield) using copper(II) chloride to give **59j** where this had not been possible in our hands with mercury(II) chloride. Furthermore, the guanidylation of *para*-nitroaniline to give **59i**, which was not achieved in other studies,²¹⁹ was effectively carried out using our method (41% yield).

Table 3.6.1. Copper(II) chloride coupling of amines with **8**.

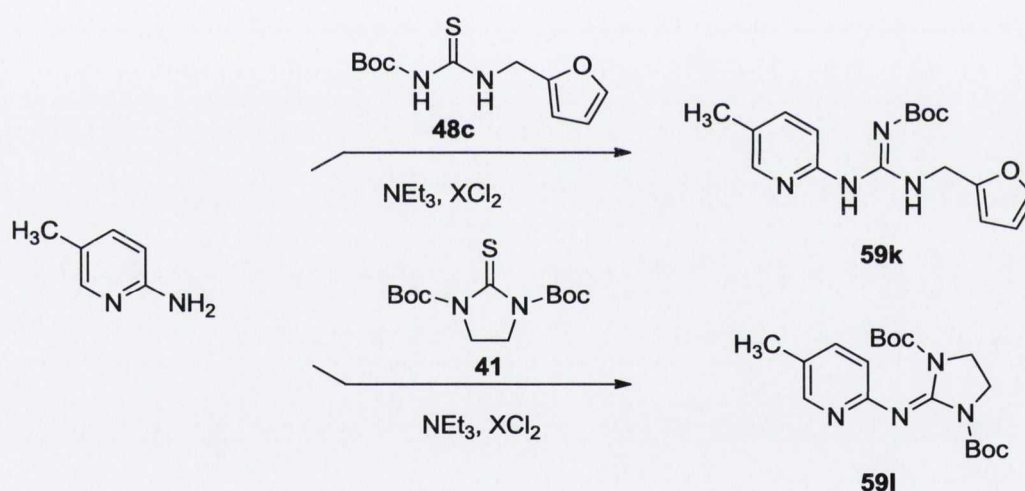
R	Conditions	Product	% Yield
	CH ₂ Cl ₂ , RT, 3 h	59a	89
	CH ₂ Cl ₂ , RT, 3 h	59b	89
	CH ₂ Cl ₂ , RT, 3 h	59c	83
	CH ₂ Cl ₂ , RT, 5 h	59d	76
	CH ₂ Cl ₂ , RT, 16 h	59e	73
	CH ₂ Cl ₂ , RT, 16 h	59f	72
	CH ₂ Cl ₂ , RT, 16 h	59g	67
	CH ₂ Cl ₂ , RT, 16 h	59h	62
	DMF, 60 °C, 16 h	59i	41
	DMF, 60 °C, 16 h	59j	26

Based on these results we suggest that this method is at least as efficient and high yielding as mercury(II) chloride promoted guanidylation and vastly more desirable as it

negates the use of mercury salts. It is also applicable to both aliphatic and aryl amines, which is essential to its practicality.

Having shown that the method was successful in generating *N,N'*-di-Boc-protected guanidines we wanted to extend it to the synthesis of *N,N'*-di-Boc-protected 2-iminoimidazolidines and *N*-Boc,*N'*-substituted guanidines such as those from families **C1**, **C2** and **D** respectively (Table 3.6.2). The test amine used for these reactions was 2-amino-5-methylpyridine as its yield in the guanidylation reaction with copper(II) chloride was moderate and both of the products resulting from this amine had previously been prepared using mercury(II) chloride as coupling agent (**59k** = **49h**, **59l** = **42c**), allowing for direct comparison of yields.

Table 3.6.2. Application of copper(II) chloride mediated coupling of aryl amines with thiourea derivatives and comparison of yields with mercury(II)chloride coupling.



Product	Coupling Agent	Conditions	% Yield
59k	CuCl_2	RT, 16 h	41
	HgCl_2	RT, 16 h	32
59l	CuCl_2	RT, 16 h	74
	HgCl_2	RT, 16 h	75

For both classes of reaction, favourable yields were obtained. Using copper(II) chloride, coupling of the amine with **41** resulted in almost exactly the same yield as the mercury(II) chloride promoted reaction. For **48c**, the reaction went with poor yield but this can be explained by the fact that electron-withdrawing substituents are normally needed on the thiourea nitrogen atoms to improve the electrophilicity of the thiourea carbon and facilitate the reaction. That said, the yield was still slightly higher than it was for the analogous mercury(II) chloride reaction and this also proves that the method performs well for the coupling of troublesome amine and thiourea derivatives.

3.6.4. Conclusions

Overall, yields were on a par with the mercury(II) chloride promoted guanidylation to such a point that we now carry out these reactions with copper(II) chloride instead of mercury(II) chloride in our lab, due to the cleanliness and reduced toxicity of the reagents involved. The relevant amines are readily available and the restrictions imposed by the necessary synthesis of the thiourea-containing reagent are minimal, far outweighed by avoiding the use of mercury(II) salts.

Chapter 4 – Conclusions and Future Work

4.1. Conclusions

Computational and Structural Studies

The complexes of 5-substituted-pyridin-2-ylguanidiniums with benzene and hexafluorobenzene were investigated to model the π -cation and π - π interactions which are thought to be important in the binding of this type of ligand to the α_2 -AR. For this study, the M06-2X functional, which is known to describe weak interactions well, was utilised at the 6-311++G(d,p) level in PCM-water. The inclination for formation of either complex type was elucidated; electron-poor pyridyl rings led to π - π complexes with benzene being favoured, while π -cation complexes between the guanidinium and benzene were preferred for electron-rich pyridyl rings. More importantly, the preference of pyridin-2-ylguanidiniums to form π -cation complexes could later be correlated to higher binding affinity at the α_2 -AR. As complexes of this type are of great interest elsewhere – particularly π -cation complexes – they were also characterised by means of the AIM, NBO and NICS methodologies to gain further understanding of the interactions involved. A number of BCPs were found between the stacking monomers during the AIM analysis, confirming that either π - π or π -cation complexes are being formed, while NBO analysis revealed that interactions between C-C bonding orbitals and the empty lone-pair of the guanidine carbon are essential for π -cation complex formation. The NICS analysis found that the aromaticity of benzene increases in all complexes; however, that of the pyridine ring is decreased for π -cation complexes and increased for π - π complexes.

In a separate study, pyridin-2-ylguanidiniums and 1-(pyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidines were studied using the DFT functional at the 6-31+G(d,p) level, in gas phase, as part of a structural study of initial target compounds, **A**. Their conformational preferences were found to be rooted in IMHB interactions. A network of three IMHBs in 1-(pyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidines (synthetic intermediates to **A**) was found to confer the *anti*-conformation to these molecules: this was proven using NMR spectroscopy and DFT calculations on 1-(pyridin-2-yl)-2-(*tert*-

butoxycarbonyl)-3-alkylguanidines (synthetic intermediates to **D**), which adopt the *syn*-conformation due to loss of one of these IMHBs and incorporation of a new IMHB between the pyridine nitrogen and a guanidine N-H. These intermediates were also characterised using GIAO calculations – in conjunction with low temperature NMR experiments – to elucidate their isomeric preferences and the reasoning for broad, poorly resolved NMR spectra at room temperature. This is thought to occur due to interconversion between two tautomers of the guanidine moiety: one which places the C=N in conjugation with the carbonyl group, the other which places it in conjugation with the pyridyl ring. Finally, in pyridin-2-ylguanidiniums **A**, the *syn*-orientation is induced by an IMHB between the pyridine nitrogen and a guanidinium N-H, with an almost quantitative preference for this arrangement predicted to exist in solution.

The predictions from these studies on compounds from family **A**, and their synthetic intermediates, were corroborated by NMR experiments, confirming the existence of these conformational factors in solution: Nuclear-Overhauser effect experiments, variable temperature ^1H NMR signals and the characteristic broadened and highly shifted nature of hydrogen-bonded protons were used to assign conformations. Further evidence was gained from three X-ray crystal structures which demonstrated the existence of the predicted conformations in the solid phase.

Synthesis

Thirty seven new aryl guanidine and guanidine-like hydrochlorides have been synthesised as potential antagonists of the α_2 -AR, useful in the treatment of depression. This includes families of *para*-substituted pyridin-2-yl and pyridin-3-yl guanidine hydrochlorides (**A** and **B**), *para*-substituted pyridin-2'-yl and pyridin-3'-yl 2-iminoimidazolidine hydrochlorides (**C1** and **C2**), *para*-substituted pyridin-2-yl-*N,N'*-disubstituted guanidine hydrochlorides (**D**) and *N*-substituted-1,4-dihydroquinazolin-2-amine hydrochlorides (**E**), which were chosen based on the theoretical predictions described, as well as CoMFA modelling from elsewhere in our group, and pharmacological results as they became available.

The majority of compounds from families **A-D** were synthesised using mercury(II) chloride promoted coupling of an aryl amine and a thiourea derivative, as described.

Where this was not possible, the introduction of the guanidine functionality was carried out using NAS, something which had not previously been successfully achieved in our group with this type of chemistry. In many cases, the necessary aryl amines were not commercially available and had to be synthesised using literature procedures. Furthermore, both 2-(*N*-ethylamino)-5-aminopyridine and 2-amino-5-(*N*-ethylamino)pyridine, which had not been previously reported in the literature, were successfully synthesised. During the development of this work, a new methodology was brought forth which negates the need for mercury(II) chloride in the synthesis of guanidines, 2-iminoimidazolidines and di-substituted guanidines from aryl amines. It was found that copper(II) chloride – in place of mercury(II) chloride – results in yields which are at least as high as the corresponding mercury(II) chloride promoted reactions. Compounds from family **E** were synthesised using a literature procedure for the generation of 1,4-dihydroquinazolin-2-amines and then converted to their hydrochloride salts for the purposes of pharmacological testing.

The syntheses yielded three crystal structures [two pyridin-2-ylguanidine hydrochlorides and one 1-(pyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine] that were possible to resolve using X-ray crystallography, and which became instrumental in structural studies of the ligands from this work, and in gaining SARs for our compounds at the α_2 -AR.

Pharmacology

Data on binding affinity (pK_i) at the α_2 -AR was obtained for all thirty seven synthesised hydrochloride compounds, using competition binding assays with the α_2 -AR selective radioligand [^3H]RX821002. The range of pK_i values from this study is 3.85–6.84. However, for the first time there is an indication that selectivity for a particular subtype of the α_2 -AR (α_{2A} -AR, α_{2B} -AR, α_{2C} -AR) is being attained by some molecules, as suggested by the biphasic binding curves which have been obtained for several compounds. The range of pK_i values for this suggested binding event is 7.27–11.00, giving great encouragement that high affinity molecules could be obtained in future work.

Functional activity was obtained for twenty one of these compounds (all compounds with $pK_i \geq 6.00$) using [35 S]GTP γ S binding experiments, and each one resulted in either the desired antagonistic or inverse-agonistic activity at the receptor. The presence of inverse-agonistic activity profiles was not confined to particular structural features; however, some assertions could be made. *Mono*-substituted guanidiniums gave antagonistic activity only, while some members of the families of 2-iminoimidazolidiniums, *N,N'*-di-substituted guanidiniums and *N*-substituted-1,4-dihydroquinazolin-2-ammoniums led to inverse agonistic profiles – the remaining members of these families being antagonists.

As all compounds resulted in the desired activity, SARs from this project focus mainly on binding affinity to the α_2 -AR. With this in mind, pyridin-3-yl cationic compounds (**B**, **C2**) had generally poorer pK_i values than analogous pyridin-2-yl cationic compounds (**A**, **C1**). Unlike what had been seen in phenyl derivatives, *N,N'*-di-substituted pyridin-2-ylguanidiniums (**D**) did not have lower pK_i values than their corresponding *mono*-substituted pyridin-2-ylguanidiniums (**A**). It can also be said that 2-iminoimidazolidinium derivatives (**C1**, **C2**) had higher pK_i values than either guanidiniums (**A**, **B**), *N,N'*-di-substituted pyridin-2-ylguanidiniums (**D**) or *N*-substituted-1,4-dihydroquinazolin-2-ammoniums (**E**). The low binding affinities for members of family **E** seem to indicate that a degree of flexibility is required for optimal interactions with the active site and this will have to be considered during the design of future ligands of this type.

4.2. Future Work

Having obtained pharmacological data on a broad ranging library of new compounds, future work will concentrate on optimising these compounds and investigating their potential subtype-specificity. To this end, homology models of the α_{2A} -AR, α_{2B} -AR and α_{2C} -AR will be constructed from their known amino acid sequences and the high resolution crystal structure of the homologous human β_2 -AR. The compounds from this work will then be docked into these models and interactions with the receptor will be characterised using molecular dynamics simulations. Variations in either, or each of, the

binding modes or binding energies across subtypes will hopefully suggest which subtype is being targeted.

Also based on the findings from this study, additional pharmacological testing will further investigate the utility of these compounds and, hopefully, construct more SARs to allow directed design of future ligands. Thus, assays in the presence of subtype-selective agonists for the α_{2A} -AR, α_{2B} -AR and α_{2C} -AR, respectively, will be used to elucidate the subtype-selectivity of these compounds. Those derivatives which maintain favourable pharmacological profiles will be subjected to *in-vivo* microdialysis testing to evaluate their ability to increase extracellular concentrations of NA and their performance in physiological conditions, particularly, their ability to penetrate the BBB and their stability.

In an exciting development, Prof. Martin Caffrey of the Trinity College Dublin School of Biochemistry and Immunology has begun a project aimed at resolving the crystal structure of the α_{2A} -AR in complexation with high-affinity compounds from Rozas' group. If achieved, this would give invaluable information on the binding site of the α_{2A} -AR, and in conjunction with molecular modelling would allow for optimisation of the ligand-receptor interactions of this type of molecule. Knowing that this type of molecule is capable of crossing the BBB (from microdialysis experiments) and acting as antidepressants (from animal behavioural testing), the potential is huge for developing highly useful drug candidates, acting as α_2 -AR antagonists.

In terms of optimising binding affinity, an updated CoMFA model which includes the full set of compounds from this work, as well as those previously modelled, would undoubtedly give new information on the favourable features for increased binding affinity to the α_2 -AR. The available information is set up to construct a comprehensive model which will give new insights into the interactions at the receptor; a wide range of low to high binding affinities, variations in activity, and a diverse set of ligands in terms of steric, conformational and electronic properties.

Through this combination of directed design and chemical synthesis it is certain that new high affinity antagonists of the α_2 -AR – and potentially its subtypes – can be obtained.

Future Antagonists of the α_2 -AR

While the design strategies outlined above will be implemented immediately, it is tempting to suggest which molecules might be successful α_2 -AR antagonists in future, based on the pharmacological results obtained in this project.

Given that members of family **E** were predicted to have high affinity for the α_2 -AR, while maintaining antagonistic activity, it is worth investigating this class of compounds further. As stated, the cationic moiety seems to be too rigid and thus, incorporation of an extra CH_2 would recover some of the flexibility lost on ring-closure (Fig. 4.1). Molecules of this type can be synthesised in a similar CuO catalysed manner to those of Family **E** from the suitable phenyl diamine and dithiocarbamates.

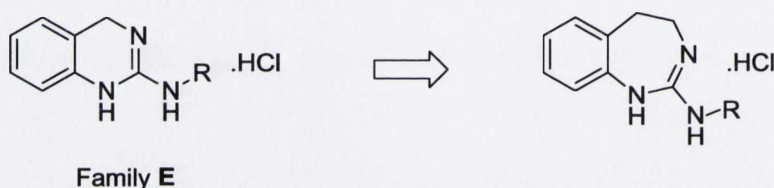


Fig. 4.1. An ethylene bridge in place of the methylene bridge of family **E** will increase flexibility and may improve interactions between the cation and the active site.

Another encouraging result from this project was that the high-affinity 2-iminoimidazolidinium chlorides had antagonistic activity, where in previous studies the majority of compounds of this class had been agonists. This means that molecules of this type also need to be investigated further. The fact that *N,N'*-disubstituted pyridin-2-ylguanidine hydrochlorides (**D**) did not suffer the losses in pK_i compared to *mono*-substituted pyridin-2-ylguanidine hydrochlorides (**A**) – that had been observed in analogous phenyl derivatives – suggests that substitution in this region can also be incorporated. This is advantageous for two reasons: this was shown to lead to antagonistic activity, and optimisation of ligand-receptor interactions in this region can potentially lead to improved binding affinity. To this end, *N,N'*-disubstituted pyridin-2'-yl-2-iminoimidazolidine hydrochlorides (Fig. 4.2) are also suggested as future antagonists of the α_2 -AR. Synthesis of these molecules can be achieved using a similar route to that employed in the synthesis of family **D**, provided the required *N*-Boc-*N'*-substituted-imidazolidine-2-thiones can be attained.

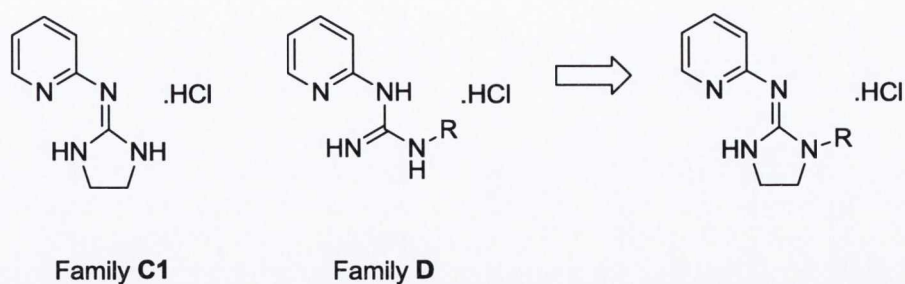


Fig. 4.2. A combination of the favourable features of families **C1** and **D** leads to *N,N'*-disubstituted-pyridin-2'-yl-2-iminoimidazolidine hydrochlorides as potential high affinity antagonists of the α_2 -AR.

Chapter 5 – Experimental

5.1. Synthetic Chemistry

5.1.1. Materials and Methods

All commercial chemicals were obtained from either Sigma-Aldrich or Fluka and used without further purification. Deuterated solvents for NMR spectroscopy use were purchased from Apollo. Dry solvents were prepared using standard procedures, according to Vogel,²²¹ with distillation prior to use. Chromatographic columns were run using a Biotage SP4 flash purification system with Biotage SNAP silica cartridges. Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using either Merck Kieselgel 60 F254 silica gel plates or Polygram Alox N/UV254 aluminium oxide plates. Visualisation was by UV light (254 nm). NMR spectra were recorded on Bruker DPX-400 Avance spectrometers, operating at 400.13 MHz and 600.1 MHz for ¹H NMR; 100.6 MHz and 150.9 MHz for ¹³C-NMR. Shifts are referenced to the internal solvent signals.²²² NMR data were processed using Bruker TOPSPIN software. HRMS spectra were measured on a Micromass LCT electrospray TOF instrument with a WATERS 2690 autosampler and methanol/acetonitrile as carrier solvent. Melting points were determined using a Stuart Scientific Melting Point SMP1 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer equipped with a Universal ATR sampling accessory.

5.1.2. Purity Assessment of Hydrochloride Salts

HPLC purity analysis was carried out using a Varian ProStar system equipped with a Varian Prostar 335 diode array detector and a manual injector (20 µl). For purity assessment, UV detection was performed at 245 nm and peak purity was confirmed using a purity channel. The stationary phase consisted of an ACE 5 C18-AR column (150 × 4.6 mm), and the mobile phase used the following gradient system, eluting at 1 mL/min: aqueous formate buffer (30 mM, pH 3.0) for 10 minutes, linear ramp to 85%

methanol buffered with the same system over 25 minutes, hold at 85% buffered methanol for 10 minutes. Minimum requirement for purity was set at 95.0%.

5.1.3. General Procedures

Method A: Preparation of 1-(pyridinyl)-2,3-di(*tert*-butoxycarbonyl)guanidines, 1-(pyridinyl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidines, 1-(pyridinyl)-2-(*tert*-butoxycarbonyl)-3-substituted guanidines.

To a solution of starting amine (1.00 eq., 1.06 mmol), the appropriate thiourea derivative (1.05 eq., 1.11 mmol) and triethylamine (3.5 eq., 3.71 mmol, 517 μ L) in CH_2Cl_2 (5.5 mL) at 0 °C was added mercury(II) chloride (1.05 eq., 1.11 mmol, 301 mg). The mixture was stirred for 30 min at 0 °C, then warmed to RT and stirred until reaction was complete, as adjudged by disappearance of starting material in TLC analysis. The reaction mixture was diluted with EtOAc (50 mL) and filtered through a pad of Celite to remove any mercury by-products. The filtrate was washed with brine (20 mL) and water (20 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure to yield a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture. Where necessary, recrystallisation was carried out as described below for specific compounds.

Method B: Preparation of *N*-(*tert*-butoxycarbonyl)-*N'*-substituted thioureas.²⁰³

Thiourea (1.0 eq., 6.58 mmol, 501 mg) was dissolved in dry tetrahydrofuran (70 mL) under argon at 0 °C, to which 4.5 equivalents of sodium hydride (60% suspension in mineral oil, 4.5 eq., 29.61 mmol, 788 mg) was added. The reaction was stirred at RT for 45 min to complete formation of the anion and re-cooled to 0 °C prior to the addition of di-*tert*-butyl dicarbonate (2.2 eq., 14.48 mmol, 3.160 g). After stirring at RT for 8 h, the reaction was re-cooled to 0 °C and sodium hydride (60% suspension in mineral oil, 1.7 eq., 11.19 mmol, 298 mg) was added. After 1 h trifluoroacetic anhydride (1.54 eq., 10.13 mmol, 1.431 mL) was added and the reaction was stirred for 1 h before the appropriate amine (1.54 eq., 10.13 mmol) was added and the reaction was allowed to come to RT overnight. The reaction was then cooled to 0 °C and dropwise H_2O (20 mL) was added to quench the reaction. The product was extracted with EtOAc (3 $\times$ 20 mL) and the combined organic phases were washed with brine (sat. 30 mL) and H_2O (30

mL), dried over anhydrous MgSO_4 , filtered and concentrated under vacuum. The resulting residue was purified by silica gel chromatography using gradient elution (hexane:EtOAc), followed by removal of solvents under vacuum to afford the product.

Method C: Preparation of *S*-methyl-*N*-substituted dithiocarbamates.

To a solution of carbon disulphide (1.1 eq., 3.72 mmol, 225 μL) and triethylamine (1.1 eq., 3.72 mmol, 519 μL) in CH_2Cl_2 (4 mL) at 0 °C was added starting amine (1.0 eq., 3.38 mmol). After 15 min methyl iodide (1.1 eq., 3.72 mmol, 232 μL) was added dropwise. The reaction was warmed to RT and stirred for 2 h. It was then diluted with EtOAc (30 mL) and added to a 1 M solution of H_2SO_4 (30 mL). The aqueous layer was extracted with EtOAc (2×30 mL) and the combined organic layers were washed with water (2×20 mL) and dried over MgSO_4 . Filtration and removal of solvents yielded a residue which was purified either by recrystallisation from hexane or silica gel column chromatography, eluting with hexane:EtOAc, as appropriate.

Method D: Preparation of *N*-substituted-1,4-dihydroquinazolin-2-amines.²¹²

To a suspension of 2-aminobenzylamine (1.0 eq., 1.19 mmol, 145 mg) and the appropriate *S*-methyl-*N*-substituted dithiocarbamate (1.1 eq., 1.31 mmol) in dimethylformamide (4 mL) at RT were added Cu(II)O (0.2 eq., 0.24 mmol, 19 mg) and K_2CO_3 (2.0 eq., 2.37 mmol, 328 mg). The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. It was then cooled to RT, diluted with EtOAc (60 mL) and filtered through Celite. The filtrate was washed with brine (30 mL) and water (4×30 mL) to remove traces of dimethylformamide, dried over MgSO_4 , filtered and concentrated under vacuum. The resulting residue was purified using silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

Method E: Generation of guanidine hydrochlorides from *N*-(di-*tert*-butoxycarbonyl) protected guanidines using trifluoroacetic acid and Amberlite resin.

The corresponding 1-(pyridinyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (1.0 eq., 0.75 mmol) was treated with a 25% v/v solution of TFA in CH₂Cl₂ (10 mL). Once disappearance of starting material was observed by TLC (3 h), the excess TFA and CH₂Cl₂ were removed under vacuum. The resulting residue was dissolved in H₂O (10 mL) and Amberlite resin in its chloride-activated form (5 g per mmol) was added. The mixture was stirred at RT for 24 h, when the Amberlite was removed by filtration and the aqueous filtrate was washed with CH₂Cl₂ (2 × 5 mL). Removal of H₂O under vacuum yielded the crude guanidine hydrochloride. Disappearance of the trifluoroacetate salt was checked by ¹⁹F NMR spectroscopy and, if necessary, stirring in Amberlite was repeated. The crude salt was then purified by reverse phase chromatography (C-8 silica) using 100% H₂O as mobile phase. Removal of solvent yielded the pure guanidine hydrochloride which was recrystallised if deemed necessary.

Method F: Generation of guanidine hydrochlorides from *N*-(*tert*-butoxycarbonyl) protected guanidines using hydrochloric acid in 1,4-dioxane.

To either *N*-(di-*tert*-butoxycarbonyl) protected guanidine or *N*-(*tert*-butoxycarbonyl)-*N'*-substituted guanidine (1.0 eq., 0.5 mmol) was added 4 M HCl/1,4-dioxane (6.0 eq. per Boc group) and a 1:1 solution of ⁱPrOH:CH₂Cl₂, such as to maintain a reaction concentration of 0.2 M. The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 5 mL) and then purified using reverse phase chromatography (C-8 silica) using 100% H₂O as mobile phase. Removal of solvent yielded the pure guanidine hydrochloride which was recrystallised if deemed necessary.

Method G: Protonation of *N*-substituted-1,4-dihydroquinazolin-2-amines using hydrochloric acid in 1,4-dioxane.

To a solution of *N*-substituted-1,4-dihydroquinazolin-2-amine (1.0 eq., 0.5 mmol) in CH₂Cl₂ (0.5 mL) was added excess 4 M HCl/dioxane (3.0 eq., 1.5 mmol, 375 µL). Where necessary CH₃OH (1 mL) was added dropwise as needed to aid solubility. Stirring was continued for 1 h after which solvent and excess HCl were removed under

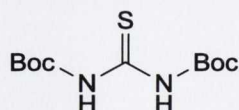
vacuum. The crude salt was then dissolved in a minimum volume of water, washed with CH_2Cl_2 (2×5 mL) and purified using reverse phase chromatography (C-8 silica) with 100% H_2O as mobile phase. Where necessary, further purification was carried out as described.

Method H: Copper(II) chloride promoted coupling of amines and thiourea derivatives.

To a solution of starting amine (1.0 eq., 1 mmol) in CH_2Cl_2 (5 mL) at RT were added *N,N'*-di-(*tert*-butoxycarbonyl)thiourea (1.05 eq., 1.05 mmol, 290 mg) and triethylamine (3.0 eq., 3.00 mmol, 418 μL). After 15 minutes copper(II) chloride (1.05 eq., 1.05 mmol, 141 mg) was added and the reaction was stirred at RT until adjudged complete by TLC analysis. At this stage the reaction was diluted with EtOAc (30 mL) and filtered through a pad of celite. The filtrate was washed with brine (30 mL) and water (30 mL) and dried using anhydrous MgSO_4 . Filtration and removal of solvent under reduced pressure yielded a residue which was purified by silica gel chromatography using gradient elution (hexane:EtOAc). Removal of solvents under vacuum afforded the product. Where stated, reaction conditions with dimethylformamide and heating at 60 °C were employed.

5.1.4. Synthesis and Characterisation

N,N'-Di-(*tert*-butoxycarbonyl)thiourea (8)

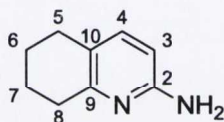


To a cooled solution of thiourea (5.00 g, 65.62 mmol) in dry tetrahydrofuran (40 ml) under argon was added NaH as a 60% suspension in mineral oil (4.5 eq., 11.82 g, 295.29 mmol). After 5 min, the ice-bath was removed and the reaction was stirred for 10 min at RT. The mixture was re-cooled to 0 °C and di-*tert*-butyldicarbonate (2.2 eq., 31.51 g, 144.36 mmol) was added. After 30 min, the ice-bath was removed and the reaction mixture was stirred for 16 h at RT. The reaction was quenched by the dropwise

addition of saturated NaHCO_3 solution (25 mL), diluted with EtOAc (50 mL) and filtered through a bed of celite. The filtrate was extracted with EtOAc (3×40 mL) and the combined organic phases were washed with brine (20 mL) and H_2O (20 mL), dried over MgSO_4 , filtered and concentrated under vacuum. The product was purified by trituration in cold hexane to yield product (14.51 g, 80%) as a white solid. **M.p.** 136-138 °C, lit. 136-138 °C.¹⁹⁶

δ_{H} (400 MHz, CDCl_3): 1.57 (s, 18H, Boc CH_3), 3.93 (broad s, 2H, NH).

2-Amino-5,6,7,8-tetrahydroquinoline (10e)



To 3-(2-(hydroxyimino)cyclohexyl)propanenitrile **13** (1.92 g, 11.58 mmol) were added acetic anhydride (1.1 eq., 1.30 g, 12.73 mmol) and acetyl chloride (1.5 eq., 1.24 mL, 17.36 mmol). The mixture was stirred at 55 °C for 8 h. After cooling to RT NaOH (excess, 1.75 g, 43.75 mmol) in H_2O (10 mL) was added and the mixture was refluxed for a further 2 h. The aqueous layer was then extracted with EtOAc (4×20 mL) and the combined organic layers were washed with 1 M acetic acid (2×20 mL) and H_2O (20 mL), dried over anhydrous MgSO_4 , filtered and concentrated *in-vacuo* to yield a residue which was purified by silica-gel chromatography (gradient 1:1 hexane:EtOAc – EtOAc) to give **10e** (718 mg, 42%) as a white crystalline solid. **M.p.** 87-89 °C, clean melt, lit. 87-90 °C.¹⁸³

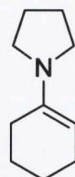
δ_{H} (400 MHz, CDCl_3): 1.75 (m, 2H, CH_2 , H-6), 1.83 (m, 2H, CH_2 , H-7), 2.60 (t, 2H, J 6.4, CH_2 , H-5), 2.72 (t, 2H, J 6.3, CH_2 , H-8), 4.34 (broad s, 2H, NH_2), 6.30 (d, 1H, J 8.2, H-3), 7.13 (d, 1H, J 8.2, H-4).

δ_{C} (100 MHz, CDCl_3): 22.9 (CH_2 , 6), 23.1 (CH_2 , 7), 27.7 (CH_2 , 5 or 8), 32.0 (CH_2 , 5 or 8), 106.3 (CH Ar, 3), 121.8 (q Ar, 5), 139.0 (CH Ar, 4), 154.6 (q Ar, 2 or 9), 155.8 (q Ar, 2 or 9).

ν_{max} (ATR)/ cm^{-1} : 3452 (NH), 3149 (NH), 2923, 1639, 1570, 1485, 1416, 1357, 1121, 987, 818.

HRMS (m/z GC.EI): Found: 148.1007 (M^+ , $C_9H_{12}N_2$ Requires: 148.1000).

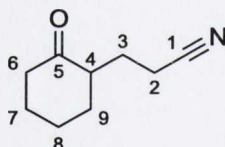
1-(Cyclohex-1-en-1-yl)pyrrolidine (11)



Cyclohexanone (5.17 mL, 49.92 mmol) and pyrrolidine (1.5 eq., 6.15 mL, 74.89 mmol) were added to toluene (20 mL) in a RBF fitted with a Dean-Stark apparatus and a reflux condenser. The reaction was stirred under reflux (87 °C) for 21 h. Solvent and excess pyrrolidine were removed under reduced pressure. As per the procedure, **11** was used directly in the synthesis of 3-(oxocyclohexyl)propanenitrile **12**.¹⁸³

R_f 0.44 in hexane:EtOAc 2:1

3-(Oxocyclohexyl)propanenitrile (12)



To a solution of 1-(cyclohex-1-en-1-yl)pyrrolidine **11** (7.50 g, 49.59 mmol) in dry dioxane (15 mL) was added acrylonitrile (1.1 eq., 3.70 mL, 56.53 mmol). The mixture was refluxed (101 °C) for 16 h, then cooled and H_2O (10 mL) was added. After a further 2 h reflux, solvent was removed under reduced pressure and the product was extracted with Et_2O (4×25 mL). The combined organic layers were washed with 1 M HCl solution (2×10 mL) and water (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in-vacuo*. The resulting liquid was purified by distillation at 160 °C to give **12** (5.34 g, 71%) as a viscous brown liquid identified as. The molecule, though reported, had not previously been fully characterised and so a series of NMR experiments were carried out to assign signals.¹⁸³

δ_H (600 MHz, $CDCl_3$): 1.36 (m, 1H, CH_2 , H-9), 1.47 (m, 1H, CH_2 , H-3), 1.61 (m, 1H, CH_2 , H-7 or H-8), 1.68 (m, 1H, CH_2 , H-7 or H-8), 1.85 (m, 1H, CH_2 , H-7 or H-8), 2.00

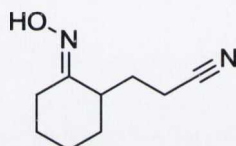
(m, 1H, CH₂, H-3), 2.05 (m, 1H, CH₂, H-7 or H-8), 2.10 (m, 1H, CH₂, H-9), 2.29 (dt, 2H, J 13.3, 6.3, CH₂, H-6), 2.35 (d, 1H, J 13.3, CH₂, H-3), 2.41 (t, 2H, J 6.9, CH₂, H-2), 2.43 (m, 1H, CH, H-4).

δ_C (150 MHz, CDCl₃): 15.0 (CH₂, 2), 24.9 (CH₂, 7 or 8), 25.4 (CH₂, 3), 27.8 (CH₂, 7 or 8), 34.0 (CH₂, 9), 42.0 (CH₂, 6), 48.7 (CH, 4), 119.6 (q, 1), 211.6 (q, 5).

$\nu_{\max}$ (ATR)/cm⁻¹: 2936, 2862, 2245 (C≡N), 1704 (C=O), 1449, 1429, 1376, 1339, 1312, 1002, 1025, 1229, 1131, 1076, 1043, 1009, 885, 828, 792, 716, 665.

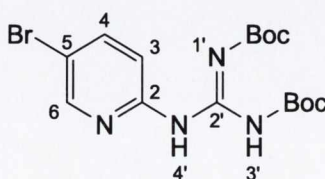
HRMS (*m/z* ESI⁺): Found: 180.1381 (M⁺ + C₂H₅. C₁₁H₁₈NO Requires: 180.1388).

3-(2-(Hydroxyimino)cyclohexyl)propanenitrile (**13**)



To a solution of hydroxylamine hydrochloride (1.3 eq., 1.806 g, 25.990 mmol) in H₂O (6 mL) at 0 °C was added dropwise a solution of 3-(oxocyclohexyl)propanenitrile **12** (1.0 eq., 3.00 g, 19.84 mmol) in Et₂O (40 mL). A solution of NaOH (1.35 eq., 1.07 g, 26.78 mmol) in H₂O (6 mL) was then added at such a rate as to maintain a temperature below 10 °C. The mixture was then brought to RT and stirred for 5 h. The organic layer was then separated and the aqueous layer was extracted with Et₂O (4 × 20 mL). The combined organic layers were washed with H₂O (2 × 20 mL), dried over anhydrous MgSO₄, filtered and concentrated *in-vacuo* to yield **13** as a colourless oil (2.04 g, 62%). As per Vijn *et al.*, the material was immediately used in the synthesis of 2-amino-5,6,7,8-tetrahydroquinoline (**10e**).¹⁸³

1-(5-Bromopyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**14a**)



Following Method A, HgCl_2 (330 mg, 1.21 mmol) was added over a solution of 2-amino-5-bromopyridine (200 mg, 1.16 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (352 mg, 1.21 mmol), and triethylamine (564 μL , 4.05 mmol) in CH_2Cl_2 (6 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 15 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **14a** as a white powder which was recrystallised from hexane:EtOAc to give the product (384 mg, 80%) as transparent crystals. **Mp.** 125-126 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.47 (s, 9H, CH_3 , Boc), 1.49 (s, 9H, CH_3 , Boc), 7.75 (dd, 1H, J 8.3, 2.1, H-4), 8.28 (d, 1H, J 2.1, H-6), 8.31 (d, 1H, J 8.3, H-3), 10.86 (broad s, 1H, H-3'), 11.49 (broad s, 1H, H-4').

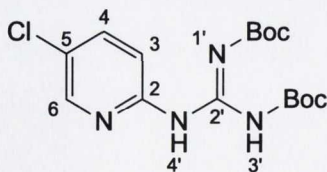
δ_{H} (400 MHz, $\text{DMSO}-d_6$): 1.44 (s, 9H, CH_3 , Boc), 1.50 (s, 9H, CH_3 , Boc), 8.13 (d, 1H, J 8.8, H-4), 8.22 (d, 1H, J 8.8, H-3), 8.44 (d, 1H, J 1.9, H-6), 10.64 (broad s, 1H, H-3'), 11.36 (broad s, 1H, H-4').

δ_{C} (100 MHz, CDCl_3): 27.8 (CH_3 , Boc), 27.9 (CH_3 , Boc), 79.9 (q ^tBu , Boc), 83.9 (q ^tBu , Boc), 114.7 (CH Ar, 3), 117.2 (q Ar, 5), 140.3 (CH Ar, 4), 148.6 (CH Ar, 6), 149.1 (q Ar, 2), 152.5 (q CO, Boc), 152.7 (q, 2'), 162.8 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3247 (NH), 2980, 1715 (C=O), 1632 (C=N), 1593, 1553, 1453, 1404, 1367, 1324, 1287, 1252, 1232, 1148, 1129, 1101, 1057, 1028, 1004, 878, 841, 793, 745.

HRMS (m/z ESI^+): Found: 415.0990 ($\text{M}^+ + \text{H}$. $\text{C}_{16}\text{H}_{24}^{79}\text{BrN}_4\text{O}_4$ Requires: 415.0981).

1-(5-Chloropyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**14b**)



Following Method A, HgCl_2 (443 mg, 1.63 mmol) was added over a solution of 2-amino-5-chloropyridine (200 mg, 1.56 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (474 mg, 1.63 mmol), and triethylamine (759 μL , 5.45 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 18 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **14b**

as an off-white powder which was recrystallised from hexane:EtOAc to give product (202 mg, 35%) as colourless crystals. **Mp.** 122-124 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.53 (s, 9H, CH_3 , Boc), 1.54 (s, 9H, CH_3 , Boc), 7.67 (dd, 1H, J 8.9, 2.6, H-4), 8.24 (d, 1H, J 2.6, H-6), 8.40 (d, 1H, J 8.9, H-3), 10.92 (broad s, 1H, H-3'), 11.51 (broad s, 1H, H-4').

δ_{H} (400 MHz, $\text{DMSO}-d_6$): 1.44 (s, 9H, CH_3 , Boc), 1.50 (s, 9H, CH_3 , Boc), 8.02 (d, 1H, J 8.8, H-4), 8.27 (d, 1H, J 8.9, H-3), 8.38 (d, 1H, J 2.4, H-6), 10.65 (broad s, 1H, H-3'), 11.36 (broad s, 1H, H-4').

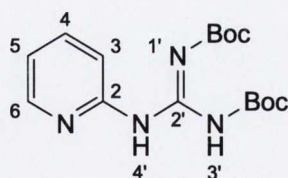
δ_{C} (100 MHz, CDCl_3): 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 80.1 (q ^tBu , Boc), 84.1 (q ^tBu , Boc), 116.8 (CH Ar, 3), 126.9 (q Ar, 5), 137.7 (CH Ar, 4), 146.6 (CH Ar, 6), 148.9 (q Ar, 2), 152.6 (q CO, Boc), 152.9 (q, 2'), 163.0 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3249 (NH), 2981, 1741, 1716 (C=O), 1633 (C=N), 1576, 1559, 1476, 1455, 1407, 1383, 1367, 1322, 1287, 1252, 1235, 1219, 1142, 1123, 1101, 1059, 1028, 1006, 967, 917, 880, 848, 837, 801, 782, 743, 728, 685.

HRMS (m/z ESI^+): Found: 371.1489 ($\text{M}^+ + \text{H}$, $\text{C}_{16}\text{H}_{24}^{35}\text{ClN}_4\text{O}_4$ Requires: 371.1486).

Crystal structure: See Fig. 3.2.8 and CCDC 822416.

1-(Pyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**14c**)



Following Method A, HgCl_2 (303 mg, 1.12 mmol) was added over a solution of 2-aminopyridine (100 mg, 1.06 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (324 mg, 1.12 mmol), and triethylamine (518 μL , 3.72 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 8 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **14c** as an off-white powder which was recrystallised from hexane:EtOAc to give product (282 mg, 79%) as colourless crystals. **Mp.** 138-140 °C, clean melt.

δ_{H} (600 MHz, CDCl_3): 1.53 (s, 18H, CH_3 , Boc), 7.01 (app. t, 1H, J 5.4, H-5), 7.70 (app. t, 1H, J 7.3, H-4), 8.29 (d, 1H, J 4.0, H-6), 8.37 (broad s, 1H, H-3), 10.89 (broad s, 1H, NH, H-3'), 11.53 (broad s, 1H, NH, H-4').

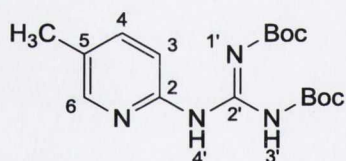
δ_{H} (600 MHz, $\text{DMSO}-d_6$): 1.44 (s, 9H, CH_3 , Boc), 1.51 (s, 9H, CH_3 , Boc), 7.16 (app. t, 1H, J 5.7, H-5), 7.87 (app. t, 1H, J 7.7, 7.2, H-4), 8.22 (d, 1H, J 7.9, H-3), 8.32 (d, 1H, J 3.8, H-6), 10.59 (broad s, 1H, NH, H-3'), 11.44 (broad s, 1H, NH, H-4').

δ_{C} (150 MHz, CDCl_3): 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 79.9 (q ^tBu , Boc), 83.9 (q ^tBu , Boc), 116.0 (CH Ar, 3), 119.7 (CH Ar, 5), 138.1 (CH Ar, 4), 148.0 (CH Ar, 6), 150.6 (q Ar, 2), 152.6 (q, CO, Boc), 153.1 (q, 2'), 163.2 (q, CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3253 (NH), 2979, 1725 (C=O), 1621 (C=N), 1586, 1570, 1479, 1398, 1368, 1332, 1302, 1293, 1256, 1229, 1147, 1121, 1089, 1030, 994, 883, 848, 809, 763, 733, 710, 669.

HRMS (m/z ESI $^+$): Found: 337.1882 ($\text{M}^+ + \text{H}$. $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_4$ Requires: 337.1876).

1-(5-Methylpyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**14d**)



Following Method A, HgCl_2 (527 mg, 1.94 mmol) was added over a solution of 2-amino-5-methylpyridine (200 mg, 1.85 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (564 mg, 1.94 mmol), and triethylamine (902 μL , 6.47 mmol) in CH_2Cl_2 (9 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 8 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **14d** (460 mg, 71%) as a white powder. **Mp.** 139 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, CH_3 , Boc), 1.49 (s, 9H, CH_3 , Boc), 2.23 (s, 3H, CH_3), 7.45 (dd, 1H, J 8.4, 1.9, H-4), 8.07 (d, 1H, J 1.9, H-6), 8.20 (broad s, 1H, H-3), 10.78 (broad s, 1H, NH, H-3'), 11.52 (broad s, 1H, NH, H-4').

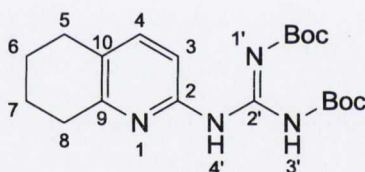
δ_{H} (400 MHz, DMSO- D_6): 1.44 (s, 9H, CH_3 , Boc), 1.51 (s, 9H, CH_3 , Boc), 2.23 (s, 3H, CH_3), 7.45 (dd, 1H, J 8.4, 1.9, H-4), 8.07 (d, 1H, J 1.9, H-6), 8.20 (broad s, 1H, H-3), 10.54 (broad s, 1H, NH, H-3'), 11.46 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 17.8 (CH_3), 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 79.7 (q ^tBu , Boc), 83.7 (q ^tBu , Boc), 115.6 (CH Ar, 3), 129.1 (q Ar, 5), 138.6 (CH Ar, 4), 147.9 (CH Ar, 6), 148.4 (q Ar, 2), 152.7 (q CO, Boc), 153.0 (q 2'), 163.3 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3244 (NH), 2978, 1720 (C=O), 1632 (C=N), 1585, 1560, 1475, 1454, 1404, 1374, 1324, 1305, 1289, 1268, 1252, 1230, 1151, 1136, 1107, 1058, 1025, 882, 856, 838, 803, 758, 749, 709.

HRMS (m/z ESI $^+$): Found: 351.2036 ($\text{M}^+ + \text{H}$, $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_4$ Requires: 351.2032).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**14e**)



Following Method A, HgCl_2 (308 mg, 1.13 mmol) was added over a solution of 2-amino-5,6,7,8-tetrahydroquinoline (160 mg, 1.08 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (329 mg, 1.13 mmol), and triethylamine (527 μL , 3.78 mmol) in CH_2Cl_2 (5 mL) at 0 $^\circ\text{C}$. The mixture was stirred at 0 $^\circ\text{C}$ for 30 min and a further 5 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **14e** (327 mg, 90%) as a white powder. **Mp.** 164-165 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.52 (s, 18H, CH_3 , Boc), 1.78 (m, 2H, CH_2 , H-6), 1.84 (m, 2H, CH_2 , H-7), 2.70 (t, 2H, J 6.3, 6.1, CH_2 , H-5), 2.78 (t, 2H, J 6.1, 6.3, CH_2 , H-8), 7.35 (d, 1H, J 8.3, H-4), 8.04 (broad s, 1H, H-3), 10.64 (broad s, 1H, NH, H-3'), 11.54 (broad s, 1H, NH, H-4').

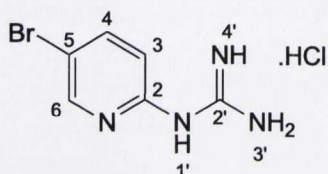
δ_{H} (400 MHz, DMSO- D_6): 1.43 (s, 9H, CH_3 , Boc), 1.44 (s, 9H, CH_3 , Boc), 1.73 (m, 2H, CH_2 , H-6), 1.80 (m, 2H, CH_2 , H-7), 2.71 (m, 2H, CH_2 , H-5), 2.78 (m, 2H, CH_2 , H-8), 7.53 (d, 1H, J 8.7, H-4), 7.93 (broad s, 1H, H-3), 10.49 (broad s, 1H, NH, H-3'), 11.53 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 22.7 (CH_2 , 6), 22.9 (CH_2 , 7), 28.00 (CH_3 , Boc), 28.02 (CH_2 , 5 or 8), 28.04 (CH_3 , Boc), 32.0 (CH_2 , 5 or 8), 79.6 (q ^tBu , Boc), 83.6 (q ^tBu , Boc), 113.6 (CH Ar, 3), 128.3 (q Ar, 10), 138.7 (CH Ar, 4), 147.6 (q Ar, 2), 152.6 (q CO, Boc), 152.0 (q, 2'), 155.4 (q Ar, 9), 163.3 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3251 (NH), 2979, 1714 (C=O), 1645 (C=N), 1629, 1588, 1565, 1449, 1395, 1369, 1354, 1325, 1313, 1279, 1247, 1231, 1151, 1112, 1058, 1029, 996, 941, 899, 874, 864, 840, 804, 755, 713.

HRMS (m/z ESI $^+$): Found: 391.2342 ($\text{M}^+ + \text{H}$, $\text{C}_{20}\text{H}_{31}\text{N}_4\text{O}_4$ Requires: 391.2345).

1-(5-Bromopyridin-2-yl)guanidine hydrochloride (**15a**)



Following Method E, a 25% v/v solution of TFA in CH_2Cl_2 (10 mL, 30 mmol TFA) was added to **14a** (300 mg, 0.72 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H_2O (9 mL) and Amberlite in its chloride activated form (6.0 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H_2O under reduced pressure yielded the crude hydrochloride salt. ^{19}F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H_2O as mobile phase. Removal of solvent yielded the pure guanidine hydrochloride **15a** (135 mg, 74%) as a white solid. **M.p.** 76-77 $^{\circ}\text{C}$, clean melt.

δ_{H} (600 MHz, $\text{DMSO}-d_6$): 7.06 (d, 1H, J 8.8, H-3), 8.09 (dd, 1H, J 8.8, 2.5, H-4), 8.23 (broad s, 4H, H-3' and H4'), 8.43 (d, 1H, J 2.5, H-6), 11.53 (broad s, 1H, H-1').

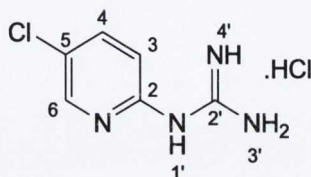
δ_{C} (150 MHz, $\text{DMSO}-d_6$): 114.7 (q Ar, 5), 116.1 (CH Ar, 3), 142.9 (CH Ar, 4), 148.1 (CH Ar, 6), 151.7 (q Ar, 2), 155.9 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3313 (NH), 3218 (NH), 3011 (NH), 1684.0 (C=N), 1618, 1582, 1551, 1465, 1360, 1308, 1275, 1234, 1137, 1094, 1006, 925, 874, 825, 732.

HRMS (m/z ESI⁺): Found: 214.9926 ($M^+ + H$. $C_6H_8^{79}\text{BrN}_4$ Requires: 214.9932).

Purity by HPLC: 99.1% (t_R 23.88 min).

1-(5-Chloropyridin-2-yl)guanidine hydrochloride (**15b**)



Following Method E, a 25% v/v solution of TFA in CH_2Cl_2 (10 mL, 30 mmol TFA) was added to **14b** (200 mg, 0.54 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H_2O (7 mL) and Amberlite in its chloride activated form (4.0 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H_2O under reduced pressure yielded the crude hydrochloride salt. ^{19}F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H_2O as mobile phase. Removal of solvent yielded the pure guanidine hydrochloride **15b** (95 mg, 85%) as a white solid. **M.p.** 162-164 °C, clean melt.

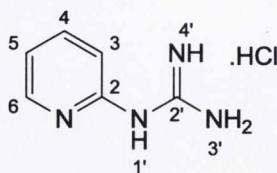
δ_{H} (600 MHz, DMSO- D_6): 7.13 (d, 1H, J 8.8, H-3), 7.98 (dd, 1H, J 8.8, 2.6, H-4), 8.28 (broad s, 4H, H-3' and H-4'), 8.35 (d, 1H, J 2.6, H-6), 11.70 (broad s, 1H, H-1').

δ_{C} (150 MHz, DMSO- D_6): 115.0 (CH Ar, 3), 126.6 (q Ar, 5), 139.2 (CH Ar, 4), 144.7 (CH Ar, 6), 151.0 (q Ar, 2), 155.7 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3313 (NH), 3178 (NH), 2953, 1685 (C=N), 1617, 1587, 1551, 1465, 1364, 1310, 1274, 1236, 1136, 1113, 1022, 1009, 924, 876, 828, 757, 734, 718.

HRMS (m/z ESI⁺): Found: 171.0433 ($M^+ + H$. $C_6H_8^{35}\text{ClN}_4$ Requires: 171.0437).

Purity by HPLC: 99.0% (t_R 22.68 min).

1-(Pyridin-2-yl)guanidine hydrochloride (15c)

Following Method E, a 25% v/v solution of TFA in CH₂Cl₂ (10 mL, 30 mmol TFA) was added to **14c** (250 mg, 0.74 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H₂O (8 mL) and Amberlite in its chloride activated form (5.0 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H₂O under reduced pressure yielded the crude hydrochloride salt. ¹⁹F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H₂O as mobile phase. Removal of solvent yielded an off-white solid which was recrystallised from CH₃OH and Et₂O to give the pure guanidine hydrochloride **15c** (91 mg, 71%) as a white, crystalline solid. **M.p.** 77-78 °C, clean melt.

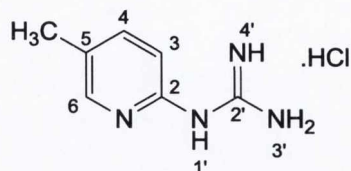
δ_{H} (600 MHz, DMSO-D₆): 7.07 (d, 1H, J 8.2, H-3), 7.18 (dd, 1H, J 6.9, 5.4, H-5), 7.88 (m, 1 H, H-4), 8.30 (broad s, 4H, H-3' and H-4'), 8.32 (d, 1H, J 5.4 Hz, H-6), 11.39 (broad s, 1H, H-1').

δ_{C} (150 MHz, DMSO-D₆): 113.2 (CH Ar, 3), 119.3 (CH Ar, 5), 139.5 (CH Ar, 4), 146.7 (CH Ar, 6), 151.9 (q Ar, 2), 155.3 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3312 (NH), 3180 (NH), 3130 (NH), 1679 (C=N), 1622, 1596, 1561, 1462, 1416, 1319, 1274, 1244, 1154, 1054, 1020, 998, 874, 775.

HRMS (m/z ESI⁺): Found: 137.0827 (M⁺ + H. C₆H₉N₄ Requires: 137.0827).

Purity by HPLC: 97.2% (*t*R 16.81 min).

1-(5-Methylpyridin-2-yl)guanidine hydrochloride (15d)

Following Method E, a 25% v/v solution of TFA in CH₂Cl₂ (10 mL, 30 mmol TFA) was added to **14d** (210 mg, 0.60 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H₂O (8 mL) and Amberlite in its chloride activated form (5.0 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H₂O under reduced pressure yielded the crude hydrochloride salt. ¹⁹F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H₂O as mobile phase. Removal of solvent yielded a white solid which was recrystallised from ¹PrOH and Et₂O to give the pure guanidine hydrochloride **15d** (94 mg, 84%) as rod-shaped colourless crystals. **M.p.** 188-192 °C, clean melt.

δ_{H} (600 MHz, DMSO-D₆): 2.27 (s, 3H, CH₃), 6.98 (d, 1H, J 8.3, H-3), 7.71 (dd, 1H, J 8.3, 1.9, H-4), 8.15 (d, 1H, J 1.9, H-6), 8.21 (broad s, 4H, H-3' and H-4'), 11.17 (broad s, 1H, H-1').

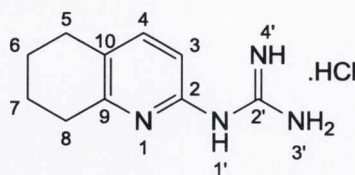
δ_{C} (150 MHz, DMSO-D₆): 17.1 (CH₃), 112.7 (CH Ar, 3), 128.5 (q Ar, 5), 140.1 (CH Ar, 4), 146.1 (CH Ar, 6), 149.7 (q Ar, 2), 155.2 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3268 (NH), 2889, 1677 (C=N), 1621, 1601, 1563, 1489, 1376, 1315, 1285, 1242, 1086, 1035, 1023, 1002, 909, 873, 832, 798, 738, 718.

HRMS (m/z ESI⁺): Found: 151.0979 (M⁺ + H. C₇H₁₁N₄ Requires: 151.0984).

Purity by HPLC: 98.3% (*t*R 21.57 min).

Crystal structure: See Fig. 3.2.8 and CCDC 822417.

1-(5,6,7,8-Tetrahydroquinolin-2-yl)guanidine hydrochloride (15e)

Following Method E, a 25% *v/v* solution of TFA in CH_2Cl_2 (6 mL, 18 mmol TFA) was added to **14e** (144 mg, 0.37 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H_2O (5 mL) and Amberlite in its chloride activated form (3.0 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H_2O under reduced pressure yielded the crude hydrochloride salt. ^{19}F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H_2O as mobile phase. Removal of solvent yielded a white solid which was recrystallised from methanol and Et_2O to give the pure guanidine hydrochloride **15e** (72 mg, 87%) as rod-shaped colourless crystals. **M.p.** 224–228 °C, decomposition.

δ_{H} (600 MHz, $\text{DMSO}-d_6$): 1.73 (m, 2H, CH_2 , H-6), 1.80 (m, 2H, CH_2 , H-7), 2.68 (app. t, 2H, J 6.1, 6.0, CH_2 , H-5), 2.78 (app. t, 2H, J 6.1, 6.0, CH_2 , H-8), 6.80 (d, 1H, J 8.2, H-3), 7.55 (d, 1H, J 8.2, H-4), 8.22 (broad s, 4H, H-3' and H-4'), 11.08 (broad s, 1H, H-1').

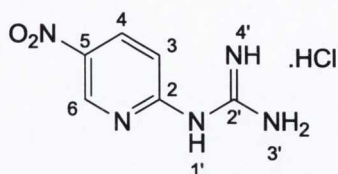
δ_{C} (150 MHz, $\text{DMSO}-d_6$): 22.1 (CH_2 , 6), 22.2 (CH_2 , 7), 27.2 (CH_2 , 5), 31.5 (CH_2 , 8), 110.5 (CH Ar, 3), 127.3 (q Ar, 10), 140.1 (CH Ar, 4), 149.2 (q Ar, 2), 154.0 (q Ar, 9), 155.2 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3323 (NH), 3149 (NH), 2961, 1679 ($\text{C}=\text{N}$), 1634, 1597, 1566, 1465, 1031, 813.

HRMS (m/z ESI^+): Found: 191.1293 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_{15}\text{N}_4$ Requires: 191.1297).

Purity by HPLC: 98.1% (t_{R} 27.00 min).

Crystal structure: See Fig. 3.2.8 and CCDC 822187.

1-(5-Nitropyridin-2-yl)guanidine hydrochloride (15f)

To guanidine hydrochloride (1.1 eq, 133 mg, 1.39 mmol) and ^tBuOH (12 mL) was added finely ground NaOH (3.0 eq., 151 mg, 3.79 mmol) was added. The mixture was stirred for 30 min at RT after which 2-nitro-5-chloropyridine **17** (1.0 eq, 200 mg, 1.26 mmol) was added. Reflux at 120 °C was continued for 6 h. The resulting solution was extracted with ⁱPrOH/CH₂Cl₂ (20%, 4 × 30 mL) and washed with 2 M NaOH solution (30 mL). The organic extracts were combined, dried over MgSO₄, and concentrated *in vacuo* to give the neutral guanidine (153 mg, 67%). This was stirred at RT in a 1.25 M solution of HCl in CH₃OH (6.0 eq, 5.98 mL, 7.47 mmol) for 3 h. Removal of solvent and purification by reverse phase chromatography (C-8 silica) with 100% H₂O as mobile phase yielded **15f** (156 mg, 85%) as a yellow crystalline material. **M.p.** 93-95 °C, clean melt.

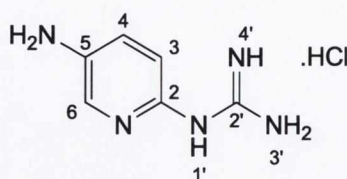
δ_{H} (600 MHz, DMSO-D₆): 7.27 (d, 1H, J 9.1, H-3), 8.49 (broad s, 4H, NH, H-3' and H-4'), 8.62 (dd, 1H, J 9.1, 2.7, H-4), 9.12 (d, 1H, J 2.7, H-6), 12.17 (broad s, 1H, NH, H-1').

δ_{C} (150 MHz, DMSO-D₆): 113.7 (CH Ar, 3), 135.0 (CH Ar, 4), 140.3 (q Ar, 5), 144.1 (CH Ar, 6), 155.2 (q Ar, 2), 156.0 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3426 (NH), 3318 (NH), 3185, 2924, 1687 (C=N), 1620, 1601, 1552, 1516, 1474, 1340, 1246, 1121, 1079, 1013, 984, 947, 864, 840, 758, 708.

HRMS (*m/z* ESI⁺): Found: 182.0679 (M⁺ + H. C₆H₈N₅O₂ Requires: 182.0678).

Purity by HPLC: 96.8% (tR 18.97 min).

1-(5-Aminopyridin-2-yl)guanidine hydrochloride (15g)

To a solution of neutral 1-(5-nitropyridin-2-yl)guanidine **18** (100 mg, 0.55 mmol), in CH₃OH (6 mL) was added 10% Pd/C (29 mg, 0.028 mmol, 5 mol%). The mixture was stirred under a hydrogen atmosphere (3 atm.) for 7 h. It was then diluted with CH₃OH (30 mL), filtered through filter paper and concentrated under reduced pressure. Purification of the neutral guanidine proved unsuccessful so it was converted to the hydrochloride salt and subsequently purified. The residue was stirred in a 1.25 M methanolic HCl solution (6.0 eq, 2.6 mL, 3.30 mmol) for 3 h. Excess solvent and HCl were removed under reduced pressure and the residue was dissolved in a minimum volume of H₂O. The aqueous layer was washed with CH₂Cl₂ (2 × 2 mL) and concentrated under reduced pressure to yield a red solid, which was purified by reverse phase chromatography (C-8 silica) with 100% H₂O as mobile phase. Recrystallisation from CH₃OH/Et₂O afforded the pure hydrochloride salt **15g** (71 mg, 69%) as a red solid. **M.p.** 200-202 °C, burned.

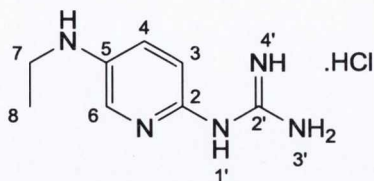
δ_{H} (600 MHz, DMSO-D₆): 3.82 (broad s, 2H, NH₂), 7.03 (d, 1H, J 8.8, H-3), 7.56 (d, 1H, J 8.8, H-4), 8.08 (s, 1H, H-6), 8.20 (broad s, 4H, NH, H-3' and H-4'), 11.39 (broad s, 1H, NH, H-1').

δ_{C} (150 MHz, DMSO-D₆): 113.9 (CH Ar, 3), 127.2 (CH Ar, 4), 133.8 (q Ar, 5), 137.5 (CH Ar, 6), 143.7 (q Ar, 2), 154.7 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3308 (NH), 2795, 2544, 2025, 1682 (C=N), 1627, 1602, 1560, 1488, 1396, 1287, 1252, 1226, 1137, 1099, 1033, 877, 825, 807, 731.

HRMS (m/z ESI⁺): Found: 152.0934 ($M^+ + H$. C₆H₁₀N₅ Requires: 152.0936).

Purity by HPLC: 97.4% (tR 4.84 min).

1-[5-(Ethylamino)pyridin-2-yl]guanidine hydrochloride (15h)

To a solution of neutral 1-(5-aminopyridin-2-yl)guanidine **19** (111 mg, 0.73 mmol) in a mixture of CH₃CN/CH₃OH (5:1, 3.0 mL) was added acetaldehyde (1.0 eq., 0.73 mmol, 41 μ L). The solution was stirred under an argon atmosphere and sodium triacetoxyborohydride (2.0 eq., 311 mg, 1.47 mmol) was added. Reaction was continued for 6 h, after which excess solvent and acetaldehyde was removed under reduced pressure. The resulting residue was dissolved in solution of ⁱPrOH/CH₂Cl₂ (20%, 10 mL), washed with NaHCO₃ (sat., 5 mL) and water (3 mL), dried over MgSO₄, filtered and concentrated under vacuum. The residue was stirred in a 1.25 M methanolic HCl solution (4.0 eq., 2.4 mL, 2.94 mmol) for 3 h. Excess solvent and HCl were removed under reduced pressure and the residue was dissolved in a minimum volume of H₂O. The aqueous layer was washed with CH₂Cl₂ and concentrated under vacuum to yield a red solid, which was purified by reverse phase chromatography (C-8 silica) with 100% H₂O as mobile phase (115.5 mg, 73%) to yield a dark red solid identified by ¹H NMR spectroscopy as a mixture of salts. Normal phase chromatography eluting with a gradient of CH₂Cl₂/CMA isolated **15h** (51 mg, 32%) which was obtained as a red solid after re-stirring in 1.25 M methanolic HCl (2.4 mL). **M.p.** 154-157 °C, clean melt.

δ_{H} (400 MHz, DMSO-D₆): 1.22 (t, 3H, J 7.2, CH₃, H-8), 3.43 (q, 2H, J 7.2, CH₂, H-7), 7.12 (d, 1H, J 9.4, H-3), 7.66 (broad s, 4H, NH, H-3' and H-4'), 7.75 (d, 1H, J 9.4, H-4), 7.92 (s, 1H, H-6), 9.78 (broad s, 1H, NH, H-1').

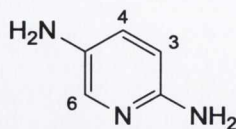
δ_{C} (100 MHz, DMSO-D₆): 13.7 (CH₃, 8), 37.0 (CH₂, 7), 114.3 (CH Ar, 3), 120.7 (q Ar, 5), 135.0 (CH Ar, 4), 142.0 (CH Ar, 6), 142.0 (q Ar, 2), 157.2 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3313, 3181 (NH), 3130 (NH), 3037 (NH), 2973, 2810, 1702, 1666 (C=N), 1608, 1584, 1498, 1469, 1456, 1432, 1337, 1302, 1272, 1230, 1183, 1123, 1102, 1057, 1037, 1003, 942, 909, 875, 857, 821, 781, 730, 694, 672.

HRMS (m/z ESI⁺): Found: 180.1244 (M⁺ + H. C₈H₁₄N₅ Requires: 180.1249).

Purity by HPLC: 99.5% (tR 3.88 min).

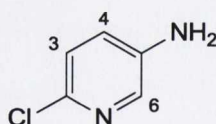
2,5-Diaminopyridine (**16**)



To a solution of 2-amino-5-nitropyridine **43** (1.0 eq., 400 mg, 2.88 mmol), in EtOAc (15 mL) was added 10% Pd/C (61 mg, 0.06 mmol, 2 mol%). The mixture was stirred under a hydrogen atmosphere (3 atm.) for 16 h. It was then filtered through filter paper, concentrated under reduced pressure and purified by silica gel column chromatography, eluting with 4:1 CH₂Cl₂:CH₃OH. Removal of solvent yielded **16** (191 mg, 61%) as a red/black solid. **M.p.** 96-97 °C, clean melt, lit. 95-98 °C.²²³

δ_{H} (400 MHz, CDCl₃): 4.28 (broad s, 2H, NH₂), 4.95 (broad s, 2H, NH₂), 6.27 (d, 1H, J 8.5, H-3), 6.79 (dd, 1H, 8.5, 2.7, H-4), 7.39 (d, 1H, J 2.7, H-6).

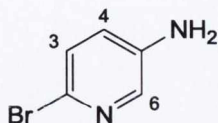
2-Chloro-5-aminopyridine (**23**)



To a suspension of 2-chloro-5-nitropyridine (250 mg, 1.58 mmol) in a mixture of H₂O (2.8 mL) and acetic acid (1.2 mL) at 45 °C was added iron powder (3.0 eq., 264 mg, 4.73 mmol). Stirring was continued for 2 h after which full conversion of starting material was observed by TLC. The reaction mixture was diluted with EtOAc (30 mL) and filtered through a bed of celite. The aqueous layer was extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with saturated NaHCO₃ solution (15 mL) and H₂O (15 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to give pure 2-chloro-5-aminopyridine **23** (199 mg, 98%) as a white solid. **M.p.** 83-85 °C, clean melt, lit. 82-83 °C.²²⁴

δ_{H} (400 MHz, CDCl_3): 3.75 (broad s, 2H, NH_2), 6.94 (dd, 1H, J 8.5, 2.5, H-4), 7.04 (d, 1H, J 8.5, H-3), 7.80 (s, 1H, H-6).

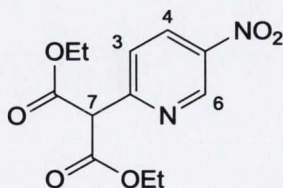
2-Chloro-5-aminopyridine (24)



To a suspension of 2-bromo-5-nitropyridine (155 mg, 0.76 mmol) in a mixture of H_2O (1.4 mL) and acetic acid (0.6 mL) at 45 °C was added iron powder (3.0 eq., 128 mg, 2.29 mmol). Stirring was continued for 2 h after which full conversion of starting material was observed by TLC. The reaction mixture was diluted with EtOAc (20 mL) and filtered through a bed of celite. The aqueous layer was extracted with EtOAc (2 × 20 mL) and the combined organic layers were washed with saturated NaHCO_3 solution (15 mL) and H_2O (15 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to give pure 2-bromo-5-aminopyridine **24** (127 mg, 96%) as a white solid. **M.p.** 77-78 °C, clean melt, lit. 77 °C.²²⁵

δ_{H} (400 MHz, CDCl_3): 3.68 (broad s, 2H, NH_2), 6.88 (dd, 1H, J 8.5, 3.0, H-4), 7.21 (d, 1H, J 8.5, H-3), 7.85 (d, 1H, J 3.0, H-6).

Diethyl 2-(5-nitropyridin-2-yl)malonate (25)



To a suspension of sodium hydride (60% suspension in mineral oil, 2.2 eq., 371 mg, 5.55 mmol) in dry tetrahydrofuran (20 mL) over 10 mins was added diethylmalonate (2.0 eq., 770 μL , 5.05 mmol). After 10 min stirring, a solution of 2-chloro-5-nitropyridine (1.0 eq., 400 mg, 2.52 mmol) in tetrahydrofuran (5 mL) was added. The mixture was brought to 66 °C and stirred for 16 h. The reaction was then diluted with EtOAc (40 mL) and washed with H_2O (3 × 20 mL). The organic layer was dried using

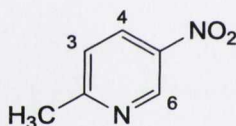
MgSO₄, filtered, and concentrated under reduced pressure to give a yellow solid which was purified by silica gel chromatography, eluting with hexane:EtOAc. The product, **25** (677 mg, 95%), was obtained as a bright yellow solid after removal of solvents. **M.p.** 97-99 °C, clean melt.

δ_{H} (400 MHz, CDCl₃): 1.32 (app. t, 6H, J 6.8, 2 CH₃), 4.30 (m, 4H, 2 CH₂), 5.08 (s, 1H, CH, H-7), 7.79 (d, 1H, J 8.8, H-3), 8.54 (dd, 1H, J 8.8, 2.4, H-4), 9.40 (d, 1H, J 2.4, H-6).

δ_{C} (100 MHz, CDCl₃): 14.0 (2 CH₃), 60.4 (CH, 7), 62.6 (2 CH₂), 124.4 (CH Ar, 3), 131.8 (CH Ar, 4), 143.7 (q Ar, 5), 144.6 (CH Ar, 6), 158.9 (q Ar, 2), 166.4 (q, CO).

HRMS (*m/z* ESI): Found: 281.0779 (*M*⁻ - H. C₁₂H₁₃N₂O₆ Requires: 281.0774).

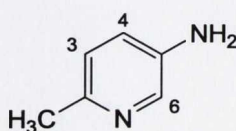
2-Methyl-5-nitropyridine (**26**)



Aqueous sulfuric acid (20%, 3.0 eq., 5.10 mL, 5.10 mmol) was added to **25** (1.0 eq., 480 mg, 1.70 mmol) and the mixture was heated at 100 °C for 2 h. The mixture was diluted with H₂O (20 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H₂O (2 × 20 mL), dried using MgSO₄, filtered, and concentrated under reduced pressure to yield **26** (232 mg, 99%) as a yellow solid. **M.p.** 104-106 °C, lit. 104-105 °C.²²⁶

δ_{H} (400 MHz, CDCl₃): 2.73 (s, 3H, CH₃), 7.38 (d, 1H, J 8.0, H-3), 8.40 (dd, 1H, J 8.0, 2.5, H-4), 9.36 (s, 1H, H-6).

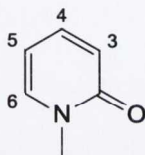
2-Methyl-5-aminopyridine (**27**)



To a solution of 2-methyl-5-nitropyridine **26** (1.0 eq., 220 mg, 1.59 mmol), in EtOAc (15 mL) was added 10% Pd/C (34 mg, 0.03 mmol, 2 mol%). The mixture was stirred under a hydrogen atmosphere (3 atm.) for 4 h. It was then filtered through filter paper and concentrated under reduced pressure to yield **27** (167 mg, 97%) as a red solid. **M.p.** 97-98 °C, clean melt, lit. 92-96 °C.²²⁷

δ_{H} (400 MHz, CDCl_3): 2.30 (s, 3H, CH_3), 4.03 (broad s, 2H, NH_2), 6.79 (s, 2H, H-4 and H-3), 7.87 (s, 1H, H-6).

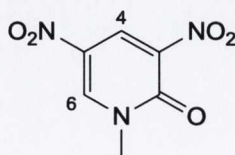
N-Methylpyridin-2-one (29)



Sodium hydroxide (1.2 eq., 0.38 g, 9.39 mmol) was dissolved in H_2O (26 mL) and 2-chloro-1-methylpyridinium iodide (1.0 eq., 2.00 g, 7.83 mmol) was added slowly. The resulting brown solution was stirred at 90 °C for 5 h. The mixture was then extracted with CH_2Cl_2 (3 $\times$ 30 mL) and the combined organic layers were washed with H_2O (2 $\times$ 15 mL). The organic layer was dried over MgSO_4 , filtered, and concentrated under vacuum to yield **29** (803 mg, 94%) as a brown/white solid. **M.p.** 33-35 °C, clean melt, lit. 31-32.²²⁸

δ_{H} (400 MHz, CDCl_3): 3.52 (s, 3H, CH_3), 6.13 (t, 1H, J 6.7, H-5), 6.53 (d, 1H, J 9.1, H-3), 7.29 (m, 2H, H-4 and H-6).

3,5-Dinitro-*N*-methylpyridin-2-one (30)

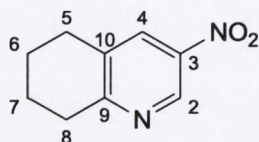


To fuming nitric acid (15.4 eq., 8 mL, 192.09 mmol) was added **29** (1.0 eq., 1.36 g, 12.46 mmol). The mixture was heated to 80 °C for 5h. The majority of the nitric acid

was removed under reduced pressure and the mixture was poured onto crushed ice (30 g). The organic material was then extracted with EtOAc (3 × 30 mL), washed with H₂O (2 × 10 mL), dried over MgSO₄, filtered, and concentrated under vacuum to yield **30** (966 mg, 39%) as a yellow solid. **M.p.** 181-184 °C, decomposition, lit. 178-179 °C.²²⁹

δ_{H} (400 MHz, DMSO-D₆): 3.65 (s, 3H, CH₃), 8.93 (s, 1H, H-6), 9.52 (s, 1H, H-4).

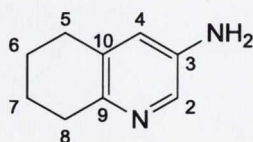
3-Nitro-5,6,7,8-tetrahydroquinolone (**31**)



Cyclohexanone (2.0 eq., 499 μ L, 4.82 mmol), **30** (1.0 eq., 480 mg, 2.41 mmol) and concentrated ammonia solution (22.0 eq., 2.93 mL, 53.03 mmol, 18 M) were dissolved in CH₃OH (25 mL) and heated to 65 °C. Stirring was continued for 3 h after which the methanol and ammonia were removed under reduced pressure. The mixture was extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with H₂O (2 × 10 mL), dried over MgSO₄, filtered, and concentrated under vacuum to give a residue which was purified by silica gel chromatography, eluting with hexane:EtOAc. The product, **31** (352 mg, 82 %), was afforded as an off-white solid. **M.p.** 76-78 °C, decomposition, lit., 74-75 °C.¹⁸⁶

δ_{H} (400 MHz, CDCl₃): 1.86-1.89 (m, 2H, CH₂, H-6), 1.91-1.97 (m, 2H, CH₂, H-7), 2.88 (app.t, 2H, J 6.4, H-5), 3.01 (app. t, 2H, J 6.4, H-8), 8.14 (d, 1H, J 4.0, H-4), 9.14 (d, 1H, J 4.0, H-2).

3-Amino-5,6,7,8-tetrahydroquinolone (**32**)

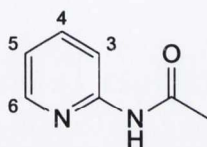


To a solution of **31** (1.0 eq., 150 mg, 0.84 mmol) in EtOAc (16 mL) was added 10% Pd/C (18 mg, 0.02 mmol, 2 mol%). The mixture was stirred under a hydrogen

atmosphere (3 atm.) for 3 h. It was then filtered through filter paper and concentrated under reduced pressure to yield **32** (124 mg, 99%) as a red solid. **M.p.** 91-92 °C, clean melt, lit. 97-98 °C.²³⁰

δ_{H} (400 MHz, CDCl_3): 1.77-1.91 (m, 4H, 2 CH_2 , H-6 and H-7), 2.73 (app. t, 2H, J 6.0, CH_2 , H-5), 2.93 (app. t, 2H, J 7.6, H-8), 4.14 (broad s, 2H, NH_2), 6.90 (d, 1H, J 2.6, H-4), 8.06 (d, 1H, J 2.6, H-2).

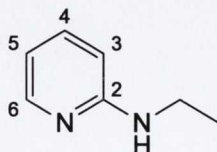
N-(Pyridin-2-yl)acetamide (**33**)



To a solution of 2-aminopyridine (1.0 eq., 500 mg, 5.31 mmol) in CH_2Cl_2 at RT were added triethylamine (3.0 eq., 2.22 mL, 15.94 mmol) and acetic anhydride (2.0 eq., 1.00 mL, 10.63 mmol). Stirring was continued for 6 h, after which the mixture was diluted with EtOAc (30 mL). The organic layer was washed with H_2O (2×15 mL), dried over MgSO_4 , filtered, and concentrated under vacuum to yield a colourless oil which was purified by silica gel chromatography, eluting with hexane:EtOAc. Removal of solvent yielded **33** (650 mg, 90%) as a yellow/white solid. **M.p.** 75-76 °C, clean melt, lit., 70-71 °C.²³¹

δ_{H} (400 MHz, CDCl_3): 2.14 (s, 3H, CH_3), 6.96-6.99 (m, 1H, H-5), 7.63-7.66 (m, 1H, H-3), 8.18-8.21 (m, 2H, H-4 and H-6), 9.84 (broad s, 1H, NH).

2-(*N*-Ethylamino)pyridine (**34**)



To a solution of **33** (1.0 eq., 400 mg, 2.94 mmol) in dry tetrahydrofuran (30 mL) at 0 °C was added lithium aluminium hydride (5.0 eq., 557 mg, 14.69 mmol). The mixture was

stirred at RT for 16 h, after which H₂O (30 mL) was added to quench the reaction. The mixture was filtered through celite and the organic phase was extracted with EtOAc (3 × 30 mL), washed with H₂O (2 × 15 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure to yield **34** (266 mg, 74%) as a red liquid.

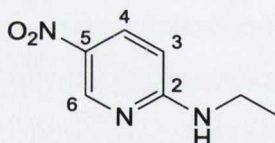
δ_{H} (400 MHz, CDCl₃): 1.24 (t, 3H, J 7.2, CH₃), 3.28 (q, 2H, J 7.2, CH₂), 4.65 (broad s, 1H, NH), 6.37 (d, 1H, J 8.4, H-3), 6.54 (m, 1H, H-5), 7.41 (app. dt, 1H, J 8.4, 1.9, H-4), 8.06 (dd, 1H, J 5.0, 1.9, H-6).

δ_{C} (100 MHz, CDCl₃): 14.8 (CH₃), 36.9 (CH₂), 106.3 (CH Ar, 3), 112.6 (CH Ar, 5), 137.5 (CH Ar, 4), 148.1 (CH Ar, 6), 158.9 (q Ar, 2).

ν_{max} (ATR)/cm⁻¹: 2959 (NH), 1610, 1571, 1482, 1443, 1320, 1273, 1253, 1150, 1046, 989, 846, 631, 550, 519.

HRMS (m/z ESI⁺): Found: 123.0921 (M⁺ + H. C₇H₁₁N₂ Requires: 123.0922).

2-(*N*-Ethylamino)-5-nitropyridine (**35**)



Aqueous sulfuric acid (20%, 2.5 eq., 5.10 mL, 5.10 mmol) and potassium nitrate (1.1 eq., 228 mg, 2.25 mmol) were added to **34** (1.0 eq., 250 mg, 2.05 mmol) and the mixture was stirred at RT for 5 h. The reaction was cooled to 0 °C and aqueous ammonia was added dropwise until a pH of 12 was reached. The resulting precipitate was extracted with EtOAc (3 × 30 mL), washed with brine (20 mL) and water (2 × 20 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure to yield a yellow/brown solid which was purified by silica gel column chromatography, eluting with 1:1 hexane:EtOAc. Removal of solvent yielded **35** (171 mg, 50%) as a yellow solid. **M.p.** 115-116 °C, lit., 117-119 °C.²³²

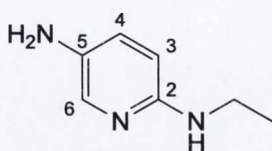
δ_{H} (400 MHz, CDCl₃): 1.26 (t, 3H, J 7.0, CH₃), 3.40 (broad s, 2H, CH₂), 6.13 (broad s, 1H, NH), 6.35 (d, 1H, J 9.3, H-3), 8.10 (broad s, 1H, H-4), 8.93 (s, 1H, H-6).

δ_{C} (100 MHz, CDCl_3): 14.2 (CH_3), 36.9 (CH_2), 132.6 (CH Ar , 4), 146.8 (CH Ar , 6), 161.1 (q Ar, 5), 135.1 (CH Ar , 3), 105.3 (q Ar, 2).

ν_{max} (ATR)/ cm^{-1} : 3343 (NH), 2980, 2934, 1601, 1550 (NO_2), 1469, 1465, 1408, 1367, 1321, 1279, 1248, 1163, 1133, 1109, 1100, 1060, 994, 973, 951, 861, 824, 766, 730.

HRMS (m/z ESI $^+$): Found: 168.0772 ($\text{M}^+ + \text{H}$, $\text{C}_7\text{H}_{10}\text{N}_3\text{O}_2$ Requires: 168.0773).

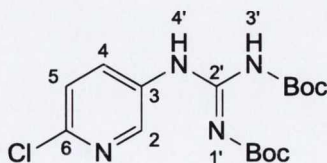
2-(*N*-Ethylamino)-5-aminopyridine (**36**)



To a solution of **35** (1.0 eq., 200 mg, 1.20 mmol) in EtOAc (12 mL) was added 10% Pd/C (25 mg, 0.02 mmol, 2 mol%). The mixture was stirred under a hydrogen atmosphere (3 atm.) for 5 h. It was then filtered through filter paper and concentrated under reduced pressure to yield **36** (157 mg, 95%) as a dark red solid. **M.p.** 96-97 °C, clean melt, lit. 95-98 °C.²³³

δ_{H} (400 MHz, DMSO- D_6): 1.08 (t, 3H, J 8.0, CH_3), 3.10 (m, 2H, CH_2), 4.26 (broad s, 2H, NH_2), 5.44 (broad s, 1H, NH), 6.26 (d, 1H, J 8.0, H-3), 6.81 (dd, 1H, J 8.0, 1.9, H-4), 7.45 (s, 1H, H-6).

1-(6-Chloropyridin-3-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**37a**)



Following Method A, HgCl_2 (333 mg, 1.23 mmol) was added over a solution of 2-chloro-5-aminopyridine (150 mg, 1.17 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (356 mg, 1.23 mmol), and triethylamine (569 μL , 4.08 mmol) in CH_2Cl_2 (6 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 4 h at

RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **37a** (342 mg, 79%) as a white powder. **Mp.** 118-120 °C, clean melt.

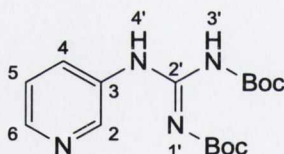
δ_{H} (400 MHz, CDCl_3): 1.50 (s, 9H, CH_3 , Boc), 1.55 (s, 9H, CH_3 , Boc), 7.31 (d, 1H, J 8.7, H-5), 8.22 (dd, 1H, J 8.7, 2.8, H-4), 8.47 (d, 1H, J 2.8, H-6), 10.44 (broad s, 1H, H-3'), 11.60 (broad s, 1H, H-4').

δ_{C} (100 MHz, CDCl_3): 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 80.1 (q ^tBu , Boc), 84.4 (q ^tBu , Boc), 124.1 (CH Ar, 5), 132.5 (CH Ar, 4), 133.0 (q Ar, 3), 142.7 (CH Ar, 2), 146.3 (q Ar, 6), 153.3 (q CO, Boc), 153.6 (q, 2'), 163.1 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3249 (NH), 2981, 1741, 1716 (C=O), 1633 (C=N), 1576, 1559, 1476, 1455, 1407, 1383, 1367, 1322, 1287, 1252, 1235, 1219, 1142, 1123, 1101, 1059, 1028, 1006, 967, 917, 880, 848, 837, 801, 782, 743, 728, 685.

HRMS (m/z ESI^+): Found: 371.1488 ($\text{M}^+ + \text{H}$, $\text{C}_{16}\text{H}_{24}^{35}\text{ClN}_4\text{O}_4$ Requires: 371.1486).

1-(Pyridin-3-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**37b**)



Following Method A, HgCl_2 (242 mg, 0.89 mmol) was added over a solution of 3-aminopyridine (80 mg, 0.85 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methyliothiourea (259 mg, 0.89 mmol), and triethylamine (415 μL , 2.98 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 5 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **37b** (255 mg, 89%) as a beige powder. **Mp.** 136-138 °C, clean melt.

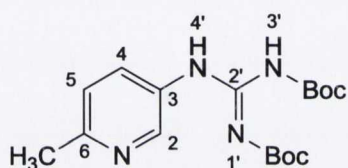
δ_{H} (400 MHz, CDCl_3): 1.50 (s, 9H, Boc CH_3), 1.54 (s, 9H, Boc CH_3), 7.28 (dd, 1H, J 8.3, 4.1, H-5), 8.21 (dd, 1H, J 8.3, 1.5, H-4), 8.34 (d, 1H, J 4.1, H-6), 8.67 (d, 1H, J 1.5, H-2), 10.41 (broad s, 1H, NH, H-3'), 11.61 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 27.6 (CH_3 , Boc), 27.6 (CH_3 , Boc), 79.6 (q ^tBu , Boc), 83.7 (q ^tBu , Boc), 123.1 (CH Ar, 5), 129.2 (CH Ar, 4), 133.4 (q Ar, 3), 142.7 (CH Ar, 2), 145.0 (q Ar, 6), 152.8 (q CO, Boc), 153.3 (q, 2'), 162.8 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3146 (NH), 2981, 1725 (C=O), 1636 (C=N), 1587, 1561, 1477, 1396, 1367, 1329, 1280, 1252, 1226, 1151, 1132, 1101, 1057, 1029, 942, 883, 845, 801, 773, 746, 719, 702, 656.

HRMS (m/z ESI $^+$): Found: 337.1870 ($\text{M}^+ + \text{H}$. $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_4$ Requires: 337.1876).

1-(6-Methylpyridin-3-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**37c**)



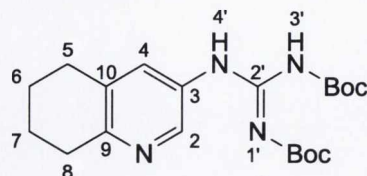
Following Method A, HgCl_2 (395 mg, 1.46 mmol) was added over a solution of 2-methyl-5-aminopyridine (150 mg, 1.39 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiourea (423 mg, 1.46 mmol), and triethylamine (677 μL , 4.86 mmol) in CH_2Cl_2 (7 mL) at 0 $^\circ\text{C}$. The mixture was stirred at 0 $^\circ\text{C}$ for 30 min and a further 3 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **37c** (428 mg, 88%) as a white powder. **Mp.** 102 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, Boc CH_3), 1.53 (s, 9H, Boc CH_3), 2.50 (s, 3H, CH_3), 7.12 (d, 1H, J 8.0, H-5), 8.03 (d, 1H, J 8.0, H-4), 8.53 (s, 1H, H-2), 10.28 (broad s, 1H, NH, H-3'), 11.61 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 23.8 (CH_3), 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 79.8 (q ^tBu , Boc), 84.0 (q ^tBu , Boc), 123.0 (CH Ar, 5), 130.3 (CH Ar, 4), 131.1 (q Ar, 3), 142.7 (CH Ar, 2), 153.3 (q Ar, 6), 153.8 (q CO, Boc), 154.4 (q, 2'), 163.3 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3244 (NH), 2978, 1720 (C=O), 1632 (C=N), 1585, 1560, 1475, 1454, 1404, 1374, 1324, 1305, 1289, 1268, 1252, 1230, 1151, 1136, 1107, 1058, 1025, 882, 856, 838, 803, 758, 749, 709.

HRMS (m/z ESI $^+$): Found: 351.2028 ($\text{M}^+ + \text{H}$. $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_4$ Requires: 351.2032).

1-(5,6,7,8-tetrahydroquinolin-3-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (37d)

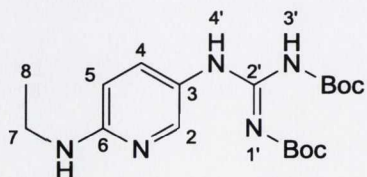
Following Method A, HgCl_2 (93 mg, 0.32 mmol) was added over a solution of 3-amino-5,6,7,8-tetrahydroquinoline (45 mg, 0.30 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (93 mg, 0.32 mmol), and triethylamine (148 μL , 1.06 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 2 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **37d** (103 mg, 87%) as a white powder. **Mp.** 124-125 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.50 (s, 9H, CH_3 , Boc), 1.55 (s, 9H, CH_3 , Boc), 1.77-1.83 (m, 2H, CH_2 , H-6), 1.86-1.92 (m, 2H, CH_2 , H-7), 2.80 (app. t, 2H, J 6.4, 6.1, CH_2 , H-5), 2.89 (app. t, 2H, J 6.4, CH_2 , H-8), 7.79 (d, 1H, J 2.0, H-4), 8.44 (d, 1H, J 2.0, H-2), 10.25 (broad s, 1H, NH, 3'), 11.63 (broad s, 1H, NH, 4').

δ_{C} (100 MHz, CDCl_3): 22.5 (CH_2 , 6), 23.1 (CH_2 , 7), 28.1 (CH_3 , Boc), 28.1 (CH_3 , Boc), 28.9 (CH_2 , 5), 32.0 (CH_2 , 8), 79.8 (q ^tBu , Boc), 84.0 (q ^tBu , Boc), 130.7 (CH Ar, 4), 131.1 (q Ar, 3), 132.4 (q Ar, 10), 140.8 (CH Ar, 2), 153.3 (q CO, Boc), 153.7 (q Ar, 9), 153.9 (q, 2'), 163.3 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3250 (NH), 2982 (NH), 2933, 1725 (C=O), 1634 (C=N), 1596, 1552, 1467, 1400, 1368, 1328, 1280, 1249, 1228, 1153, 1105, 1059, 1032, 943, 909, 877, 855, 808, 760, 721, 711.

HRMS (m/z ESI^+): Found: 391.2350 ($\text{M}^+ + \text{H}$. $\text{C}_{20}\text{H}_{31}\text{N}_4\text{O}_4$ Requires: 391.2345).

1-[6-(Ethylamino)pyridin-3-yl]-2,3-di(*tert*-butoxycarbonyl)guanidine (37e)

Following Method A, HgCl_2 (270 mg, 1.00 mmol) was added over a solution of 2-(*N*-ethylamino)-5-aminopyridine (130 mg, 0.95 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiurea (289 mg, 1.00 mmol), and triethylamine (462 μL , 3.32 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 3 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **37e** (327 mg, 91%) as a white, crystalline solid. **Mp.** 136-138 °C, clean melt.

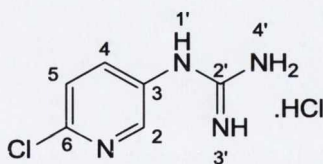
δ_{H} (400 MHz, CDCl_3): 1.27 (t, 3H, J 7.0, CH_3 , H-8), 1.50 (s, 9H, CH_3 Boc), 1.56 (s, 9H, CH_3 Boc), 3.31 (m, 2H, CH_2 , H-7), 4.44 (t, 1H, J 5.3, NH, NH₂Et), 6.40 (d, 1H, J 8.9, H-5), 7.77 (dd, 1H, J 8.9, 2.6, H-4), 8.15 (d, 1H, J 2.6, H-2), 10.03 (broad s, 1H, NH, H-3'), 11.65 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 14.9 (CH_3 , 8), 28.1 (CH_3 , Boc), 28.2 (CH_3 , Boc), 37.2 (CH_2 , 7), 79.5 (q ^{*t*}Bu, Boc), 83.6 (q ^{*t*}Bu, Boc), 106.5 (CH Ar, 5), 123.7 (q Ar, 3), 133.6 (CH Ar, 4), 142.7 (CH Ar, 2), 153.4 (q CO, Boc), 154.2 (q, 2'), 156.5 (q Ar, 6), 163.6 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3285 (NH), 3269 (NH), 3124 (NH), 2976, 2932, 1792, 1722 (C=O), 1605 (C=N), 1561, 1517, 1475, 1448, 1389, 1325, 1280, 1246, 1227, 1150, 1122, 1105, 1059, 954, 883, 843, 814, 763.

HRMS (m/z ESI⁺): Found: 380.2291 ($\text{M}^+ + \text{H}$, $\text{C}_{18}\text{H}_{30}\text{N}_5\text{O}_4$ Requires: 380.2298).

1-(6-Chloropyridin-3-yl)guanidine hydrochloride (**38a**)



Following Method F 4M HCl/dioxane (12.0 eq., 3.64 mL, 14.57 mmol) was added to **37a** (1.0 eq., 450 mg, 1.21 mmol) in 50% ^{*i*}ProH/ CH_2Cl_2 (2.43 mL). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase.

Compound **38a** (231 mg, 92%) was obtained as a white solid. **M.p.** 190-196 °C, clean melt.

δ_{H} (600 MHz, DMSO- D_6): 7.59 (d, 1H, J 8.5, H-5), 7.73 (broad s, 4H, H-3' and H-4'), 7.76 (dd, 1H, J 8.5, 2.5, H-4), 8.33 (d, 1H, J 2.5, H-2), 10.16 (broad s, 1H, H-1').

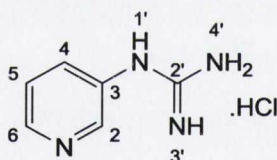
δ_{C} (150 MHz, DMSO- D_6): 125.0 (CH Ar, 5), 132.0 (q Ar, 3), 136.4 (CH Ar, 4), 146.3 (CH Ar, 6), 147.4 (q Ar, 2), 156.3 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3278 (NH), 3092 (NH), 3050 (NH), 2839, 2163, 2012, 1910, 1667 (C=N), 1632, 1603, 1584, 1454, 1417, 1362, 1287, 1266, 1132, 1106, 1029, 1010, 934, 873, 833, 745, 707.

HRMS (m/z ESI $^+$): Found: 171.0433 ($\text{M}^+ + \text{H}$. $\text{C}_6\text{H}_8\text{N}_4^{35}\text{Cl}$ Requires: 171.0437).

Purity by HPLC: 96.0% (t_{R} 7.43 min).

1-(Pyridin-3-yl)guanidine hydrochloride (**38b**)



Following Method E, a 25% v/v solution of TFA in CH_2Cl_2 (5.4 mL, 16 mmol TFA) was added to **37b** (130 mg, 0.39 mmol). Stirring at RT was continued for 3 h, when solvent and excess TFA were removed under vacuum to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H_2O (5 mL) and Amberlite in its chloride activated form (3.5 g) was added. Stirring at RT was continued for 36 h after which the Amberlite was removed by filtration and removal of H_2O under reduced pressure yielded the crude hydrochloride salt. ^{19}F NMR spectroscopy confirmed the absence of fluorinated compounds and the salt was purified using reverse phase silica chromatography (C-8 silica) with 100% H_2O as mobile phase. Removal of solvent yielded **38b** (57 mg, 85%) as a white, crystalline solid. **M.p.** 65-67 °C, clean melt.

δ_{H} (600 MHz, DMSO- D_6): 7.70 (m, 1H, H-5), 7.80 (broad s, 4H, H-3' and H-4'), 7.95 (d, 1H, J 7.8, H-4), 8.62 (s, 1H, H-2), 8.68 (s, 1H, H-6), 10.33 (broad s, 1H, H-1').

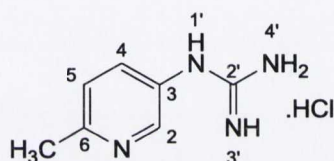
δ_{C} (150 MHz, DMSO-D6): 125.5 (CH Ar, 5), 133.6 (q Ar, 3), 135.2 (CH Ar, 4), 143.2 (CH Ar, 6), 144.4 (CH Ar, 2), 156.2 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3237 (NH), 3115 (NH), 3057 (NH), 2587, 2390, 2331, 2164, 2032, 1658 (C=N), 1570, 1507, 1478, 1424, 1385, 1261, 1179, 1131, 1047, 1013, 933, 838, 810, 721, 687.

HRMS (m/z ESI⁺): Found: 137.0829 ($M^+ + H$. C₆H₉N₄ Requires: 137.0827).

Purity by HPLC: 99.2% (t_R 2.19 min).

1-(6-Methylpyridin-3-yl)guanidine hydrochloride (**38c**)



Following Method F 4M HCl/dioxane (12.0 eq., 3.43 mL, 13.70 mmol) was added to **37c** (1.0 eq., 400 mg, 1.14 mmol) in 50% ⁱPrOH/CH₂Cl₂ (2.30 mL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **38c** (194 mg, 82%) was obtained as a white solid. **M.p.** 171-172 °C, clean melt.

δ_{H} (600 MHz, DMSO-D6): 2.73 (s, 3H, CH₃), 7.86 (d, 1H, J 8.7, H-5), 8.00 (broad s, 4H, H-3' and H-4'), 8.23 (dd, 1H, J 8.7, 2.2, H-4), 8.72 (d, 1H, J 2.2, H-2), 10.69 (broad s, 1H, H-1').

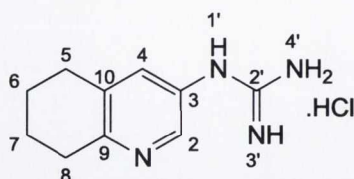
δ_{C} (150 MHz, DMSO-D6): 19.3 (CH₃), 127.6 (CH Ar, 5), 132.8 (q Ar, 3), 138.6 (CH Ar, 2), 140.1 (CH Ar, 4), 151.5 (q Ar, 6), 156.3 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3275 (NH), 2894, 1677 (C=N), 1622, 1603, 1565, 1491, 1456, 1376, 1288, 1244, 1107, 1025, 875, 832, 798, 739, 716.

HRMS (m/z ESI⁺): Found: 151.0980 ($M^+ + H$. C₇H₁₁N₄ Requires: 151.0984).

Purity by HPLC: 99.7% (*t*R 3.16 min).

1-(5,6,7,8-tetrahydroquinolin-3-yl)guanidine hydrochloride (38d)



Following Method F 4M HCl/dioxane (12.0 eq., 1.61 mL, 6.45 mmol) was added to **37d** (1.0 eq., 210 mg, 0.54 mmol) in 50% ¹PrOH/CH₂Cl₂ (0.2 mL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **38d** (106 mg, 87%) was obtained as a white solid. **M.p.** 85-88 °C, clean melt.

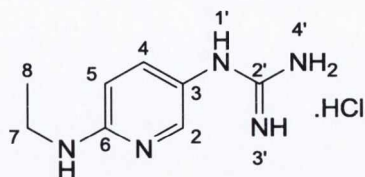
δ_H (600 MHz, DMSO-D₆): 1.78 (app. t, 2H, CH₂, J 5.9, 5.5, H-6), 1.86 (app. t, 2H, CH₂, J 5.9, 5.5, H-7), 2.85 (t, 2H, CH₂, J 6.1, H-5), 2.97 (app. t, 2H, CH₂, J 6.1, H-8), 7.76 (broad s, 4H, H-3' and H-4'), 7.85 (s, 1H, H-4), 8.48 (s, 1H, H-2), 10.25 (broad s, 1H, H-1').

δ_C (150 MHz, DMSO-D₆): 21.5 (CH₂, 6), 21.7 (CH₂, 7), 27.8 (CH₂, 5), 29.1 (CH₂, 8), 131.6 (q Ar, 10), 135.9 (q Ar, 3), 138.3 (CH Ar, 4), 139.3 (CH Ar, 2), 152.4 (q Ar, 9), 155.6 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3274 (NH), 2893, 1676 (C=N), 1620, 1598, 1563, 1490, 1376, 1325, 1289, 1244, 1083, 1025, 904, 876, 831, 737, 716, 653.

HRMS (*m/z* ESI⁺): Found: 191.1292 (M⁺ + H. C₁₀H₁₅N₄ Requires: 191.1297).

Purity by HPLC: 96.4% (*t*R 10.11 min).

1-[6-(*N*-ethylamino)pyridin-3-yl]guanidine hydrochloride (38e)

Following Method F 4M HCl/dioxane (12.0 eq., 1.98 mL, 7.91 mmol) was added to **37e** (1.0 eq., 250 mg, 0.66 mmol) in 50% ¹ProH/CH₂Cl₂ (1.32 mL). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **38e** (122 mg, 100%) was obtained as a white, crystalline solid. **M.p.** 120-124 °C, decomposition.

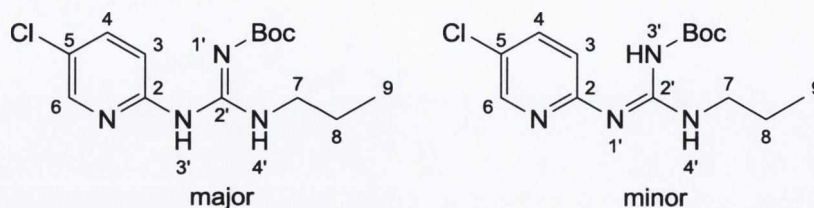
δ_{H} (600 MHz, DMSO-D₆): 1.22 (t, 3H, J 7.1, CH₃, H-8), 3.43 (q, 2H, J 7.1, CH₂, H-7), 7.13 (d, 1H, J 9.5, H-5), 7.67 (broad s, 4H, NH, H-3' and 4'), 7.75 (dd, 1H, J 1.9, 9.5, H-4), 7.91 (d, 1H, J 1.9, H-2), 9.17 (broad s, 1H, NH, NHet), 9.81 (broad s, 1H, NH, 1').

δ_{C} (150 MHz, DMSO-D₆): 14.0 (CH₃, 8), 37.3 (CH₂, 7), 114.6 (CH Ar, 5), 121.0 (q Ar, 3), 135.3 (CH Ar, 2), 142.4 (CH Ar, 4), 152.3 (q Ar, 6), 157.5 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3310, 3250 (NH), 3179 (NH), 3129 (NH), 3040 (NH), 2973, 2848, 2808, 2770, 1663 (C=N), 1605, 1582, 1502, 1439, 1376, 1336, 1302, 1273, 1230, 1166, 1103, 1055, 1021, 1005, 875, 858, 821, 781, 684.

HRMS (*m/z* ESI⁺): Found: 180.1246 (M⁺ + H. C₈H₁₄N₅ Requires: 180.1249).

Purity by HPLC: 96.3% (*t*R 2.41 min).

1-(5-Chloropyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(propyl)guanidine (39a)

Following Method A, HgCl_2 (222 mg, 0.82 mmol) was added over a solution of 2-amino-5-chloropyridine (100 mg, 0.78 mmol), thiourea **48d** (178 mg, 0.82 mmol), and triethylamine (379 μL , 2.72 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49j** (96 mg, 39%) as a colourless gum identified as a mixture of isomers. **M.p.** 79-82 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 1.00 (m, 3H, CH_3 , H-9), 1.54 (s, 9H, CH_3 , Boc), 1.65 (app. sex, 2H, J 7.3, CH_2 , H-8), 3.49 (m, 2H, CH_2 , H-7), 6.82 (d, 1H, J 8.8, H-3), 7.60 (dd, 1H, J 8.8, 2.3, H-4), 8.17 (d, 1H, J 2.3, H-6), 9.77 (broad s, 1H, NH, H-4'), 12.28 (broad s, 1H, NH, H-3').

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 1.00 (m, 3H, CH_3 , H-9), 1.53 (s, 9H, CH_3 , Boc), 1.65 (app. sex, 2H, J 7.3, CH_2 , H-8), 3.41 (broad s, 2H, CH_2 , H-7), 6.88 (broad s, 1H, H-3), 7.46 (d, 1H, J 6.9, H-4), 7.87 (broad s, 1H, NH, H-4'), 8.12 (broad s, 1H, H-6), 12.62 (broad s, 1H, NH, H-3').

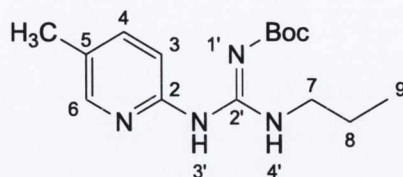
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 11.5 (CH_3 , 9), 22.5 (CH_2 , 8), 28.2 (CH_3 , Boc), 42.6 (CH_2 , 7), 78.8 (q ^tBu , Boc), 114.2 (CH Ar, 3), 122.8 (q Ar, 5), 138.4 (CH Ar, 4), 144.5 (CH Ar, 6), 151.4 (q Ar, 2), 157.2 (q, 2'), 164.4 (q C=O, Boc).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 11.6 (CH_3 , 9), 22.5 (CH_2 , 8), 28.4 (CH_3 , Boc), 42.6 (CH_2 , 7), 82.0 (q ^tBu , Boc), 121.8 (CH Ar, 3), 124.9 (q Ar, 5), 137.6 (CH Ar, 4), 143.8 (CH Ar, 6), 149.8 (q Ar, 2), 153.7 (q CO, Boc), 160.1 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3359 (NH), 2968, 1730 (C=O), 1645 (C=N), 1585, 1560, 1476, 1455, 1406, 1380, 1329, 1322, 1288, 1259, 1234, 1170, 1156, 1120, 1032, 980, 832, 799, 734, 689.

HRMS (m/z ESI⁺): Found: 335.1246 ($M^+ + Na$. $C_{14}H_{21}N_4O_2^{35}ClNa$ Requires: 335.1251).

1-(5-Methylpyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(propyl)guanidine (39b)



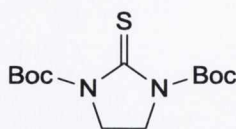
Following Method A, $HgCl_2$ (264 mg, 0.97 mmol) was added over a solution of 2-amino-5-methylpyridine (100 mg, 0.93 mmol), thiourea **48d** (212 mg, 0.97 mmol), and triethylamine (451 μ L, 3.24 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded a mixture of isomers which were separable on further silica gel chromatography to give **49k** (76 mg, 28%) as a white solid. **M.p.** 102–104 °C.

δ_H (600 MHz, $CDCl_3$): 0.98 (t, 3H, J 7.4, CH_3 , H-9), 1.51 (s, 9H, CH_3 , Boc), 1.62 (app. sex, 2H, J 7.6, 7.2, CH_2 , H-8), 2.24 (s, 3H, CH_3), 3.46 (m, 2H, CH_2 , H-7), 6.72 (d, 1H, J 8.4, H-3), 7.42 (dd, 1H, J 8.4, 2.1, H-4), 7.98 (d, 1H, J 2.1, H-6), 10.17 (broad s, 1H, NH, H-4'), 11.97 (broad s, 1H, NH, H-1').

δ_C (150 MHz, $CDCl_3$): 11.5 (CH_3 , 9), 17.5 (CH_3), 22.5 (CH_2 , 8), 28.4 (CH_3 , Boc), 42.4 (CH_2 , 7), 78.4 (q t Bu, Boc), 112.7 (CH Ar, 3), 126.7 (q Ar, 5), 139.2 (CH Ar, 4), 145.3 (CH Ar, 6), 150.9 (q Ar, 2), 157.5 (q CO, Boc), 164.2 (q, 2').

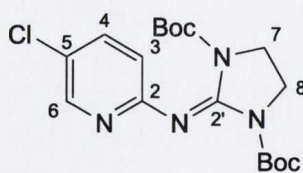
ν_{max} (ATR)/ cm^{-1} : 3355 (NH), 2965, 2930, 2875, 1712 (C=O), 1638 (C=N), 1597, 1562, 1495, 1474, 1347, 1300, 1245, 1172, 1154, 1125, 1056, 1026, 961, 909, 821, 805, 774, 739, 665.

HRMS (m/z ESI⁺): Found: 315.1792 ($M^+ + Na$. $C_{15}H_{24}N_4O_2Na$ Requires: 315.1797).

***N,N'*-di-(*tert*-butoxycarbonyl)imidazolidine-2-thione (41)**

To a cooled solution of imidazolidine-2-thione (4.21 g, 41.21 mmol) in dry tetrahydrofuran (40 mL) under argon was added a 60% suspension of NaH in mineral oil (4.5 eq., 7.42 g, 185.44 mmol). After 5 min, the ice-bath was removed and the reaction was stirred for 10 min at RT. The mixture was re-cooled to 0 °C and di-*tert*-butyldicarbonate (2.2 eq., 19.79 g, 90.66 mmol) was added. After 30 min, the ice-bath was removed and the reaction mixture was stirred for 16 h at RT. The reaction was quenched by the dropwise addition of saturated NaHCO₃ solution (25 mL), diluted with EtOAc (50 mL) and filtered through a bed of celite. The filtrate was extracted with EtOAc (3 × 40 mL) and the combined organic phases were washed with brine (20 mL) and H₂O (20 mL), dried over MgSO₄, filtered and concentrated under vacuum. The product, **41** (9.82 g, 79%), was purified by trituration in cold hexane to yield a yellow needle-like solid. **M.p.** 118-120 °C.¹⁹⁶

δ_{H} (400 MHz, CDCl₃): 1.52 (s, 18H, CH₃, Boc), 3.88 (s, 4H, CH₂).

1-(5-Chloropyridin-2'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (42a)

Following Method A, HgCl₂ (443 mg, 1.63 mmol) was added over a solution of 2-amino-5-chloropyridine (200 mg, 1.56 mmol), **41** (494 mg, 1.63 mmol), and triethylamine (759 μ L, 5.45 mmol) in CH₂Cl₂ (8 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 8 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **42a** (347 mg, 56%) as a colourless oil.

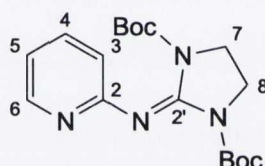
δ_{H} (400 MHz, CDCl₃): 1.38 (s, 18H, CH₃, Boc), 3.84 (s, 4H, CH₂, H-7 and H-8), 6.95 (d, 1H, J 8.7, H-3), 7.53 (dd, 1H, J 2.6, 8.7, H-4), 8.11 (d, 1H, J 2.6, H-6).

δ_{C} (100 MHz, CDCl_3): 27.8 (CH_3 , Boc), 43.0 (CH_2 , 7 and 8), 82.9 (q ^tBu , Boc), 119.4 (CH Ar, 3), 124.7 (q Ar, 5), 137.3 (CH Ar, 4), 141.8 (q Ar, 2), 145.9 (CH Ar, 6), 149.7 (q CO, Boc), 159.2 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 2980, 2933, 2251, 1765, 1712 ($\text{C}=\text{O}$), 1654 ($\text{C}=\text{N}$), 1579, 1548, 1460, 1367, 1291, 1251, 1146, 1039, 1007, 980, 910, 843, 769, 727.

HRMS (m/z ESI^+): Found: 419.1453 ($\text{M}^+ + \text{Na}$. $\text{C}_{18}\text{H}_{25}\text{N}_4\text{O}_4^{35}\text{ClNa}$ Requires: 419.1462).

1-(Pyridin-2'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (**42b**)



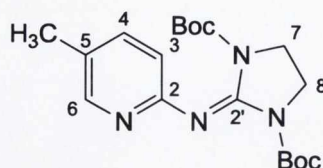
Following Method A, HgCl_2 (606 mg, 2.23 mmol) was added over a solution of 2-aminopyridine (200 mg, 2.13 mmol), **41** (675 mg, 2.23 mmol), and triethylamine (1037 μL , 7.44 mmol) in CH_2Cl_2 (11 mL) at 0 $^\circ\text{C}$. The mixture was stirred at 0 $^\circ\text{C}$ for 30 min and a further 6 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **42b** (431 mg, 56%) as a white, crystalline solid. **Mp.** 140 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.47 (s, 18H, CH_3 , Boc), 3.71 (s, 4H, CH_2 , H-7 and H-8), 6.81 (ddd, 1H, J 7.3, 5.0, 1.0, H-5), 7.54 (app. td, 1H, J 7.3, 2.0, H-4), 7.99 (broad s, 1H, H-3), 8.17 (dd, 1H, J 2.0, 5.0, H-6).

δ_{C} (100 MHz, CDCl_3): 28.2 (CH_3 , Boc), 45.1 (CH_2 , 7 and 8), 82.6 (q ^tBu , Boc), 113.1 (CH Ar, 3), 117.6 (CH Ar, 5), 137.7 (CH Ar, 4), 147.7 (CH Ar, 6), 149.8 (q Ar, 2), 150.3 (q CO, Boc), 152.3 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3266, 2981, 2934, 2884, 1805, 1696 ($\text{C}=\text{O}$), 1649 ($\text{C}=\text{N}$), 1576, 1545, 1479, 1462, 1437, 1376, 1365, 1329, 1306, 1254, 1232, 1151, 1117, 1088, 852, 841, 769, 760, 748, 677.

HRMS (m/z ESI^+): Found: 363.2026 ($\text{M}^+ + \text{H}$. $\text{C}_{18}\text{H}_{27}\text{N}_4\text{O}_4$ Requires: 363.2032).

1-(5-Methylpyridin-2'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (42c)

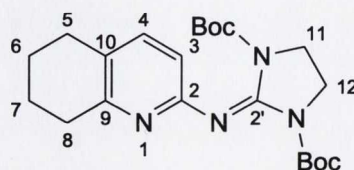
Following Method A, HgCl_2 (527 mg, 1.94 mmol) was added over a solution of 2-amino-5-methylpyridine (200 mg, 1.85 mmol), **41** (587 mg, 1.94 mmol), and triethylamine (902 μL , 6.47 mmol) in CH_2Cl_2 (9 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 15 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **42c** (523 mg, 75%) as a white, crystalline solid. **Mp.** 89-91 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.44 (s, 18H, CH_3 , Boc), 2.15 (s, 3H, CH_3), 3.68 (s, 4H, CH_2 , H-7 and H-8), 7.34 (dd, 1H, J 8.4, 2.0, H-4), 7.85 (broad s, 1H, H-3), 7.97 (d, 1H, H-6).

δ_{C} (100 MHz, CDCl_3): 17.6 (CH_3), 28.1 (CH_3 , Boc), 45.2 (CH_2 , 7 and 8), 82.4 (q ^tBu , Boc), 112.6 (CH Ar, 3), 126.6 (q Ar, 5), 138.4 (CH Ar, 4), 147.6 (CH Ar, 6), 149.8 (q CO, Boc), 150.3 (q Ar, 2), 152.3 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3265, 2980, 2933, 2885, 1804, 1697 (C=O), 1647 (C=N), 1602, 1577, 1543, 1480, 1467, 1365, 1330, 1307, 1288, 1253, 1232, 1149, 1115, 1026, 879, 851, 840, 767, 748, 740, 701, 684.

HRMS (m/z ESI^+): Found: 377.2186 ($\text{M}^+ + \text{H}$. $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}_4$ Requires: 377.2189).

1-(5,6,7,8-Tetrahydroquinolin-2'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (42d)

Following Method A, HgCl_2 (385 mg, 1.42 mmol) was added over a solution of 2-amino-5,6,7,8-tetrahydroquinoline (200 mg, 1.35 mmol), **41** (428 mg, 1.42 mmol), and triethylamine (658 μL , 4.72 mmol) in CH_2Cl_2 (7 mL) at 0 °C. The mixture was stirred

at 0 °C for 30 min and a further 6 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **42d** (455 mg, 81%) as a colourless, yellow oil.

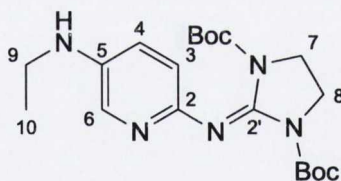
δ_{H} (400 MHz, CDCl_3): 1.44 (s, 18H, CH_3 , Boc), 1.71-1.80 (m, 4H, CH_2 , H-6 and H-7), 2.66 (app. t, 2H, J 5.9, 6.1, CH_2 , H-5), 2.72 (app. t, 2H, J 6.1, 6.3, CH_2 , H-8), 3.82 (s, 4H, CH_2 , H-11 and H-12), 6.75 (d, 1H, J 8.1, H-3), 7.26 (d, 1H, J 8.1, H-4).

δ_{C} (100 MHz, CDCl_3): 23.0 (CH_2 , 6), 23.2 (CH_2 , 7), 27.8 (CH_3 , Boc), 28.2 (CH_2 , 5), 32.5 (CH_2 , 8), 42.7 (CH_2 , 11 and 12), 82.3 (q ^tBu , Boc), 115.7 (CH Ar, 3), 125.5 (q Ar, 10), 138.4 (CH Ar, 4), 140.3 (q Ar, 9), 150.0 (q Ar, 2), 154.4 (q CO, Boc), 157.9 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 2977, 2932, 1752, 1702 (C=O), 1589, 1586, 1458, 1366, 1352, 1291, 1248, 1145, 1104, 1039, 995, 977, 936, 847, 833, 821, 767, 729, 696.

HRMS (m/z ESI $^{+}$): Found: 417.2494 ($\text{M}^{+} + \text{H}$, $\text{C}_{22}\text{H}_{33}\text{N}_4\text{O}_4$ Requires: 417.2502).

1-[5-(Ethylamino)pyridin-2'-yl]-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (42e**)**



Following Method A, HgCl_2 (104 mg, 0.38 mmol) was added over a solution of 2-amino-5-(ethylamino)pyridine (50 mg, 0.36 mmol), **41** (116 mg, 0.38 mmol), and triethylamine (178 μL , 1.28 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 5 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **42e** (52 mg, 35%) as red liquid.

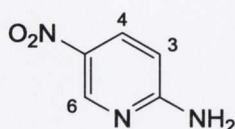
δ_{H} (400 MHz, CDCl_3): 1.21 (t, 3H, J 7.0, CH_3 , H-10), 1.52 (s, 18H, CH_3 , Boc), 3.11 (q, 2H, J 7.0, CH_2 , H-9), 3.75 (s, 4H, CH_2 , H-7 and H-8), 5.43 (broad s, 1H, NH), 6.93 (dd, 1H, J 8.9, 3.0, H-4), 7.69 (d, 1H, 8.9, H-6), 7.80 (d, 1H, J 3.0, H-3).

δ_{C} (100 MHz, CDCl_3): 14.8 (CH_3 , 10), 28.2 (CH_3 , Boc), 38.9 (CH_2 , 9), 45.3 (CH_2 , 7 and 8), 82.4 (q ^tBu , Boc), 114.3 (CH Ar, 3), 122.4 (CH Ar, 4), 132.8 (CH Ar, 6), 140.2 (q Ar, 5), 149.9 (q Ar, 2), 152.2 (q CO, Boc), 155.0 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3375 (NH), 2977, 2933, 1804, 1740, 1712, 1698 (C=O), 1676 (C=N), 1604, 1571, 1502, 1477, 1457, 1367, 1303, 1268, 1245, 1144, 1081, 973, 848, 834, 813, 765, 721, 686.

HRMS (m/z ESI⁺): Found: 406.2450 ($M^+ + H$. $C_{20}H_{32}N_5O_4$ Requires: 406.2454).

2-Amino-5-nitropyridine (**43**)



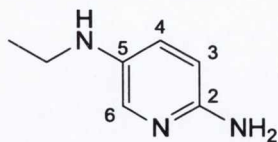
2-aminopyridine (700 mg, 7.44 mmol) was dissolved in H_2SO_4 (exc., 10 mL). Under stirring at 0 °C potassium nitrate (1.1 eq., 827 mg, 8.18 mmol,) was added. The mixture was stirred at RT for 16 h, after which it was cooled to 0 °C and quenched by adding dropwise concentrated ammonia solution until a pH of 12 was reached. The resulting precipitate was extracted with EtOAc (3 × 30 mL), washed with brine (20 mL) and water (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to yield a yellow/brown solid which was purified by silica gel column chromatography, eluting with 1:1 hexane:EtOAc. Removal of solvent yielded **43** (6447 mg, 63%) as a yellow solid. **M.p.** 183-184 °C, clean melt.

δ_H (400 MHz, $CDCl_3$): 6.50 (d, 1H, J 9.1, H-3), 7.55 (broad s, 2H, NH_2), 8.12 (dd, 1H, 9.1, 2.4, H-4), 8.84 (d, 1H, J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 107.2 (CH Ar, 3), 132.6 (CH Ar, 4), 134.3 (q Ar, 5), 147.0 (CH Ar, 6), 163.2 (q Ar, 2).

$\nu_{\max}$ (ATR)/ cm^{-1} : 3494, 3363 (NH), 3240, 3210 (NH), 3043, 2623, 1993, 1634, 1592, 1571, 1495, 1472, 1416, 1331, 1280, 1129, 999, 954, 864, 840, 763, 732.

HRMS (m/z ESI⁺): Found: 140.0467 ($M^+ + H$. $C_5H_6N_3O_2$ Requires: 140.0460).

2-Amino-5-(ethylamino)pyridine (44)

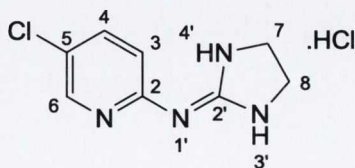
To a solution of 2,5-diaminopyridine **16** (250 mg, 2.29 mmol) in ethanol (11 mL) was added ethyl mesylate (1.00 eq., 236 μ L, 2.29 mmol). The mixture was stirred at 60 °C for 16 h. The product was extracted with a 20% solution of i PrOH/ CH_2Cl_2 (3×20 mL), washed with saturated NaHCO_3 (2×20 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure to yield a residue that was purified by silica gel column chromatography, eluting with 4:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$. Removal of solvent afforded **44** (149 mg, 47%) as a red liquid. The position of alkylation was confirmed by a NOE signal from H^3 to the NH_2 .

δ_{H} (400 MHz, CDCl_3): 1.11 (t, 3H, J 6.9, CH_3), 2.92 (q, 2H, J 6.9, CH_2), 4.66 (broad s, 1H, NH, NH^{Et}), 5.00 (broad s, 2H, NH_2), 6.34 (d, 1H, J 8.9, H-3), 6.82 (dd, 1H, 8.9, 3.0, H-4), 7.36 (d, 1H, J 3.0, H-6).

δ_{C} (100 MHz, CDCl_3): 14.6 (CH_3), 38.9 (CH_2), 108.8 (CH Ar, 3), 124.1 (CH Ar, 4), 131.5 (CH Ar, 6), 137.1 (q Ar, 5), 151.8 (q Ar, 2).

ν_{max} (ATR)/ cm^{-1} : 3314 (NH), 2968, 2871, 1625, 1578, 1496, 1392, 1279, 1240, 1144, 1100, 1059, 1015, 820.

HRMS (m/z ESI^+): Found: 138.1027 ($\text{M}^+ + \text{H}$, $\text{C}_7\text{H}_{12}\text{N}_3$ Requires: 138.1031).

1-(5-Chloropyridin-2'-yl)-2-iminoimidazolidine hydrochloride (45a)

Following Method F 4M HCl/dioxane (12.0 eq., 854 μ L, 3.42 mmol) was added to **42a** (1.0 eq., 113 mg, 0.29 mmol) in 50% i PrOH/ CH_2Cl_2 (570 μ L). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl

were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **45a** (64 mg, 96%) was obtained as a white solid. **M.p.** 95-96 °C, clean melt.

δ_{H} (600 MHz, DMSO- D_6): 3.70 (s, 4H, CH_2 , H-7 and H-8), 7.23 (d, 1H, J 8.8, H-3), 7.96 (dd, 1H, J 8.8, 2.7, H-4), 8.30 (d, 1H, J 2.7, H-6), 8.86 (broad s, 2H, NH, H-3' and H-4'), 12.31 (broad s, 1H, NH, H-1').

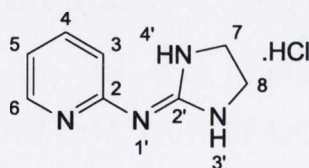
δ_{C} (150 MHz, DMSO- D_6): 42.5 (CH_2 , 7 and 8), 114.3 (CH Ar, 3), 125.7 (q Ar, 5), 139.1 (CH Ar, 4), 145.2 (CH Ar, 6), 149.5 (q Ar, 2), 155.5 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3295, 3230 (NH), 3165 (NH), 3112 (NH), 3035, 2951, 1694, 1640 (C=N), 1628, 1588, 1569, 1501, 1468, 1442, 1389, 1365, 1301, 1276, 1231, 1180, 1137, 1111, 1047, 1017, 959, 930, 875, 832, 757, 687.

HRMS (m/z ESI $^+$): Found: 197.0596 ($\text{M}^+ + \text{H}$. $\text{C}_8\text{H}_{10}\text{N}_4^{35}\text{Cl}$ Requires: 197.0594).

Purity by HPLC: 98.7% (t_{R} 2.36 min).

1-(Pyridin-2'-yl)-2-iminoimidazolidine hydrochloride (**45b**)



Following Method F 4M HCl/dioxane (12.0 eq., 1076 μL , 4.30 mmol) was added to **42b** (1.0 eq., 130 mg, 0.36 mmol) in 50% i ProH/ CH_2Cl_2 (717 μL). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **45b** (65 mg, 92%) was obtained as a white solid. **M.p.** 155-160 °C, decomposition.

δ_{H} (600 MHz, DMSO- D_6): 3.73 (s, 4H, CH_3 , H-7 and H-8), 7.12 (d, 1H, J 8.4, H-3), 7.20 (dd, 1H, J 7.0, 5.3, H-5), 7.87 (dt, 1H, J 7.0, 2.3, H-4), 8.33 (d, 1H, J 5.3, H-6), 8.90 (broad s, 2H, NH, H-3' and H-4'), 11.60 (broad s, 1H, NH, H-1').

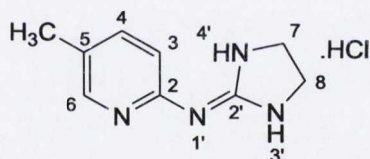
δ_{C} (150 MHz, DMSO- D_6): 42.4 (CH_2 , 7 and 8), 112.7 (CH Ar, 3), 119.6 (CH Ar, 5), 139.4 (CH Ar, 4), 147.1 (CH Ar, 6), 150.7 (q Ar, 2), 155.8 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3451, 3401, 3159 (NH), 3059 (NH), 2957, 2906, 2526, 2384, 2270, 1632 (C=N), 1594, 1476, 1448, 1426, 1366, 1344, 1279, 1247, 1199, 1158, 1102, 1053, 1017, 952, 932, 869, 772, 724, 693.

HRMS (m/z ESI $^{+}$): Found: 163.0979 ($\text{M}^{+} + \text{H}$. $\text{C}_8\text{H}_{11}\text{N}_4$ Requires: 163.0984).

Purity by HPLC: 97.2% (t_{R} 18.80 min).

1-(5-Methylpyridin-2'-yl)-2-iminoimidazolidine hydrochloride (45c)



Following Method F 4M HCl/dioxane (12.0 eq., 1195 μL , 4.78 mmol) was added to **42c** (1.0 eq., 150 mg, 0.40 mmol) in 50% $^i\text{PrOH}/\text{CH}_2\text{Cl}_2$ (797 μL). Stirring was continued for 3 h at 55 $^{\circ}\text{C}$ and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **45c** (79 mg, 93%) was obtained as a white solid. **M.p.** 140-145 $^{\circ}\text{C}$, decomposition.

δ_{H} (600 MHz, DMSO- D_6): 2.27 (s, 3H, CH_3), 3.71 (s, 4H, CH_2 , H-7 and H-8), 7.06 (d, 1H, J 8.3, H-3), 7.70 (dd, 1H, J 8.3, 2.0, H-4), 8.15 (d, 1H, J 2.0, H-6), 8.84 (broad s, 2H, NH, H-3' and H-4'), 11.76 (broad s, 2H, NH, H-1').

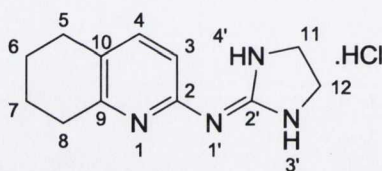
δ_{C} (150 MHz, DMSO- D_6): 17.2 (CH_3), 42.4 (CH_2 , 7 and 8), 112.3 (CH Ar, 3), 128.7 (q Ar, 5), 139.9 (CH Ar, 4), 146.6 (CH Ar, 6), 148.6 (q Ar, 2), 155.8 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3287 (NH), 3123 (NH), 2909, 2702, 2473, 2373, 2073, 1642 (C=N), 1599, 1578, 1486, 1454, 1373, 1297, 1280, 1257, 1238, 1222, 1141, 1077, 1032, 989, 935, 918, 875, 860, 831, 790, 741, 721.

HRMS (m/z ESI⁺): Found: 177.1145 ($M^+ + H$. $C_9H_{13}N_4$ Requires: 177.1140).

Purity by HPLC: 96.4% (t_R 22.31 min).

1-(5,6,7,8-Tetrahydroquinolin-2'-yl)-2-iminoimidazolidine hydrochloride (45d)



Following Method F 4M HCl/dioxane (12.0 eq., 3.24 mL, 12.97 mmol) was added to **42d** (1.0 eq., 450 mg, 1.08 mmol) in 50% ⁱPrOH/ CH_2Cl_2 (2.16 mL). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **45d** (254 mg, 93%) was obtained as a white solid. **M.p.** 208-212 °C, decomposition.

δ_H (600 MHz, DMSO- D_6): 1.72-1.76 (m, 2H, CH_2 , H-6), 1.79-1.83 (m, 2H, CH_2 , H-7), 2.69 (app. t, 2H, J 5.9, 6.3, H-5), 2.84 (app. t, 2H, J 6.1, 6.4, H-8), 3.73 (s, 4H, CH_2 , H-11 and H-12), 6.92 (d, 1H, J 8.2, H-3), 7.54 (d, 1H, J 8.2, H-4), 8.79 (broad s, 2H, NH, H-3' and H-4'), 11.87 (broad s, 1H, NH, H-1').

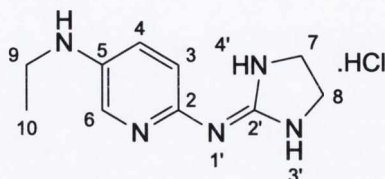
δ_C (150 MHz, DMSO- D_6): 22.2 (CH_2 , 6), 22.2 (CH_2 , 7), 27.3 (CH_2 , 5), 31.5 (CH_2 , 8), 42.5 (CH_2 , 11 and 12), 110.1 (CH Ar, 3), 127.5 (q Ar, 10), 139.9 (CH Ar, 4), 148.1 (q Ar, 9), 154.8 (q Ar, 2), 155.9 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3344, 3296, 3169 (NH), 3114 (NH), 3058 (NH), 2934, 2848, 1642 (C=N), 1595, 1583, 1474, 1464, 1415, 1377, 1351, 1309, 1286, 1264, 1204, 1156, 1137, 1111, 1089, 1066, 1018, 949, 938, 927, 896, 864, 835, 817, 759, 736.

HRMS (m/z ESI⁺): Found: 217.1458 ($M^+ + H$, C₁₂H₁₇N₄ Requires: 217.1453).

Purity by HPLC: 97.0% (t_R 26.65 min).

1-[5-(Ethylamino)pyridin-2'-yl]2-iminoimidazolidine hydrochloride (45e)



Following Method F 4M HCl/dioxane (12.0 eq., 252 μ L, 1.01 mmol) was added to **42e** (1.0 eq., 34 mg, 0.08 mmol) in 50% ⁱPrOH/CH₂Cl₂ (200 μ L). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 $\times$ 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **45e** (19 mg, 93%) was obtained as a colourless gum. **M.p.** Above 220 °C, decomposition.

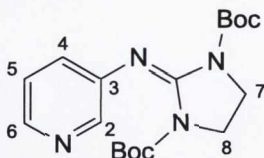
δ_H (400 MHz, DMSO-D₆): 1.22 (m, 3H, CH₃, H-10), 3.44 (m, 2H, CH₂, H-9), 3.64 (s, 4H, CH₂, H-7 and H-8), 7.16 (d, 1H, J 8.0, H-3), 7.77 (d, 1H, J 8.0, H-4), 7.91 (s, 1H, H-6), 8.58 (broad s, 2H, NH, H-3' and H-4'), 9.32 (s, 1H, NH, NH₂), 10.65 (broad s, 1H, NH, H-1').

δ_C (100 MHz, DMSO-D₆): 14.1 (CH₃, 10), 37.6 (CH₂, 9), 43.2 (CH₂, 7 and 8), 114.6 (CH Ar, 3), 122.0 (q Ar, 5), 134.1 (CH Ar, 4), 141.6 (CH Ar, 6), 152.2 (q Ar, 2), 159.4 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3375 (NH), 3198 (NH), 3057 (NH), 2860, 1675, 1619 (C=N), 1590, 1482, 1458, 1407, 1375, 1321, 1280, 1213, 1154, 1128, 1110, 1085, 1003, 938, 935, 785, 746, 717, 658.

HRMS (m/z ESI⁺): Found: 206.1401 ($M^+ + H$, C₁₀H₁₆N₅ Requires: 206.1406).

Purity by HPLC: 100.0% (t_R 5.07 min).

1-(Pyridin-3'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (46a)


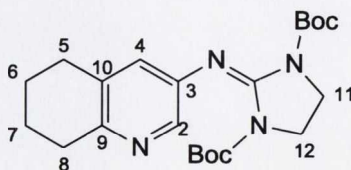
Following Method A, HgCl_2 (606 mg, 2.23 mmol) was added over a solution of 3-aminopyridine (200 mg, 2.13 mmol), **41** (675 mg, 2.23 mmol), and triethylamine (1037 μL , 7.44 mmol) in CH_2Cl_2 (11 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 5 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **46a** (670 mg, 87%) as an off-white crystalline solid. **Mp.** 99–101 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.11 (s, 18H, CH_3 , Boc), 3.62 (s, 4H, CH_2 , H-7 and H-8), 6.93 (dd, 1H, J 7.9, 4.6, H-5), 7.04 (d, 1H, J 7.9, H-4), 7.94 (d, 1H, J 3.9, H-6), 8.04 (broad s, 1H, H-2).

δ_{C} (100 MHz, CDCl_3): 27.2 (CH_3 , Boc), 42.7 (CH_2 , 7 and 8), 82.3 (q ^tBu , Boc), 122.6 (CH Ar, 5), 127.1 (CH Ar, 4), 140.5 (q Ar, 3), 142.5 (CH Ar, 2 or 6), 142.5 (CH Ar, 2 or 6), 144.5 (q CO, Boc), 149.1 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 2979, 2934, 1758, 1702 (C=O), 1670 (C=N), 1579, 1476, 1458, 1412, 1367, 1297, 1248, 1144, 1041, 1020, 977, 913, 846, 808, 767, 727, 709.

HRMS (m/z ESI^+): Found: 363.2033 ($\text{M}^+ + \text{H}$, $\text{C}_{18}\text{H}_{27}\text{N}_4\text{O}_4$ Requires: 363.2032).

1-(5,6,7,8-Tetrahydroquinolin-3'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (46b)


Following Method A, HgCl_2 (269 mg, 0.99 mmol) was added over a solution of 3-amino-5,6,7,8-tetrahydroquinoline **32** (140 mg, 0.95 mmol), **41** (300 mg, 0.99 mmol), and triethylamine (461 μL , 3.31 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was

stirred at 0 °C for 30 min and a further 4 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **46b** (335 mg, 85%) as a white solid. **Mp.** 128-131 °C, clean melt.

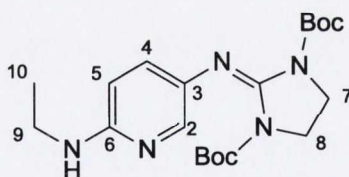
δ_{H} (400 MHz, CDCl_3): 1.21 (s, 18H, CH_3 , Boc), 1.60-1.66 (m, 2H, CH_2 , H-6), 1.68-1.75 (m, 2H, CH_2 , H-7), 2.57 (app. t, 2H, J 6.0, 6.3, CH_2 , H-5), 2.71 (app. t, 2H, J 6.0, 6.3, CH_2 , H-8), 3.71 (s, 4H, CH_2 , H-11 and H-12), 6.83 (d, 1H, J 2.4, H-4), 7.94 (d, 1H, J 2.4, H-2).

δ_{C} (100 MHz, CDCl_3): 22.7 (CH_2 , 6), 23.3 (CH_2 , 7), 27.8 (CH_3 , Boc), 28.7 (CH_2 , 5), 31.7 (CH_2 , 8), 43.1 (CH_2 , 11 and 12), 82.8 (q ^tBu , Boc), 128.1 (CH Ar, 4), 131.3 (q Ar, 10), 140.2 (q Ar, 3), 140.5 (CH Ar, 2), 142.2 (q Ar, 9), 149.9 (q CO, Boc), 150.6 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 2979, 2921, 2858, 1752, 1754, 1719(C=O), 1649 (C=N), 1594, 1560, 1465, 1368, 1333, 1293, 1257, 1233, 1202, 1187, 1107, 1037, 1005, 977, 947, 921, 844, 823, 786, 767, 746, 735, 712, 700, 682.

HRMS (m/z ESI^+): Found: 417.2496 ($\text{M}^+ + \text{H}$, $\text{C}_{22}\text{H}_{33}\text{N}_4\text{O}_4$ Requires: 417.2502).

1-(6-(Ethylamino)pyridin-3'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (46c)



Following Method A, HgCl_2 (561 mg, 2.07 mmol) was added over a solution of 2-(ethylamino)-5-aminopyridine **36** (270 mg, 1.97 mmol), **41** (621 mg, 2.07 mmol), and triethylamine (960 μL , 6.89 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 6 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **46c** (511 mg, 64%) as a white crystalline solid. **Mp.** 121-123 °C, clean melt.

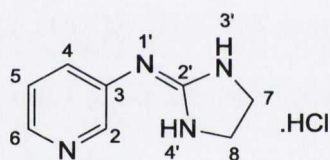
δ_{H} (400 MHz, CDCl_3): 0.98 (t, 3H, J 7.1, CH_3 , H-10), 1.14 (s, 18H, CH_3 , Boc), 3.04 (q, 2H, J 7.1, CH_2 , H-9), 3.59 (s, 4H, CH_2 , H-7 and H-8), 4.43 (broad s, 1H, NH), 6.12 (d, 1H, J 8.8, H-5), 6.95 (dd, 1H, 8.8, 2.6, H-4), 7.63 (dd, 1H, J 2.6, 0.3, H-2).

δ_{C} (100 MHz, CDCl_3): 14.7 (CH_3 , 10), 27.7 (CH_3 , Boc), 37.0 (CH_2 , 9), 43.0 (CH_2 , 7 and 8), 82.4 (q ^tBu , Boc), 106.1 (CH Ar, 5), 130.6 (CH Ar, 4), 135.2 (q Ar, 6), 139.0 (q Ar, 3), 141.0 (CH Ar, 2), 150.0 (q CO, Boc), 154.9 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3375 (NH), 2977, 2933, 1804, 1740, 1712 (C=O), 1698, 1676 (C=N), 1604, 1571, 1502, 1477, 1457, 1367, 1303, 1268, 1245, 1144, 1081, 973, 848, 834, 813, 765, 721, 686.

HRMS (m/z ESI^+): Found: 406.2445 ($\text{M}^+ + \text{H}$, $\text{C}_{20}\text{H}_{32}\text{N}_5\text{O}_4$ Requires: 406.2454).

1-(Pyridin-3'-yl)-2-iminoimidazolidine hydrochloride (**47a**)



Following Method F 4M HCl/dioxane (12.0 eq., 4.97 mL, 19.87 mmol) was added to **46a** (1.0 eq., 600 mg, 1.66 mmol) in 50% $^i\text{PrOH}/\text{CH}_2\text{Cl}_2$ (3.31 mL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **47a** (322 mg, 98%) was obtained as a white, crystalline solid. **M.p.** 112-115 °C, decomposition.

δ_{H} (400 MHz, $\text{DMSO}-d_6$): 3.69 (s, 4H, H-7 and H-8), 7.67 (app. t, 1H, J 6.6, H-5), 7.94 (d, 1H, J 8.3, H-4), 8.59 (d, 1H, J 4.4, H-6), 8.66 (s, 1H, H-2), 8.69 (broad s, 2H, NH, H-3' and H-4'), 11.08 (broad s, 1H, NH, H-1').

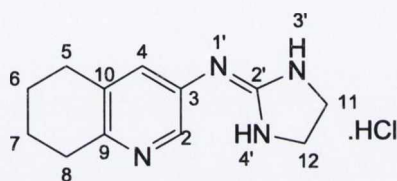
δ_{C} (150 MHz, $\text{DMSO}-d_6$): 42.7 (CH_2 , 7 and 8), 127.1 (CH Ar, 5), 137.4 (CH Ar, 4), 138.1 (CH Ar, 2), 140.1 (CH Ar, 6), 135.7 (q Ar, 3), 157.5 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3343 (NH), 3070 (NH), 3040 (NH), 2975, 2864, 2744, 2444, 2067, 1639 (C=N), 1608, 1556, 1514, 1468, 1420, 1366, 1339, 1328, 1284, 1252, 1190, 1089, 1046, 1021, 928, 885, 859, 815, 748, 671.

HRMS (m/z ESI⁺): Found: 163.0987 ($M^+ + H$. $C_8H_{11}N_4$ Requires: 163.0984).

Purity by HPLC: 99.2% (t_R 4.00 min).

1-(5,6,7,8-Tetrahydroquinolin-3'-yl)-2-iminoimidazolidine hydrochloride (47b)



Following Method F 4M HCl/dioxane (12.0 eq., 2.02 mL, 8.07 mmol) was added to **46b** (1.0 eq., 280 mg, 0.67 mmol) in 50% ¹ProH/CH₂Cl₂ (1.35 mL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **47b** (155 mg, 91%) was obtained as a white, crystalline solid. **M.p.** 122-126 °C, clean melt.

δ_H (600 MHz, DMSO-D₆): 1.79 (app. p, 2H, CH₂, H-6), 1.86 (app. p, 2H, CH₂, H-7), 2.87 (app. t, 2H, J 6.1, H-5), 3.01 (app. t, 2H, J 6.2, H-8), 3.70 (broad s, 4H, H-11 and H-12), 7.99 (s, 1H, H-4), 8.54 (s, 1H, H-2), 8.81 (broad s, 2H, NH, H-3' and H-4'), 11.35 (broad s, 1H, NH, H-1').

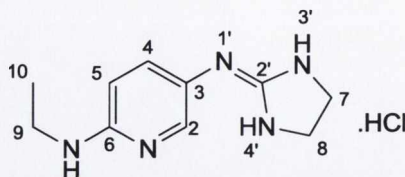
δ_C (150 MHz, DMSO-D₆): 21.3 (CH₂, 6), 21.4 (CH₂, 7), 27.7 (CH₂, 5), 28.3 (CH₂, 8), 43.0 (CH₂, 11 and 12), 132.7 (q Ar, 10), 136.7 (CH Ar, 2), 138.0 (CH Ar, 4), 151.3 (q Ar, 3), 158.1 (q, 2'), 164.6 (q Ar, 9).

$\nu_{\max}$ (ATR)/ cm^{-1} : 3418 (NH), 3108 (NH), 3020 (NH), 2955, 2932, 2898, 2868, 2812, 2418, 2035, 1622 (C=N), 1560, 1504, 1445, 1418, 1322, 1262, 1215, 1087, 1027, 1000, 947, 932, 898, 826, 779, 729, 688.

HRMS (m/z ESI⁺): Found: 217.1446 ($M^+ + H$. $C_{12}H_{17}N_4$ Requires: 217.1453).

Purity by HPLC: 96.1% (*t*R 17.20 min).

1-(6-(Ethylamino)pyridin-3'-yl)-2-iminoimidazolidine hydrochloride (47c)



Following Method F 4M HCl/dioxane (12.0 eq., 3.33 mL, 13.32 mmol) was added to **46c** (1.0 eq., 450 mg, 1.11 mmol) in 50% ⁱPrOH/CH₂Cl₂ (2.22 mL). Stirring was continued for 3 h at 55 °C and precipitation of a white solid was observed. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **47c** (256 mg, 95%) was obtained as a white, crystalline solid. **M.p.** Above 210 °C, decomposition.

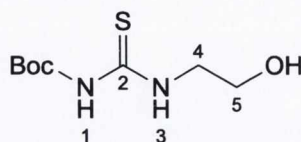
δ_H (600 MHz, DMSO-D₆): 1.22 (t, 3H, J 7.1, CH₃, H-10), 3.45 (q, 2H, CH₂, J 7.1, H-9), 3.65 (s, 4H, CH₂, H-7 and H-8), 7.15 (d, 1H, J 9.4, H-5), 7.76 (d, 1H, J 9.4, H-4), 7.92 (s, 1H, H-2), 8.55 (broad s, 2H, NH, H-3' and H-4'), 10.63 (broad s, 1H, NH, H-1').

δ_C (150 MHz, DMSO-D₆): 13.7 (CH₃, 10), 37.0 (CH₂, 9), 42.7 (CH₂, 7 and 8), 113.9 (CH Ar, 5), 121.5 (q Ar, 6), 133.8 (CH Ar, 2), 141.0 (CH Ar, 4), 151.9 (q Ar, 3), 159.0 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3199 (NH), 3057 (NH), 2861, 1674, 1619 (C=N), 1589, 1482, 1458, 1407, 1375, 1320, 1279, 1213, 1154, 1128, 1108, 1085, 1066, 1020, 1002, 989, 934, 835, 786, 745, 717, 657.

HRMS (*m/z* ESI⁺): Found: 206.1401 (M⁺ + H. C₁₀H₁₆N₅ Requires: 206.1406).

Purity by HPLC: 97.9% (*t*R 3.21 min).

***N*-(*tert*-Butoxycarbonyl)-*N'*-ethoxy thiourea (48a)**

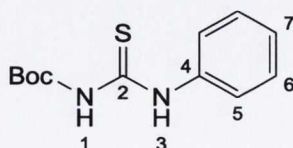
Following Method B thiourea (600 mg, 7.88 mmol) was dissolved in dry THF (60 mL) under argon at 0 °C and NaH (60% suspension in mineral oil, 4.5 eq., 851 mg, 35.47 mmol) was added. After 45 min stirring at RT and re-cooling to 0 °C di-*tert*-butyl dicarbonate (2.2 eq., 3785 mg, 17.34 mmol) was added and stirring continued at RT for 8 h. The mixture was cooled to 0 °C and further NaH (60% suspension in mineral oil, 1.7 eq., 322 mg, 13.40 mmol) was added. After 1 h trifluoroacetic anhydride (1.54 eq., 1.72 mL, 12.14 mmol) was added and the reaction was stirred for 1 h before ethanolamine (1.54 eq., 733 μ L, 12.14 mmol) was added and stirring was continued for 14 h. Usual workup and purification by trituration in cold hexane yielded **48a** (855 mg, 43%) as a white powder. **M.p.** 100-102 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, Boc CH_3), 2.82 (broad s, 1H, OH), 3.82-3.88 (m, 4H, H-4 and H-5), 8.36 (broad s, 1H, NH, H-3), 10.01 (broad s, 1H, NH, H-1).

δ_{C} (100 MHz, CDCl_3): 28.0 (CH_3 , Boc), 47.5 (CH_2 , 4), 60.7 (CH_2 , 5), 83.7 (q ^tBu , Boc), 151.9 (q CO, Boc), 180.3 (q 2).

ν_{max} (ATR)/ cm^{-1} : 3217 (NH), 2981, 2937, 2881, 1709 (C=O), 1559, 1526, 1449, 1388, 1373, 1364, 1327, 1251, 1148 (C=S), 1045, 1004.

HRMS (m/z ESI $^{+}$): Found: 243.0781 ($\text{M}^{+} + \text{Na}$. $\text{C}_8\text{H}_{16}\text{N}_2\text{O}_3\text{SNa}$ Requires: 243.0779).

***N*-(*tert*-Butoxycarbonyl)-*N'*-phenyl thiourea (48b)**

Following Method B thiourea (600 mg, 7.88 mmol) was dissolved in dry THF (60 mL) under argon at 0 °C and NaH (60% suspension in mineral oil, 4.5 eq., 851 mg, 35.47 mmol) was added. After 45 min stirring at RT and re-cooling to 0 °C di-*tert*-butyl

dicarbonate (2.2 eq., 3785 mg, 17.34 mmol) was added and stirring continued at RT for 8 h. The mixture was cooled to 0 °C and further NaH (60% suspension in mineral oil, 1.7 eq., 322 mg, 13.40 mmol) was added. After 1 h trifluoroacetic anhydride (1.54 eq., 1.72 mL, 12.14 mmol) was added and the reaction was stirred for 1 h before aniline (1.54 eq., 1.11 mL, 12.14 mmol) was added and stirring was continued for 14 h. Usual workup and purification by silica gel chromatography eluting with hexane:EtOAc yielded **48b** (1070 mg, 54%) as a white, crystalline solid. **M.p.** 105-107 °C, clean melt.

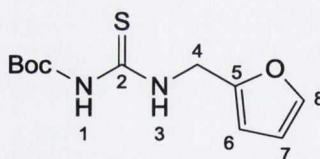
δ_{H} (400 MHz, CDCl_3): 1.56 (s, 9H, Boc CH_3), 7.28 (t, 1H, J 7.7, H-7), 7.42 (t, 2H, J 7.7, H-6), 7.67 (d, 1H, J 7.7, H-5), 8.10 (broad s, 1H, NH, H-3), 11.54 (broad s, 1H, NH, H-1).

δ_{C} (100 MHz, CDCl_3): 28.0 (CH_3 , Boc), 84.3 (q ^tBu , Boc), 124.3 (CH Ar, 7), 126.7 (CH Ar, 6), 128.8 (CH Ar, 5), 137.7 (q Ar, 4), 151.9 (q CO, Boc), 178.3 (q, 2).

ν_{max} (ATR)/ cm^{-1} : 3172 (NH), 3006 (NH), 2984, 1709 (C=O), 1591, 1528, 1477, 1451, 1391, 1366, 1321, 1252, 1196, 1143, 1075, 1048, 1016, 1004.

HRMS (m/z ESI^+): Found: 275.0828 ($\text{M}^+ + \text{Na}$. $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2\text{SNa}$ Requires: 275.0830).

N-(*tert*-Butoxycarbonyl)-*N'*-(2-furanylmethyl) thiourea (**48c**)



Following Method B thiourea (400 mg, 5.25 mmol) was dissolved in dry THF (40 mL) under argon at 0 °C and NaH (60% suspension in mineral oil, 4.5 eq., 567 mg, 23.65 mmol) was added. After 45 min stirring at RT and re-cooling to 0 °C di-*tert*-butyl dicarbonate (2.2 eq., 2523 mg, 11.56 mmol) was added and stirring continued at RT for 8 h. The mixture was cooled to 0 °C and further NaH (60% suspension in mineral oil, 1.7 eq., 215 mg, 8.93 mmol) was added. After 1 h trifluoroacetic anhydride (1.54 eq., 1.15 mL, 8.09 mmol) was added and the reaction was stirred for 1 h before furfurylamine (1.54 eq., 715 μL , 8.09 mmol) was added and stirring was continued for

14 h. Usual workup and purification by trituration in cold hexane yielded **48c** (764 mg, 58%) as a white powder. **M.p.** 99-100 °C, clean melt.

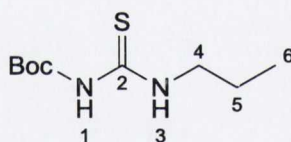
δ_{H} (400 MHz, CDCl_3): 1.51 (s, 9H, Boc CH_3), 4.88 (m, 2H, CH_2 , H-4), 6.37 (d, 2H, app. J 2.9, H-6 and H-7), 7.43 (s, 1H, H-8), 7.94 (broad t, 1H, J 5.4, NH, H-3), 9.66 (broad s, 1H, NH, H-1).

δ_{C} (100 MHz, CDCl_3): 27.5 (CH_3 , Boc), 42.0 (CH_2 , 4), 83.4 (q ^tBu , Boc), 108.1 (CH Ar, 6), 110.0 (CH Ar, 7), 142.2 (CH Ar, 8), 149.0 (q CO, Boc), 151.2 (q Ar, 5), 179.1 (q 2).

ν_{max} (ATR)/ cm^{-1} : 3279, 3251 (NH), 3174 (NH), 2983, 1711 (C=O), 1642, 1535, 1443, 1368, 1321, 1252, 1132 (C=S), 1011.

HRMS (m/z ESI $^+$): Found: 279.0793 ($\text{M}^+ + \text{Na}$. $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_3\text{SNa}$ Requires: 279.0779).

***N*-(*tert*-Butoxycarbonyl)-*N'*-propyl thiourea (**48d**)**



Following Method B thiourea (600 mg, 7.88 mmol) was dissolved in dry THF (60 mL) under argon at 0 °C and NaH (60% suspension in mineral oil, 4.5 eq., 851 mg, 35.47 mmol) was added. After 45 min stirring at RT and re-cooling to 0 °C di-*tert*-butyl dicarbonate (2.2 eq., 3785 mg, 17.34 mmol) was added and stirring continued at RT for 8 h. The mixture was cooled to 0 °C and further NaH (60% suspension in mineral oil, 1.7 eq., 322 mg, 13.40 mmol) was added. After 1 h trifluoroacetic anhydride (1.54 eq., 1.72 mL, 12.14 mmol) was added and the reaction was stirred for 1 h before *n*-propylamine (1.54 eq., 1.00 mL, 12.14 mmol) was added and stirring was continued for 14 h. Usual workup and purification by silica gel chromatography eluting with hexane:EtOAc yielded **48d** (1218 mg, 71%) as a white, crystalline solid. **M.p.** 58-60 °C, clean melt.

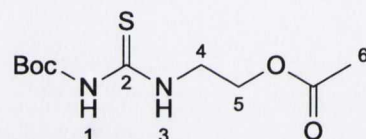
δ_{H} (400 MHz, CDCl_3): 1.01 (t, 3H, J 7.3, CH_2 , H-6), 1.52 (s, 9H, Boc CH_3), 1.71 (app. q, 2H, J 7.3, CH_2 , H-5), 3.63 (m, 2H, CH_2 , H-4), 7.97 (broad s, 1H, NH, H-3), 9.74 (broad s, 1H, NH, H-1).

δ_{C} (100 MHz, CDCl_3): 11.3 (CH_3 , 6), 21.5 (CH_2 , 5), 27.8 (CH_3 , Boc), 47.1 (CH_2 , 4), 83.4 (q ^tBu , Boc), 151.8 (q CO, Boc), 179.4 (q, 2).

ν_{max} (ATR)/ cm^{-1} : 3245 (NH), 3175 (NH), 2961, 2934, 2875, 1720 ($\text{C}=\text{O}$), 1523, 1243, 1206, 1142 ($\text{C}=\text{S}$), 1073, 1008.

HRMS (m/z ESI^+): Found: 219.1167 ($\text{M}^+ + \text{H}$. $\text{C}_9\text{H}_{19}\text{N}_2\text{O}_2\text{S}$ Requires: 219.1167).

***N*-(*tert*-Butoxycarbonyl)-*N'*-(ethylacetate) thiourea (**48e**)**



Over a solution of **48a** (600 mg, 2.73 mmol) in CH_2Cl_2 (6 mL) at 0 °C were added pyridine (3.0 eq., 658 μL , 8.18 mmol), acetic anhydride (1.5 eq., 386 μL , 4.09 mmol) and 4-(dimethylamino)pyridine (0.05 eq., 17 mg, 0.14 mmol). The mixture was stirred at RT for 3 h, after which NaHCO_3 (saturated, 15 mL) was added to quench the reaction. The mixture was stirred for 30 min and then extracted with Et_2O (3×20 mL). The combined organic layers were washed with 0.5 M HCl (15 mL), brine (15 mL) and water (15 mL). The organic extract was dried over MgSO_4 , filtered and concentrated under vacuum to yield **48e** (650 mg, 91%) as a white, crystalline solid. **M.p.** 95-97 °C, clean melt.

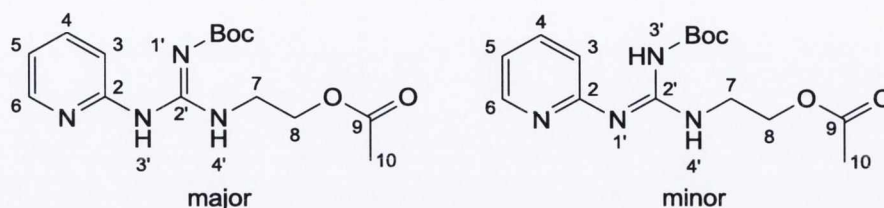
δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, Boc CH_3), 2.08 (s, 3H, CH_3 , H-6), 3.93 (q, 2H, J 5.1, CH_2 , H-4), 4.27 (t, 2H, J 5.1, CH_2 , H-5), 8.39 (broad s, 1H, NH, H-3), 9.93 (broad s, 1H, NH, H-1).

δ_{C} (100 MHz, CDCl_3): 20.4 (CH_3 , 6), 27.5 (CH_3 , Boc), 43.9 (CH_2 , 4), 61.6 (CH_2 , 5), 83.5 (q ^tBu , Boc), 151.3 (q CO, Boc), 170.4 (q CO, Ac), 179.8 (q 2).

ν_{max} (ATR)/ cm^{-1} : 3259 (NH), 3179 (NH), 2988, 2957, 1738, 1718 ($\text{C}=\text{O}$), 1558, 1526, 1391, 1365, 1329, 1240, 1195, 1133, 1050, 1039.

HRMS (m/z ESI⁺): Found: 285.0876 ($M^+ + Na$. C₁₀H₁₈N₂O₄SNa Requires: 285.0885).

1-(Pyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(ethylacetate)guanidine (49a)



Following Method A, HgCl₂ (454 mg, 1.67 mmol) was added over a solution of 2-aminopyridine (150 mg, 1.59 mmol), thiourea **48e** (439 mg, 1.67 mmol), and triethylamine (778 μ L, 5.58 mmol) in CH₂Cl₂ (8 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49a** (221 mg, 43%) as a colourless oil identified as a mixture of isomers.

δ_H (400 MHz, CDCl₃): *Major isomer*; 1.53 (s, 9H, CH₃, Boc), 2.10 (s, 3H, CH₃, H-10), 3.80 (t, 2H, J 5.4, CH₂, H-7), 4.26 (t, 2H, J 5.4, CH₂, H-8), 6.84 (d, 1H, J 8.3, H-3), 6.95 (dd, 1H, J 6.9, 5.5, H-5), 7.64 (app. td, 1H, J 7.8, 1.6, H-4), 8.18 (d, 1H, J 5.2, H-6), 10.46 (broad s, 1H, NH, H-4'), 12.09 (broad s, 1H, NH, H-3').

δ_H (400 MHz, CDCl₃): *Minor isomer*; 1.53 (s, 9H, CH₃, Boc), 2.08 (s, 3H, CH₃, H-10), 3.74 (m, 2H, CH₂, H-7), 4.27 (m, 2H, CH₂, H-8), 6.78 (t, 1H, J 6.0, H-3), 7.52 (t, 1H, J 7.1, H-5), 8.03 (broad s, 1H, NH, H-4'), 8.17 (m, 2H, H-4 and H-6), 13.17 (broad s, 1H, NH, H-3').

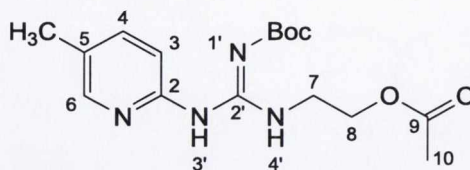
δ_C (100 MHz, CDCl₃): *Major isomer*; 20.8 (CH₃, 10), 28.4 (CH₃, Boc), 39.6 (CH₂, 7), 63.2 (CH₂, 8), 78.8 (q ^tBu, Boc), 113.3 (CH Ar, 3), 117.8 (CH Ar, 5), 138.5 (CH Ar, 4), 145.8 (CH Ar, 6), 152.9 (q Ar, 2), 157.7 (q, 2'), 164.1 (q CO, Boc), 170.8 (q CO, 9).

δ_C (100 MHz, CDCl₃): *Minor isomer*; 20.8 (CH₃, 10), 28.4 (CH₃, Boc), 78.7 (q ^tBu, Boc), 116.3 (CH Ar), 121.0 (CH Ar), 137.9 (CH Ar), 145.4 (CH Ar), 153.8 (q, 2'), not all quaternary carbons seen.

ν_{max} (ATR)/cm⁻¹: 2979, 1742 (C=O), 1642 (C=N), 1590, 1559, 1453, 1353, 1311, 1234, 1140, 1046.

HRMS (m/z ESI⁺): Found: 345.1537 ($M^+ + Na$. $C_{15}H_{22}N_4O_4Na$ Requires: 345.1539).

1-(5-Methylpyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(ethylacetate)guanidine (49b)



Following Method A, $HgCl_2$ (395 mg, 1.46 mmol) was added over a solution of 2-amino-5-methylpyridine (150 mg, 1.39 mmol), thiourea **48e** (382 mg, 1.46 mmol), and triethylamine (677 μ L, 4.86 mmol) in CH_2Cl_2 (7 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49b** (252 mg, 54%) as a colourless oil.

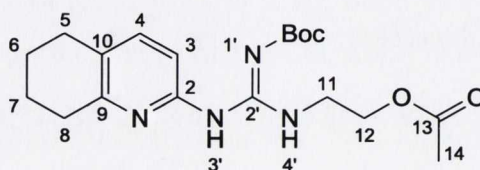
δ_H (400 MHz, $CDCl_3$): 1.56 (s, 9H, CH_3 , Boc), 2.13 (s, 3H, CH_3 , H-10), 2.31 (s, 3H, CH_3), 3.83 (app. q, 2H, J 5.4, 5.1, CH_2 , H-7), 4.29 (app. t, 2H, J 5.5, 5.3, CH_2 , H-8), 6.80 (d, 1H, J 8.4, H-3), 7.49 (dd, 1H, J 8.4, 2.2, H-4), 8.02 (d, 1H, J 2.2, H-6), 10.45 (broad s, 1H, NH, H-4'), 12.02 (broad s, 1H, NH, H-3').

δ_C (100 MHz, $CDCl_3$): 17.6 (CH_3), 20.9 (CH_3 , 10), 28.4 (CH_3 , Boc), 39.5 (CH_2 , 7), 63.3 (CH_2 , 8), 78.7 (q t Bu, Boc), 112.9 (CH Ar, 3), 127.2 (q Ar, 5), 139.4 (CH Ar, 4), 145.4 (CH Ar, 6), 150.8 (q Ar, 2), 157.8 (q, 2'), 164.1 (q CO, Boc), 170.9 (q CO, 9).

ν_{max} (ATR)/ cm^{-1} : 2926, 1742 (C=O), 1641 (C=N), 1602, 1496, 1476, 1352, 1301, 1232, 1172, 1137, 1046, 808, 741.

HRMS (m/z ESI⁺): Found: 337.1884 ($M^+ + H$. $C_{16}H_{25}N_4O_4$ Requires: 337.1876).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(Ethylacetate)guanidine (49c)



Following Method A, HgCl_2 (231 mg, 0.85 mmol) was added over a solution of 2-amino-5,6,7,8-tetrahydroquinoline (120 mg, 0.81 mmol), thiourea **48e** (223 mg, 0.85 mmol), and triethylamine (395 μL , 2.83 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49c** (210 mg, 69%) as a colourless oil.

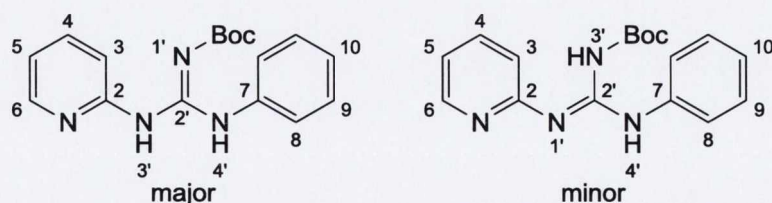
δ_{H} (400 MHz, CDCl_3): 1.52 (s, 9H, CH_3 , Boc), 2.10 (s, 3H, CH_3 , H-14), 1.75-1.82 (m, 2H, CH_2 , H-6 or H-7), 1.82-1.90 (m, 2H, CH_2 , H-6 or H-7), 2.67 (app. t, 2H, J 6.3, 6.2, CH_2 , H-5), 2.77 (app. t, 2H, J 6.5, 6.2, CH_2 , H-8), 3.81 (app. q, 2H, J 5.4, 5.4, 5.0, H-11), 4.24 (t, 2H, J 5.4, CH_2 , H-12), 6.59 (d, 1H, J 8.3, H-3), 7.30 (d, 1H, J 8.3, H-4), 10.72 (broad t, 1H, J 5.0, NH, H-4'), 11.90 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 20.9 (CH_3 , 14), 22.7 (CH_2 , 6), 22.7 (CH_2 , 7), 27.8 (CH_2 , 5), 28.4 (CH_3 , Boc), 32.2 (CH_2 , 8), 39.5 (CH_2 , 11), 63.7 (CH_2 , 12), 78.6 (q ^tBu , Boc), 110.6 (CH Ar, 3), 126.1 (q Ar, 10), 139.5 (CH Ar, 4), 150.2 (q Ar, 9), 153.4 (q Ar, 2), 157.9 (q, 2'), 164.0 (q CO, Boc), 170.7 (q CO, 13).

ν_{max} (ATR)/ cm^{-1} : 2936, 1743 (C=O), 1618 (C=N), 1592, 1459, 1335, 1236, 1172, 1138, 1046, 812, 736.

HRMS (m/z ESI $^+$): Found: 377.2197 ($\text{M}^+ + \text{H}$, $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}_4$ Requires: 377.2189).

1-(Pyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidine (**49d**)



Following Method A, HgCl_2 (121 mg, 0.45 mmol) was added over a solution of 2-aminopyridine (40 mg, 0.43 mmol), thiourea **48b** (113 mg, 0.45 mmol), and triethylamine (207 μL , 1.49 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography,

eluting with hexane:EtOAc, afforded **49d** (83 mg, 63%) as a colourless oil identified as a mixture of isomers.

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 1.52 (s, 9H, CH_3 , Boc), 6.93 (d, 1H, J 8.2, H-3), 7.03 (m, 1H, H-5), 7.11 (m, 1H, H-10), 7.36 (m, 2H, H-9), 7.67 (d, 2H, J 8.0, H-8), 7.72 (m, 1H, H-4), 8.27 (s, 1H, H-6), 12.27 (broad s, 1H, NH, H-4'), 12.63 (broad s, 1H, NH, H-3').

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 1.55 (s, 9H, CH_3 , Boc), 6.88 (m, 1H, H-3), 7.03 (m, 1H, H-5), 7.11 (m, 1H, H-10), 7.36 (m, 2H, H-9), 7.62 (m, 1H, H-4), 7.78 (d, 2H, H-8), 8.27 (s, 1H, H-6), 10.11 (broad s, 1H, NH, H-4'), 13.57 (broad s, 1H, NH, H-3').

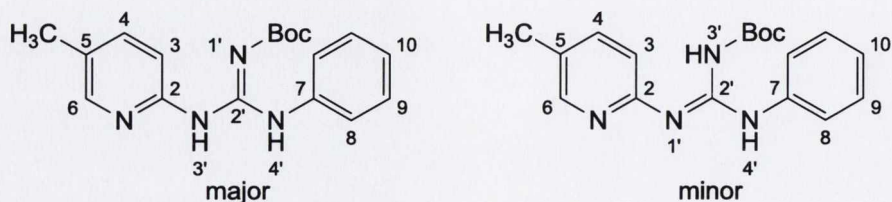
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 28.3 (CH_3 , Boc), 79.3 (q ^tBu , Boc), 113.7 (CH Ar, 3), 118.4 (CH Ar, 5), 122.4 (CH Ar, 8), 124.3 (CH Ar, 10), 129.1 (CH Ar, 9), 138.3 (q Ar, 7), 139.1 (CH Ar, 4), 145.9 (CH Ar, 6), 152.4 (q, 2'), 155.2 (q Ar, 2), 164.4 (q CO, Boc).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 28.3 (CH_3 , Boc), 82.8 (q ^tBu , Boc), 117.3 (CH Ar, 3), 120.8 (CH Ar, 8), 121.8 (CH Ar, 5), 123.3 (CH Ar, 10), 129.0 (CH Ar, 9), 137.5 (CH Ar, 4), 138.7 (q Ar, 7), 145.6 (CH Ar, 6), 146.4 (q, 2'), 154.0 (q CO, Boc), 160.5 (q Ar, 2).

ν_{max} (ATR)/ cm^{-1} : 2979, 1711 (C=O), 1646 (C=N), 1587, 1561, 1498, 1448, 1417, 1357, 1292, 1245, 1157, 1097, 1010, 809, 690, 752.

HRMS (m/z ESI $^+$): Found: 335.1494 ($\text{M}^+ + \text{Na}$. $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_2\text{Na}$ Requires: 335.1484).

1-(5-Methylpyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidine (**49e**)



Following Method A, HgCl_2 (264 mg, 0.97 mmol) was added over a solution of 2-amino-5-methylpyridine (100 mg, 0.93 mmol), thiourea **48b** (245 mg, 0.97 mmol), and triethylamine (452 μL , 3.24 mmol) in CH_2Cl_2 (5 mL) at 0 $^\circ\text{C}$. The mixture was stirred

at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49e** (269 mg, 89%) as a colourless oil identified as a mixture of isomers.

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 1.56 (s, 9H, CH_3 , Boc), 2.33 (s, 3H, CH_3), 6.87 (d, 1H, J 7.2, H-3), 7.12 (m, 1H, H-10), 7.32-7.39 (m, 2H, H-9), 7.53 (d, 1H, J 7.7, H-4), 7.69 (d, 2H, J 8.0, H-8), 8.11 (s, 1H, H-6), 12.21 (broad s, 1H, NH, H-4'), 12.41 (broad s, 1H, NH, H-3').

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 1.58 (s, 9H, CH_3 , Boc), 2.30 (s, 3H, CH_3), 7.02 (d, 1H, J 8.0, H-3), 7.08 (m, 1H, H-10), 7.32-7.39 (m, 2H, H-9), 7.44 (d, 1H, J 8.0, H-4), 7.81 (d, 2H, J 6.8, H-8), 8.11 (s, 1H, H-6), 10.00 (broad s, 1H, NH, H-4'), 13.36 (broad s, 1H, NH, H-3').

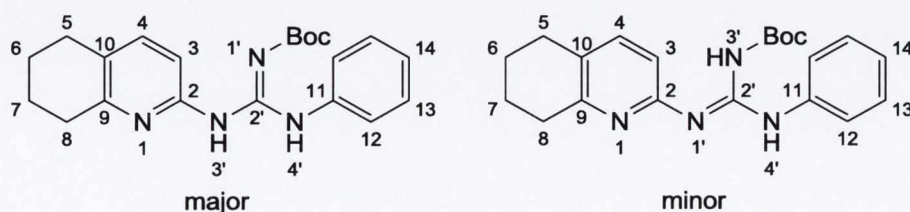
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 17.7 (CH_3), 28.4 (CH_3 , Boc), 78.9 (q ^tBu , Boc), 113.1 (CH Ar, 3), 129.0 (q Ar, 5), 122.4 (CH Ar, 8), 124.1 (CH Ar, 10), 127.5 (q Ar, 7), 128.8 (CH Ar, 9), 139.7 (CH Ar, 4), 145.5 (CH Ar, 6), 150.6 (q Ar, 2), 155.3 (q, 2'), 164.2 (q CO, Boc).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 17.8 (CH_3), 28.2 (CH_3 , Boc), 82.2 (q ^tBu , Boc), 121.1 (CH Ar, 3), 120.8 (CH Ar, 8), 126.2 (q Ar, 5), 123.0 (CH Ar, 10), 128.7 (CH Ar, 9), 137.9 (q Ar, 7), 138.9 (CH Ar, 4), 145.3 (CH Ar, 6), 145.7 (q, 2'), 153.9 (q CO, Boc), 158.6 (q Ar, 2).

ν_{max} (ATR)/ cm^{-1} : 2978, 1709 (C=O), 1643 (C=N), 1607, 1590, 1562, 1494, 1473, 1447, 1354, 1244, 1203, 1154, 1096, 1069, 1027, 985, 901, 808, 752, 690.

HRMS (m/z ESI^+): Found: 327.1828 ($\text{M}^+ + \text{H}$, $\text{C}_{18}\text{H}_{23}\text{N}_4\text{O}_2$ Requires: 327.1821).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidine (49f)



Following Method A, HgCl_2 (264 mg, 0.97 mmol) was added over a solution of 2-amino-5,6,7,8-tetrahydroquinoline (200 mg, 1.35 mmol), thiourea **48b** (358 mg, 1.42 mmol), and triethylamine (658 μL , 4.72 mmol) in CH_2Cl_2 (7 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49f** (439 mg, 88%) as a white solid identified as a mixture of isomers. **M.p.** 150-152 °C.

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 1.58 (s, 9H, CH_3 , Boc), 1.80-1.98 (m, 4H, CH_2 , H-6 and H-7), 2.74 (app. t, 2H, J 6.3, 5.8, CH_2 , H-5), 2.91 (app. t, 2H, J 6.4, 5.9, CH_2 , H-8), 6.69 (d, 1H, J 8.1, H-3), 7.10 (t, 1H, J 7.3, H-14), 7.36 (app. t, 2H, J 8.0, 7.3, H-13), 7.39 (d, 1H, J 8.1, H-4), 7.71 (d, 2H, J 8.0, H-12), 12.09 (broad s, 1H, NH, H-4'), 12.93 (broad s, 1H, NH, H-3').

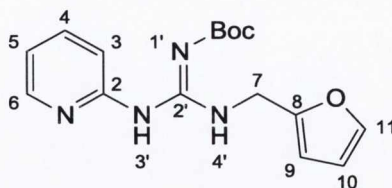
δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 1.58 (s, 9H, CH_3 , Boc), 1.80-1.98 (m, 4H, CH_2 , H-6 and H-7), 2.72 (m, 2H, CH_2 , H-5), 2.83 (t, 2H, J 6.0, CH_2 , H-8), 6.87 (d, 1H, J 8.0, H-3), 7.06 (t, 1H, J 7.1, H-14), 7.32 (m, 1H, H-4), 7.42 (app. t, 2H, J 8.2, 7.1, H-13), 7.80 (d, 1H, J 8.3, H-12), 9.89 (broad s, 1H, NH, H-4'), 14.05 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): *Major isomer*; 22.7 (CH_2 , 6), 22.7 (CH_2 , 7), 27.9 (CH_2 , 5), 28.4 (CH_3 , Boc), 32.1 (CH_2 , 8), 78.8 (q ^tBu , Boc), 110.8 (CH Ar, 3), 120.7 (q Ar, 10), 121.8 (CH Ar, 12), 123.8 (CH Ar, 14), 128.7 (q Ar, 7), 128.9 (CH Ar, 13), 139.8 (CH Ar, 4), 150.0 (q Ar, 9), 153.5 (q Ar, 2), 155.3 (q, 2'), 164.5 (q CO, Boc).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 23.0 (CH_2 , 6), 23.0 (CH_2 , 6), 28.1 (CH_2 , 5), 28.2 (CH_3 , Boc), 32.0 (CH_2 , 8), 81.9 (q ^tBu , Boc), 119.1 (CH Ar, 3), 120.5 (CH Ar, 12), 123.0 (CH Ar, 14), 126.5 (q Ar, 10), 129.3 (CH Ar, 13), 138.2 (q Ar, 7), 139.0 (CH Ar, 4), 149.8 (q, 2'), 152.9 (q CO, Boc), 154.4 (q Ar, 9), 155.6 (q Ar, 2).

ν_{max} (ATR)/ cm^{-1} : 2921, 2853, 1708 (C=O), 1687 (C=N), 1471, 1389, 1286, 716.

HRMS (m/z ESI^+): Found: 367.2140 ($\text{M}^+ + \text{H}$. $\text{C}_{21}\text{H}_{27}\text{N}_4\text{O}_2$ Requires: 367.2134).

1-(Pyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(2-furanylmethyl)guanidine (49g)

Following Method A, HgCl_2 (364 mg, 1.34 mmol) was added over a solution of 2-aminopyridine (120 mg, 1.28 mmol), thiourea **48c** (343 mg, 1.34 mmol), and triethylamine (622 μL , 4.46 mmol) in CH_2Cl_2 (6 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49g** (246 mg, 61%) as a white solid. **M.p.** 107–108 °C, clean melt.

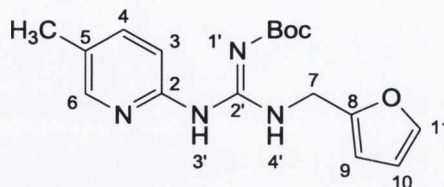
δ_{H} (400 MHz, CDCl_3): 1.57 (s, 9H, CH_3 , Boc), 4.77 (d, 1H, J 5.0, CH_2 , H-7), 6.31 (d, 1H, J 3.1, H-9), 6.35–6.38 (m, 1H, H-10), 6.87 (d, 1H, J 8.3, H-3), 6.96 (dd, 1H, J 7.2, 5.2, H-5), 7.42 (dd, 1H, J 1.7, 0.7, H-11), 7.65 (app. td, 1H, J 7.7, 1.8, H-4), 8.20 (dd, 1H, J 5.2, 1.2, H-6), 10.59 (broad s, 1H, NH, H-4'), 12.14 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 28.0 (CH_3 , Boc), 37.7 (CH_2 , 7), 78.4 (q ^tBu , Boc), 106.7 (CH Ar, 9), 109.9 (CH Ar, 10), 112.8 (CH Ar, 3), 117.3 (CH Ar, 5), 138.0 (CH Ar, 4), 141.7 (CH Ar, 11), 145.6 (CH Ar, 6), 151.0 (q Ar, 8), 152.4 (q Ar, 2), 156.9 (q, 2'), 163.6 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3062 (NH), 2976, 2926, 1646 (C=N), 1609 (C=N), 1596, 1562, 1508, 1480, 1446, 1419, 1385, 1350, 1336, 1263, 1250, 1235, 1220, 1170, 1157, 1149, 1121, 1101, 1086, 1060, 1017, 998, 967, 918, 879, 850, 802, 765, 755, 740, 696.

HRMS (m/z ESI $^+$): Found: 317.1622 ($\text{M}^+ + \text{H}$, $\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}_3$ Requires: 317.1614).

1-(5-Methylpyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(2-furanylmethyl)guanidine (49h)



Following Method A, HgCl_2 (290 mg, 1.07 mmol) was added over a solution of 2-amino-5-methylpyridine (110 mg, 1.02 mmol), thiourea **48c** (274 mg, 1.07 mmol), and triethylamine (496 μL , 3.56 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49h** (138 mg, 41%) as a white solid. **M.p.** 102–103 °C, clean melt.

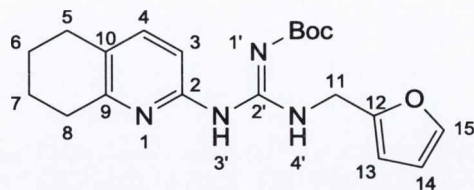
δ_{H} (400 MHz, CDCl_3): 1.57 (s, 9H, CH_3 , Boc), 2.28 (s, 3H, CH_3 , CH₃), 4.76 (d, 1H, J 4.8, CH_2 , H-7), 6.30 (d, 1H, J 3.1, H-9), 6.36 (m, 1H, H-10), 6.79 (d, 1H, J 8.4, H-3), 7.41 (s, 1H, H-11), 7.47 (d, 1H, J 8.4, H-4), 8.01 (s, 1H, H-6), 10.57 (broad s, 1H, NH, H-4'), 12.03 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 17.2 (CH_3), 28.0 (CH_3 , Boc), 37.7 (CH_2 , 7), 78.3 (q ^tBu , Boc), 106.6 (CH Ar, 9), 109.9 (CH Ar, 10), 112.3 (CH Ar, 3), 126.7 (q Ar, 5), 139.0 (CH Ar, 4), 141.6 (CH Ar, 11), 145.1 (CH Ar, 6), 150.3 (q Ar, 2), 151.1 (q Ar, 8), 157.0 (q, 2'), 163.7 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3005 (NH), 2963, 2981, 2929, 1640 (C=O), 1620 (C=N), 1599, 1566, 1506, 1493, 1482, 1427, 1386, 1348, 1336, 1299, 1262, 1252, 1236, 1214, 1167, 1153, 1122, 1085, 1059, 1036, 976, 964, 801, 772, 739, 698, 670.

HRMS (m/z ESI^+): Found: 331.1764 ($\text{M}^+ + \text{H}$, $\text{C}_{17}\text{H}_{23}\text{N}_4\text{O}_3$ Requires: 331.1770).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(2-furanylmethyl)guanidine (49i**)**



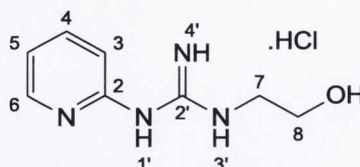
Following Method A, HgCl_2 (349 mg, 1.28 mmol) was added over a solution of 2-amino-5,6,7,8-tetrahydroquinoline (180 mg, 1.22 mmol), thiourea **48c** (329 mg, 1.28 mmol), and triethylamine (596 μL , 4.28 mmol) in CH_2Cl_2 (6 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and a further 16 h at RT. Workup and silica gel chromatography, eluting with hexane:EtOAc, afforded **49i** (294 mg, 65%) as a white solid. **M.p.** 79-82 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.56 (s, 9H, CH_3 , Boc), 1.74-1.87 (m, 4H, CH_2 , H-6 and H-7), 2.66 (m, 2H, CH_2 , H-5), 2.70 (m, 2H, CH_2 , H-8), 4.72 (d, 1H, J 4.6, CH_2 , H-11), 6.29 (d, 1H, J 2.6, H-13), 6.34-6.37 (m, 1H, H-14), 6.59 (d, 1H, J 8.2, H-3), 7.29 (d, 1H, J 8.2, H-4), 7.41 (d, 1H, J 0.9, H-15), 10.98 (broad s, 1H, NH, H-4'), 11.89 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 22.7 (CH_2 , 6), 22.8 (CH_2 , 7), 27.8 (CH_2 , 5), 28.5 (CH_3 , Boc), 31.9 (CH_2 , 8), 38.3 (CH_2 , 11), 78.6 (q ^tBu , Boc), 106.8 (CH Ar, 13), 110.4 (CH Ar, 3), 110.4 (CH Ar, 14), 126.2 (q Ar, 10), 139.4 (CH Ar, 4), 142.0 (CH Ar, 15), 150.2 (q Ar, 2), 151.5 (q Ar, 12), 153.5 (q Ar, 9), 157.4 (q, 2'), 164.0 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3119 (NH), 2979, 2938, 1713 (C=O), 1639 (C=N), 1614, 1597, 1585, 1561, 1507 1474, 1447, 1330, 1306, 1268, 1232, 117, 1092, 1007, 946, 884, 797, 778, 751, 734.

HRMS (m/z ESI $^+$): Found: 393.1893 ($\text{M}^+ + \text{Na}$. $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_3\text{Na}$ Requires: 393.1903).

1-(Pyridin-2-yl)-3-(ethoxy)guanidine hydrochloride (50a)

To **49a** (1.0 eq., 70 mg, 0.22 mmol) was added excess 1.25 M HCl/methanol (6.0 eq., 1.06 ml, 1.32 mmol). The resulting solution was stirred at 55 °C for 4 h. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50a** (39 mg, 83%) was obtained as a colourless gum. **M.p.** 140-143 °C, decomposition.

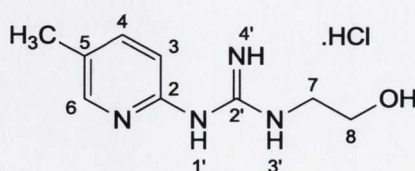
δ_{H} (400 MHz, DMSO-D₆): 3.44 (t, 2H, J 5.2, CH₂, H-8), 3.61 (t, 2H, J 5.2, CH₂, H-7), 5.19 (broad s, 1H, OH), 7.09 (d, 1H, J 8.3, H-3), 7.18 (m, 1H, H-5), 7.88 (ddd, 1H, J 8.3, 7.3, 1.1, H-4), 8.31 (dd, 1H, J 5.0, 1.1, H-6), 8.56 (broad s, 2H, NH, H-4'), 9.65 (broad s, 1H, NH, H-3'), 11.33 (broad s, 1H, NH, H-1').

δ_{C} (100 MHz, DMSO-D₆): 44.1 (CH₂, 8), 59.5 (CH₂, 7), 113.6 (CH Ar, 3), 119.5 (CH Ar, 5), 140.0 (CH Ar, 4), 146.8 (CH Ar, 6), 152.8 (q Ar, 2), 155.0 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3449 (OH), 3026 (NH), 1600, 1518, 1492, 1445, 1406, 1358, 1278, 1250, 1185, 1107, 1040, 899, 873, 797, 754, 709, 683.

HRMS (m/z ESI⁺): Found: 181.1094 (M⁺ + H. C₈H₁₃N₄O Requires: 181.1089).

Purity by HPLC: 96.4% (*t*R 18.11 min).

1-(5-Methylpyridin-2-yl)-3-(ethoxy)guanidine hydrochloride (50b)

To **49b** (1.0 eq., 70 mg, 0.21 mmol) was added excess 1.25 M HCl/methanol (6.0 eq., 1.01 mL, 1.26 mmol). The resulting solution was stirred at 55 °C for 4 h. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50b** (42 mg, 87%) was obtained as a colourless gum. **M.p.** 150-154 °C, decomposition.

δ_{H} (400 MHz, DMSO-D₆): 2.27(s, 3H, CH₃), 3.42 (t, 2H, J 5.0, CH₂, H-8), 3.60 (t, 2H, J 5.0, CH₂, H-7), 5.20 (broad s, 1H, OH), 7.01 (d, 1H, J 8.3, H-3), 7.72 (dd, 1H, J 8.3, 2.0, H-4), 8.14 (d, 1H, J 2.0, H-6), 8.53 (broad s, 2H, NH, H-4'), 9.59 (broad s, 1H, NH, H-3'), 11.41 (broad s, 1H, NH, H-1').

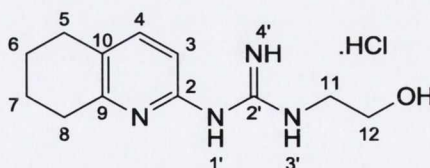
δ_{C} (100 MHz, DMSO-D₆): 17.7 (CH₃), 44.1 (CH₂, 8), 59.5 (CH₂, 7), 113.1 (CH Ar, 3), 128.7 (q Ar, 5), 140.7 (CH Ar, 4), 146.2 (CH Ar, 6), 150.6 (q Ar, 2), 154.9 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3352 (OH), 3234 (OH), 3169 (NH), 3001 (NH), 2917, 1670 (C=N), 1652, 1633, 1608, 1492, 1459, 1380, 1354, 1312, 1278, 1246, 1200, 1183, 1150, 1084, 1027, 996, 923, 887, 818, 762, 732, 718, 665.

HRMS (m/z ESI⁺): Found: 195.1245 (M⁺ + H. C₉H₁₅N₄O Requires: 195.1246).

Purity by HPLC: 98.2% (*t*R 21.83 min).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-3-(ethoxy)guanidine hydrochloride (**50c**)



To **49c** (1.0 eq., 80 mg, 0.21 mmol) was added excess 1.25 M HCl/methanol (6.0 eq., 1.01 mL, 1.26 mmol). The resulting solution was stirred at 55 °C for 3 h. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50c** (47 mg, 81%) was obtained as a white solid. **M.p.** 74-76 °C, clean melt.

δ_{H} (400 MHz, DMSO-D6): 1.70-1.82 (m, 4H, CH₂, H-6 and H-7), 2.67 (app. t, 2H, J 6.3, 5.9, CH₂, H-5), 2.76 (app. t, 2H, J 6.3, 6.0, CH₂, H-8), 3.43 (app. t, 2H, J 5.2, 4.9, CH₂, H-12), 3.61 (app. t, 2H, J 5.2, 4.9, CH₂, H-11), 5.21 (broad s, 1H, OH), 6.82 (d, 1H, J 8.2, H-3), 7.54 (d, 1H, J 8.2, H-4), 8.45 (broad s, 2H, H-4'), 10.10 (broad s, 1H, H-3'), 11.15 (broad s, 1H, H-1').

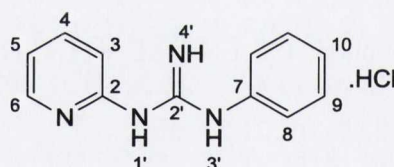
δ_{C} (100 MHz, DMSO-D6): 22.6 (CH₂, 6), 22.6 (CH₂, 7), 27.6 (CH₂, 5), 31.9 (CH₂, 8), 44.1 (CH₂, 12), 59.4 (CH₂, 11), 110.7 (CH Ar, 3), 127.5 (q Ar, 10), 140.6 (CH Ar, 4), 150.0 (q Ar, 2), 154.0 (q Ar, 9), 154.9 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3405 (OH), 3317 (OH), 2937, 1673 (C=N), 1593, 1646, 1493, 1253, 1052, 815, 749.

HRMS (m/z ESI⁺): Found: 235.1559 (M⁺ + H. C₁₂H₁₉N₄O Requires: 235.1559).

Purity by HPLC: 96.4% (t_{R} 26.89 min).

1-(Pyridin-2-yl)-3-(phenyl)guanidine hydrochloride (50d)



Following Method F 4M HCl/dioxane (6.0 eq., 408 μL , 1.63 mmol) was added to **49d** (1.0 eq., 85 mg, 0.27 mmol) in 50% ¹ProH/CH₂Cl₂ (952 μL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 $\times$ 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50d** (61 mg, 90%) was obtained as a colourless gum.

δ_{H} (400 MHz, DMSO-D6): 7.19 (d, 1H, J 8.2, H-3), 7.25 (dd, 1H, J 7.0, 5.2, H-5), 7.40 (m, 3H, H-8 and H-10), 7.53 (app. t, 2H, J 8.1, 7.4, H-9), 7.94 (app. td, 1H, 7.0, 1.6, H-4), 8.38 (d, 1H, J 5.2, H-6), 8.92 (broad s, 2H, NH, H-4'), 11.11 (broad s, 1H, NH, H-3'), 11.60 (broad s, 1H, NH, H-1').

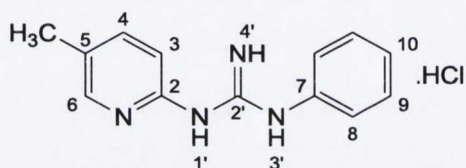
δ_C (100 MHz, DMSO-D6): 113.6 (CH Ar, 3), 119.7 (CH Ar, 5), 125.3 (CH Ar, 8), 127.4 (CH Ar, 10), 129.9 (CH Ar, 9), 134.1 (q Ar, 7), 139.8 (CH Ar, 4), 146.6 (CH Ar, 6), 151.9 (q Ar, 2), 153.5 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 3061 (NH), 2953, 1668 (C=N), 1639, 1594, 1569, 1485, 1423, 1373, 1231, 1152, 1075, 779, 758, 696.

HRMS (m/z ESI⁺): Found: 213.1145 ($M^+ + H$, C₁₂H₁₃N₄ Requires: 213.1140).

Purity by HPLC: 97.6% (t_R 24.21 min).

1-(5-Methylpyridin-2-yl)-3-(phenyl)guanidine hydrochloride (**50e**)



Following Method F 4M HCl/dioxane (6.0 eq., 368 μL , 1.47 mmol) was added to **49e** (1.0 eq., 80 mg, 0.25 mmol) in 50% ⁱProH/CH₂Cl₂ (858 μL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 $\times$ 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50e** (55 mg, 86%) was obtained as a colourless gum.

δ_H (400 MHz, DMSO-D6): 2.30 (s, 3H, CH₃), 7.10 (d, 1H, J 8.4, H-3), 7.37-7.41 (m, 3H, H-8 and H-10), 7.52 (app. t, 2H, J 8.0, 7.5, H-9), 7.78 (dd, 1H, J 8.4, 1.8, H-4), 8.21 (d, 1H, J 1.8, H-6), 8.93 (broad s, 2H, NH, H-4'), 11.43 (broad s, 2H, NH, H-1' and H-3').

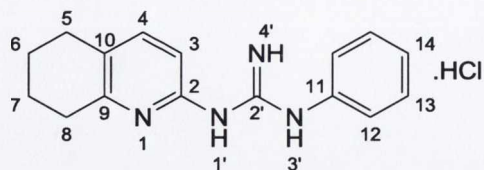
δ_C (100 MHz, DMSO-D6): 17.7 (CH₃), 113.7 (CH Ar, 3), 125.6 (CH Ar, 8), 127.7 (CH Ar, 10), 129.3 (q Ar, 5), 130.3 (CH Ar, 9), 134.8 (q Ar, 7), 140.9 (CH Ar, 4), 146.4 (CH Ar, 6), 150.3 (q Ar, 2), 153.9 (q, 2').

$\nu_{\max}$ (ATR)/ cm^{-1} : 2921, 1637 (C=N), 1608, 1595, 1572, 1483, 1369, 1281, 1233, 1140, 1029, 900, 895, 827, 752.

HRMS (m/z ESI⁺): Found: 227.1297 ($M^+ + H$, C₁₃H₁₅N₄ Requires: 227.1297).

Purity by HPLC: 98.9% (*t*R 27.09 min).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-3-(phenyl)guanidine hydrochloride (50f)



Following Method F 4M HCl/dioxane (6.0 eq., 205 μ L, 1.47 mmol) was added to **49f** (1.0 eq., 50 mg, 0.14 mmol) in 50% *i*ProH/ CH_2Cl_2 (478 μ L). Stirring was continued for 3 h at 55 $^\circ\text{C}$. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2 $\times$ 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50f** (35 mg, 86%) was obtained as a colourless gum. **M.p.** 180-183 $^\circ\text{C}$, decomposed.

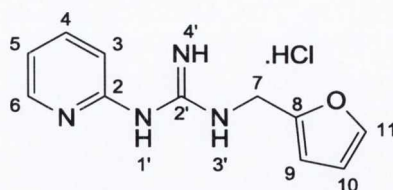
δ_{H} (600 MHz, DMSO- D_6): 1.75 (app. t, 2H, J 5.6, 5.1, CH_2 , H-6), 1.81 (app. t, 2H, J 5.8, 5.1, CH_2 , H-7), 2.71 (app. t, 2H, J 5.6, CH_2 , H-5), 2.79 (d, 2H, J 5.8, CH_2 , H-8), 6.92 (d, 1H, J 8.2, H-3), 7.35 (t, 1H, J 7.3, H-14), 7.39 (d, 2H, J 7.6, H-12), 7.50 (app. t, 2H, J 7.6, 7.3, H-13), 7.60 (d, 1H, J 8.2, H-4), 9.03 (broad s, 2H, H-4'), 11.40 (broad s, 1H, H-3'), 11.63 (broad s, 1H, H-1').

δ_{C} (150 MHz, DMSO- D_6): 22.4 (CH_2 , 6), 22.5 (CH_2 , 7), 27.5 (CH_2 , 5), 31.8 (CH_2 , 8), 111.6 (CH Ar, 3), 124.8 (CH Ar, 12), 127.1 (CH Ar, 14), 127.7 (q Ar, 10), 130.1 (CH Ar, 13), 140.6 (CH Ar, 4), 135.3 (q Ar, 11), 150.3 (q Ar, 2), 153.6 (q Ar, 9), 154.0 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3235 (NH), 2978, 2937, 1672, 1642 (C=N), 1595, 1578, 1499, 1467, 1376, 1319, 1282, 1234, 1121, 1079, 1047, 951, 835, 818, 755, 697, 664.

HRMS (m/z ESI^+): Found: 267.1605 ($\text{M}^+ + \text{H}$. $\text{C}_{16}\text{H}_{19}\text{N}_4$ Requires: 267.1610).

Purity by HPLC: 95.6% (*t*R 31.11 min).

1-(Pyridin-2-yl)-3-(2-furanylmethyl)guanidine hydrochloride (50g)

Following Method F 4M HCl/dioxane (6.0 eq., 379 μ L, 1.52 mmol) was added to **49g** (1.0 eq., 80 mg, 0.25 mmol) in 50% i PrOH/ CH_2Cl_2 (885 μ L). Stirring was continued for 3 h at 55 $^\circ\text{C}$. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50g** (52 mg, 82%) was obtained as a white solid. **M.p.** 142-144 $^\circ\text{C}$, decomposition.

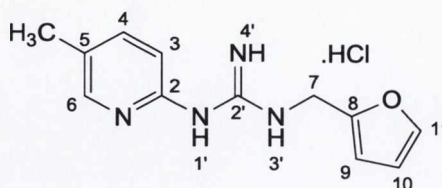
δ_{H} (400 MHz, DMSO- D_6): 4.67 (d, 2H, J 5.0, CH_2 , H-7), 6.46-6.51 (m, 2H, H-9 and H-10), 7.11 (d, 1H, J 8.2, H-3), 7.21 (dd, 1H, J 6.9, 5.2, H-5), 7.70 (dd, 1H, 1.6, 0.7, H-11), 7.90 (ddd, 1H, J 8.2, 6.9, 1.3, H-4), 8.33 (dd, 1H, J 5.2, 1.3, H-6), 8.86 (broad s, 2H, NH, H-4'), 9.71 (broad s, 1H, NH, H-3'), 11.32 (broad s, 1H, NH, H-1').

δ_{C} (100 MHz, DMSO- D_6): 38.2 (CH_2 , 7), 108.7 (CH Ar, 9 or 10), 111.1 (CH Ar, 9 or 10), 113.8 (CH Ar, 3), 119.9 (CH Ar, 5), 140.2 (CH Ar, 4), 143.7 (CH Ar, 11), 146.9 (CH Ar, 6), 150.0 (q Ar, 8), 152.5 (q Ar, 2), 154.6 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3092 (NH), 2990, 1674 (C=N), 1656, 1628, 1598, 1567, 1499, 1474, 1428, 1416, 1380, 1357 1343, 1323, 1285, 1265, 1200, 1154, 1143, 1132, 1074, 1046, 1018, 997, 929, 915, 902, 875, 834, 784, 773, 748, 715.

HRMS (m/z ESI $^+$): Found: 217.1090 ($\text{M}^+ + \text{H}$, $\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}$ Requires: 217.1089).

Purity by HPLC: 95.5% (t_{R} 24.81 min).

1-(5-Methylpyridin-2-yl)-3-(2-furanylmethyl)guanidine hydrochloride (50h)

Following Method F 4M HCl/dioxane (6.0 eq., 409 μ L, 1.64 mmol) was added to **49h** (1.0 eq., 90 mg, 0.27 mmol) in 50% i PrOH/ CH_2Cl_2 (885 μ L). Stirring was continued for 3 h at 55 $^\circ\text{C}$. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50h** (69 mg, 95%) was obtained as a white solid. **M.p.** 130-135 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, DMSO- D_6): 2.27 (s, 3H, CH_3), 4.66 (s, 2H, CH_2 , H-7), 6.46-6.49 (m, 2H, H-9 and H-10), 7.02 (d, 1H, J 8.3, H-3), 7.69 (d, 1H, J 0.6, H-11), 7.73 (dd, 1H, J 8.3, 2.0, H-4), 8.15 (d, 1H, J 2.0, H-6), 8.81 (broad s, 2H, NH, H-4'), 9.66 (broad s, 1H, NH, H-3'), 11.35 (broad s, 1H, NH, H-1').

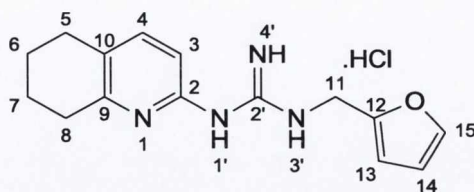
δ_{C} (100 MHz, DMSO- D_6): 17.7 (CH_3), 38.2 (CH_2 , 7), 108.7 (CH Ar, 9), 111.1 (CH Ar, 10), 113.4 (CH Ar, 3), 129.0 (q Ar, 5), 140.8 (CH Ar, 4), 143.6 (CH Ar, 11), 146.2 (CH Ar, 6), 150.0 (q Ar, 8), 150.5 (q Ar, 2), 154.6 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3102 (NH), 1664 (C=N), 1623, 1609, 1493, 1407, 1381, 1355, 1274, 1247, 1225, 1210, 1158, 1083, 1077, 1018, 925, 912, 882, 821, 771, 748, 669.

HRMS (m/z ESI $^+$): Found: 231.1241 ($\text{M}^+ + \text{H}$. $\text{C}_{12}\text{H}_{15}\text{N}_4\text{O}$ Requires: 231.1246).

Purity by HPLC: 98.4% (t_{R} 26.96 min).

1-(5,6,7,8-Tetrahydroquinolin-2-yl)-3-(2-furanylmethyl)guanidine hydrochloride (50i)



Following Method F 4M HCl/dioxane (6.0 eq., 243 μ L, 0.97 mmol) was added to **49i** (1.0 eq., 60 mg, 0.16 mmol) in 50% i PrOH/ CH_2Cl_2 (567 μ L). Stirring was continued for 3 h at 55 $^\circ\text{C}$. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2 $\times$ 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50i** (43 mg, 86%) was obtained as a white solid. **M.p.** 182-186 $^\circ\text{C}$, decomposition.

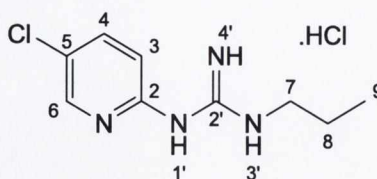
δ_{H} (400 MHz, DMSO- D_6): 1.69-1.82 (m, 4H, CH_2 , H-6 and H-7), 2.67 (app. t, 2H, J 6.0, 5.8, CH_2 , H-5), 2.73 (app. t, 2H, J 6.3, 5.8, CH_2 , H-8), 4.66 (s, 2H, CH_2 , H-11), 6.46-6.49 (m, 1H, H-14), 6.50 (d, 1H, J 2.8, H-13), 6.84 (d, 1H, J 8.2, H-3), 7.55 (d, 1H, J 8.2, H-4), 7.69 (s, 1H, H-15), 8.87 (broad s, 2H, H-4'), 9.97 (broad s, 1H, H-3'), 11.22 (broad s, 1H, H-1').

δ_{C} (100 MHz, DMSO- D_6): 22.5 (CH_2 , 6), 22.6 (CH_2 , 7), 27.6 (CH_2 , 5), 31.8 (CH_2 , 8), 38.3 (CH_2 , 11), 108.7 (CH Ar, 13), 111.0 (CH Ar, 3), 111.1 (CH Ar, 14), 127.8 (q Ar, 10), 140.74 (CH Ar, 4), 143.7 (CH Ar, 15), 149.9 (q Ar, 2), 150.0 (q Ar, 12), 154.0 (q Ar, 9), 154.6 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3150 (NH), 2938, 1643 (C=N), 1603, 1472, 1281, 1179, 1062, 1012, 802, 734.

HRMS (m/z ESI $^+$): Found: 271.1563 ($\text{M}^+ + \text{H}$, $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}$ Requires: 271.1559).

Purity by HPLC: 98.4% (t_{R} 30.64 min).

1-(5-Chloropyridin-2-yl)-3-(propyl)guanidine hydrochloride (50j)

Following Method F 4M HCl/dioxane (6.0 eq., 1.25 mL, 4.99 mmol) was added to **49j** (1.0 eq., 260 mg, 0.83 mmol) in 50% ¹ProH/CH₂Cl₂ (2.91 mL). Stirring was continued for 3 h at 55 °C. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH₂Cl₂ (2 × 2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50j** (193 mg, 93%) was obtained as a yellow, crystalline solid. **M.p.** 102-104 °C, clean melt.

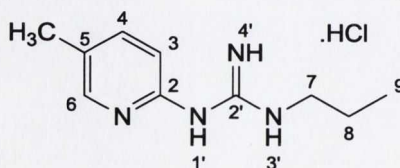
δ_H (600 MHz, DMSO-D₆): 0.94 (t, 3H, J 7.4, CH₃, H-9), 1.59 (app. sex, 2H, J 7.4, 7.2, CH₂, H-8), 3.29 (m, 2H, CH₂, H-7), 7.12 (s, 1H, H-3), 7.99 (dd, 1H, J 8.8, 2.5, H-4), 8.37 (d, 1H, J 2.5, H-6), 8.58 (broad s, 3H, NH, H-4'), 9.17 (broad s, 2H, NH, H-3'), 11.47 (broad s, 2H, NH, H-1').

δ_C (150 MHz, DMSO-D₆): 11.3 (CH₃, 9), 21.9 (CH₂, 8), 42.9 (CH₂, 7), 115.0 (CH Ar, 3), 125.7 (q Ar, 5), 139.7 (CH Ar, 4), 145.1 (CH Ar, 6), 151.0 (q Ar, 2), 154.1 (q, 2').

ν_{max} (ATR)/cm⁻¹: 3268 (NH), 3097 (NH), 3059, 2958, 2931, 2875, 1673, 1646 (C=N), 1629 (C=N), 1591, 1558, 1469, 1385, 1371, 1342, 1315, 1271, 1243, 1145, 1109, 1075, 1044, 1012, 966, 903, 869, 828, 772, 737, 658.

HRMS (*m/z* ESI⁺): Found: 211.0745 (M⁺ - H. C₉H₁₂N₄³⁵Cl Requires: 211.0750).

Purity by HPLC: 99.3% (*t*R 26.40 min).

1-(5-Methylpyridin-2-yl)-3-(propyl)guanidine hydrochloride (50k)

Following Method F 4M HCl/dioxane (6.0 eq., 282 μ L, 1.13 mmol) was added to **49k** (1.0 eq., 55 mg, 0.19 mmol) in 50% i PrOH/ CH_2Cl_2 (658 μ L). Stirring was continued for 3 h at 55 $^\circ\text{C}$. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of water. It was washed with CH_2Cl_2 (2×2 mL) and then purified by reverse phase chromatography (C-8 silica) with 100% water as mobile phase. Compound **50k** (39 mg, 90%) was obtained as a yellow, crystalline solid. **M.p.** 100-102 $^\circ\text{C}$, clean melt.

δ_{H} (600 MHz, DMSO- D_6): 0.94 (t, 3H, J 7.5, CH_3 , H-9), 1.58 (app. sex, 2H, J 7.5, 7.1, CH_2 , H-8), 2.25 (s, 3H, CH_3), 3.29 (m, 2H, CH_2 , H-7), 6.99 (broad s, 1H, H-3), 7.71 (dd, 1H, J 8.4, 1.9, H-4), 8.14 (d, 1H, J 1.9, H-6), 8.59 (broad s, 3H, NH, 4'), 9.43 (broad s, 2H, NH, 3'), 11.34 (broad s, 2H, NH, 1').

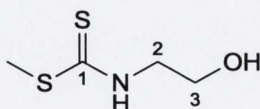
δ_{C} (150 MHz, DMSO- D_6): 11.0 (CH_3 , 10), 17.2 (CH_3 , 9), 21.6 (CH_2 , 8), 42.5 (CH_2 , 7), 112.6 (CH Ar, 3), 128.3 (q Ar, 5), 140.3 (CH Ar, 4), 145.8 (CH Ar, 6), 150.0 (q Ar, 2), 154.1 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 2965, 1674 (C=N), 1632, 1608, 1572, 1484, 1343, 1283, 1244, 1136, 1079, 1029, 968, 831, 743.

HRMS (m/z ESI^+): Found: 193.1461 ($\text{M}^+ + \text{H}$. $\text{C}_{10}\text{H}_{17}\text{N}_4$ Requires: 193.1453).

Purity by HPLC: 96.9% (t_{R} 24.57 min).

S-Methyl-N-ethoxy dithiocarbamate (**54a**)



Following Method C ethanolamine (1.0 eq., 198 μ L, 3.27 mmol) was added to a solution of carbon disulphide (1.1 eq., 217 μ L, 3.60 mmol) and triethylamine (1.1 eq., 502 μ L, 3.60 mmol) in CH_2Cl_2 (3 mL) at 0 $^\circ\text{C}$. After 15 min methyl iodide (1.1 eq., 224 μ L, 3.60 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup, followed by silica gel chromatography, eluting with hexane:EtOAc yielded **54a** (339 mg, 68%) as a colourless oil. The product, **54a**, was obtained as a mixture of isomers (7:2).

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 2.59 (s, 3H, CH_3), 3.77-3.91 (m, 4H, CH_2 , H-2 and H-3), 8.18 (broad s, 1H, NH).

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 2.64 (s, 3H, CH_3), 3.54-3.61 (m, 2H, CH_2 , H-3), 3.77-3.91 (m, 2H, CH_2 , H-2), 8.73 (broad s, 1H, NH).

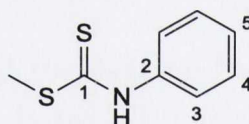
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 17.8 (CH_3), 48.8 (CH_2 , 3), 59.9 (CH_2 , 2), 199.5 (q, 1).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 18.5 (CH_3), 21.0 (CH_3 , 5), 48.0 (CH_2 , 3), 59.4 (CH_2 , 2), 201.8 (q, 1).

ν_{max} (ATR)/ cm^{-1} : 3227 (NH), 3009 (NH), 2919, 2880, 1504, 1423, 1382, 1326, 1305, 1266, 1213, 1108 (C=S), 1051, 1003, 870, 789.

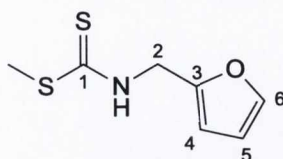
HRMS (m/z ESI⁺): Found: 150.0052 ($\text{M}^+ - \text{H}$, $\text{C}_4\text{H}_8\text{NOS}_2$ Requires: 150.0047).

S-Methyl-N-phenyl dithiocarbamate (54b)



Following Method C aniline (1.0 eq., 587 μL , 6.44 mmol) was added to a solution of carbon disulphide (1.1 eq., 428 μL , 7.09 mmol) and triethylamine (1.1 eq., 988 μL , 7.09 mmol) in CH_2Cl_2 (6 mL) at 0 °C. After 15 min methyl iodide (1.1 eq., 441 μL , 7.09 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup, followed by trituration in cold hexane yielded **54b** (803 mg, 68%) as a white, crystalline solid. **M.p.** 90-92 °C, clean melt, lit. 92-93 °C.²³⁴

δ_{H} (400 MHz, CDCl_3): 2.68 (s, 3H, CH_3), 7.35 (t, 1H, J 7.0, H-5), 7.42-7.49 (m, 4H, H-3 and H-4), 9.03 (broad s, 1H, NH).

S-Methyl-N-(2-furanylmethyl) dithiocarbamate (54c)

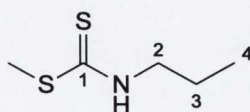
Following Method C furfurylamine (1.0 eq., 546 μL , 6.18 mmol) was added to a solution of carbon disulphide (1.1 eq., 410 μL , 6.80 mmol) and triethylamine (1.1 eq., 948 μL , 6.80 mmol) in CH_2Cl_2 (6 mL) at 0 $^\circ\text{C}$. After 15 min methyl iodide (1.1 eq., 423 μL , 6.80 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup, followed by silica gel chromatography, eluting with hexane:EtOAc, yielded **54c** (926 mg, 80%) as an off-white solid. **M.p.** 47–48 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, CDCl_3): 2.61 (s, 3H, CH_3), 4.89 (d, 2H, J 4.9, CH_2 , H-2), 6.32–6.35 (m, 2H, H-4 and H-5), 7.37 (s, 1H, H-6), 7.45 (broad s, 1H, NH).

δ_{C} (100 MHz, CDCl_3): 18.3 (CH_3), 43.8 (CH_2 , 2), 109.0 (CH Ar, 4 or 5), 110.7 (CH Ar, 4 or 5), 142.7 (CH Ar, 6), 149.3 (q Ar, 3), 199.2 (q, 1).

ν_{max} (ATR)/ cm^{-1} : 3221 (NH), 2996, 2919, 1641, 1591, 1493, 1422, 1367, 1321, 1304, 1269, 1190, 1146 (C=S), 1086, 1063, 1010, 927, 883, 864, 843, 816, 738, 694.

HRMS (m/z ESI $^-$): Found: 186.0041 ($\text{M}^- - \text{H}$, $\text{C}_7\text{H}_8\text{NOS}_2$ Requires: 186.0047).

S-Methyl-N-propyl dithiocarbamate (54d)

Following Method C *n*-propylamine (1.0 eq., 278 μL , 3.38 mmol) was added to a solution of carbon disulphide (1.1 eq., 225 μL , 3.72 mmol) and triethylamine (1.1 eq., 519 μL , 3.72 mmol) in CH_2Cl_2 (4 mL) at 0 $^\circ\text{C}$. After 15 min methyl iodide (1.1 eq., 232 μL , 3.72 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup, followed by silica gel chromatography, eluting with hexane:EtOAc, yielded **54d** (450 mg, 89%) as a colourless oil. The product, **54d**, was obtained as a mixture of isomers.

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 0.91-0.97 (m, 3H, CH_3 , H-4), 1.61-1.72 (m, 2H, CH_2 , H-3), 2.59 (s, 3H, CH_3), 3.63-3.68 (m, 2H, H-2), 7.31 (broad s, 1H, NH).

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 0.95-1.01 (m, 3H, CH_3 , H-4), 1.61-1.72 (m, 2H, CH_2 , H-3), 2.63 (s, 3H, CH_3), 3.37 (t, 2H, J 7.1, H-2), 8.21 (broad s, 1H, NH).

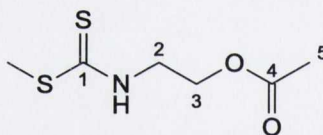
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 11.4 (CH_3 , 4), 18.1 (CH_3), 21.7 (CH_2 , 3), 49.0 (CH_2 , 2), 198.7 (q, 1).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 11.3 (CH_3 , 4), 18.8 (CH_3), 22.0 (CH_2 , 3), 48.2 (CH_2 , 2), 201.5 (q, 1).

ν_{max} (ATR)/ cm^{-1} : 3222 (NH), 2962, 2931, 2874, 1502, 1459, 1425, 1389, 1345, 1305, 1291, 1249, 1153 (C=S), 1062, 1002, 936, 883, 856, 771, 749, 727.

HRMS (m/z ESI): Found: 148.0259 ($\text{M}^- - \text{H}$, $\text{C}_5\text{H}_{10}\text{NS}_2$ Requires: 148.0255).

S-Methyl-N-(ethylacetate) dithiocarbamate (54e)



Methyl ethoxycarbamodithioate (1.0 eq., 1300 mg, 8.60 mmol), triethylamine (3.0 eq., 3.59 mL, 25.79 mmol) and acetic anhydride (1.1 eq., 892 μL , 9.46 mmol) were added to CH_2Cl_2 (21 mL) under stirring at 0 $^\circ\text{C}$. The reaction was warmed to RT and stirred for 2 h. At this point TLC showed no starting material and the reaction was diluted with CH_2Cl_2 (30 mL). The organic layer was separated, washed with brine (30 mL) and water (2×30 mL), dried over MgSO_4 and filtered. Removal of solvents under reduced pressure yielded a residue which was purified using silica-gel chromatography (hexane:EtOAc), affording **54e** (1609 mg, 97%) obtained as a colourless oil, in a mixture of isomers (85:15).

δ_{H} (400 MHz, CDCl_3): *Major isomer*; 2.06 (s, 3H, CH_3 , H-5), 2.59 (s, 3H, CH_3), 3.95-4.00 (m, 2H, CH_2 , H-2), 4.27-4.33 (m, 2H, CH_2 , H-3), 7.73 (broad s, 1H, NH).

δ_{H} (400 MHz, CDCl_3): *Minor isomer*; 2.06 (s, 3H, CH_3 , H-5), 2.60 (s, 3H, CH_3), 3.95-4.00 (m, 2H, CH_2 , H-2), 4.27-4.33 (m, 2H, CH_2 , H-3), 8.12 (broad s, 1H, NH).

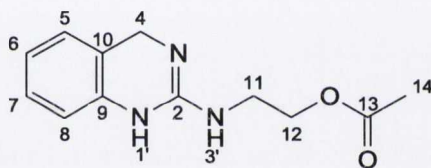
δ_{C} (100 MHz, CDCl_3): *Major isomer*; 18.1 (CH_3), 20.9 (CH_3 , 5), 46.2 (CH_2 , 3), 62.1 (CH_2 , 2), 171.3 (q, $\text{C}=\text{O}$), 199.8 (q, 1).

δ_{C} (100 MHz, CDCl_3): *Minor isomer*; 18.1 (CH_3), 21.0 (CH_3 , 5), 45.0 (CH_2 , 3), 61.7 (CH_2 , 2), 171.7 (q, $\text{C}=\text{O}$), 202.3 (q, 1).

ν_{max} (ATR)/ cm^{-1} : 3292 (NH), 2919, 1720 ($\text{C}=\text{O}$), 1508 ($\text{C}=\text{S}$), 1427, 1378, 1332, 1308, 1222, 1132, 1044, 950, 881, 808, 727.

HRMS (m/z ESI⁺): Found: 192.0146 ($\text{M}^+ - \text{H}$, $\text{C}_6\text{H}_{10}\text{NO}_2\text{S}_2$ Requires: 192.0153).

N-(Ethylacetate)-1,4-dihydroquinazolin-2-amine (**55a**)



Following Method D Cu(II)O (0.2 eq., 78 mg, 0.98 mmol) and K_2CO_3 (2.0 eq., 1349 mg, 9.76 mmol) were added to a solution of 2-aminobenzylamine (1.0 eq., 596 mg, 4.88 mmol) and **54e** (1.1 eq., 1037 mg, 5.37 mmol) in dimethylformamide (5 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification by silica gel chromatography, eluting with hexane:EtOAc, yielded **55a** (679 mg, 60%) as a white solid. **M.p.** 56-58 °C, clean melt.

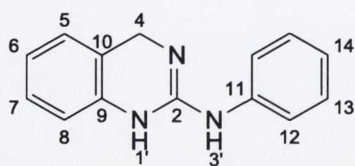
δ_{H} (400 MHz, CDCl_3): 1.94 (s, 3H, CH_3 , H-14), 3.65 (broad s, 2H, CH_2 , H-12), 4.07 (t, 2H, J 5.3, CH_2 , H-11), 4.22 (broad s, 1H, NH, H-3'), 4.54 (broad s, 2H, CH_2 , H-4), 6.61 (broad s, NH, H-1'), 6.64 (m, 1H, H-5), 6.71 (app. t, 1H, J 5.4, H-7), 6.96 (d, 1H, J 7.3, H-8), 7.06 (td, 1H, J 7.6, 1.3, H-6).

δ_{C} (100MHz, CDCl_3): 20.9 (CH_3 , 14), 43.5 (CH_2 , 12), 46.0 (CH_2 , 4), 63.1 (CH_2 , 11), 116.0 (CH Ar, 5), 121.4 (q Ar, 10), 118.4 (CH Ar, 7), 129.3 (CH Ar, 6), 130.5 (CH Ar, 8), 145.2 (q, 9), 171.5 (q CO, 13), 181.9 (q, 2).

$\nu_{\max}$ (ATR)/ cm^{-1} : 3376, 3185 (NH), 3025 (NH), 2956, 2926, 1720 (C=O), 1632 (C=N), 1607, 1586, 1545, 1494, 1458, 1445, 1382, 1361, 1335, 1315, 1282, 1253, 1216, 1163, 1145, 1085, 1065, 1044, 1023, 924, 866, 813, 790, 755, 715, 676.

HRMS: (m/z ESI^+): Found: 234.1239 ($\text{M}^+ + \text{H}$, $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_2$ Requires: 234.1243).

***N*-Phenyl-1,4-dihydroquinazolin-2-amine (55b)**



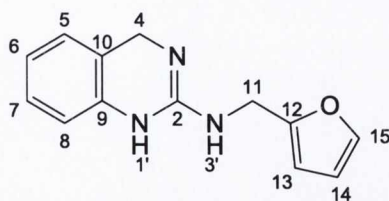
Following Method D Cu(II)O (0.2 eq., 9 mg, 0.12 mmol) and K_2CO_3 (2.0 eq., 163 mg, 1.18 mmol) were added to a solution of 2-aminobenzylamine (1.0 eq., 72 mg, 0.59 mmol) and **54b** (1.1 eq., 119 mg, 0.65 mmol) in dimethylformamide (4 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification by silica gel chromatography, eluting with hexane:EtOAc, yielded **55b** (100 mg, 76%) as an off-white solid. **M.p.** 127-128 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 4.87 (d, 2H, J 5.5, CH_2 , H-4), 6.25 (broad s, 1H, H-3'), 6.67 (m, 2H, H-5 and H-14), 7.04 (dd, J 7.7, 1.4, H-8), 7.13 (td, 1H, J 7.7, 1.4, H-6), 7.21 (dd, 2H, J 7.4, 1.1, H-12), 7.27 (t, 1H, J 7.7, H-7), 7.39 (t, 2H, J 7.4, H-13), 8.23 (broad s, 1H, H-1').

δ_{C} (100MHz, CDCl_3): 47.4 (CH_2 , 4), 115.8 (CH Ar, 5), 117.8 (CH Ar, 14), 120.5 (q Ar, 10), 125.1 (CH Ar, 12), 127.3 (CH Ar, 7), 129.5 (CH Ar, 6), 130.2 (CH Ar, 13), 130.6 (CH Ar, 8), 135.9 (q Ar, 11), 145.7 (q Ar, 9), 179.9 (q, 2).

$\nu_{\max}$ (ATR)/ cm^{-1} : 3469, 3358 (NH), 3168 (NH), 3005, 1619 (C=N), 1592, 1524, 1492 (C=N), 1455, 1425, 1352, 1315, 1299, 1266, 1242, 1197, 1175, 1112, 1071, 1028, 965, 746, 691.

HRMS: (m/z ESI^+): Found: 224.1186 ($\text{M}^+ + \text{H}$, $\text{C}_{14}\text{H}_{14}\text{N}_3$ Requires: 224.1188).

***N*-(2-Furanylmethyl)-1,4-dihydroquinazolin-2-amine (55c)**

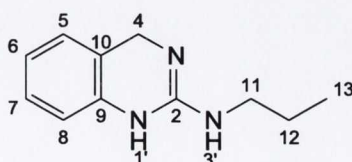
Following Method D Cu(II)O (0.2 eq., 16 mg, 0.20 mmol) and K₂CO₃ (2.0 eq., 272 mg, 1.96 mmol) were added to a solution of 2-aminobenzylamine (1.0 eq., 120 mg, 0.98 mmol) and **54c** (1.1 eq., 202 mg, 1.08 mmol) in dimethylformamide (2.5 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification by silica gel chromatography, eluting with hexane:EtOAc, yielded **55c** (142 mg, 63%) as a yellow solid. **M.p.** 69-71 °C, clean melt.

δ_{H} (400 MHz, CDCl₃): 4.57 (s, 2H, CH₂, H-4), 4.60 (s, 2H, CH₂, H-11), 6.23 (d, 1H, J 3.1, H-13), 6.30 (app. t, 1H, J 2.4, H-14), 6.32 (broad s, 1H, NH, H-1'), 6.62 (d, 1H, J 7.6, H-5), 6.68 (t, 1H, J 7.4, H-7), 6.73 (t, 1H, J 5.1, NH, H-3'), 6.94 (d, 1H, J 7.4, H-8), 7.10 (t, 1H, J 7.6, H-6), 7.32 (app. t, 1H, J 0.7, H-15).

δ_{C} (100MHz, CDCl₃): 41.4 (CH₂, 11), 46.2 (CH₂, 4), 108.1 (CH Ar, 13), 110.6 (CH Ar, 14), 116.1 (CH Ar, 5), 118.6 (CH Ar, 7), 121.6 (q Ar, 10), 129.4 (CH Ar, 6), 130.7 (CH Ar, 8), 142.3 (CH Ar, 15), 145.1 (q Ar, 9), 150.6 (q Ar, 12), 181.5 (q, 2).

ν_{max} (ATR)/cm⁻¹: 3256 (NH), 2937, 1673, 1633, 1609 (C=N), 1542, 1489, 1451, 1376, 1332, 1197, 1147, 1092, 1054, 1045, 1011, 884, 833, 751, 725, 669, 658.

HRMS: (m/z ESI⁺): Found: 228.1131 (M⁺ + H, C₁₃H₁₄N₃O Requires: 228.1137).

***N*-(*n*-Propyl)-1,4-dihydroquinazolin-2-amine (55d)**

Following Method D Cu(II)O (0.2 eq., 19 mg, 0.24 mmol) and K₂CO₃ (2.0 eq., 328 mg, 2.37 mmol) were added to a solution of 2-aminobenzylamine (1.0 eq., 145 mg, 1.19

mmol) and **54d** (1.1 eq., 195 mg, 1.31 mmol) in dimethylformamide (4 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification by silica gel chromatography, eluting with hexane:EtOAc, yielded **55d** (182 mg, 81%) as a white solid. **M.p.** 56-58 °C, clean melt.

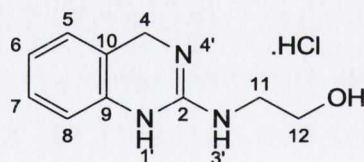
δ_{H} (400 MHz, CDCl_3): 0.88 (t, 3H, J 7.3, CH_3 , H-13), 1.53 (app. s, 2H, J 7.3, CH_2 , H-12), 3.28 (broad s, 1H, NH, H-3'), 4.23 (broad s, 2H, CH_2 , H-11), 4.64 (broad s, 2H, CH_2 , H-4), 6.34 (broad s, NH, H-1'), 6.62-6.70 (m, 2H, H-5 and H-7), 7.00 (d, 1H, J 7.3, H-8), 7.09 (t, 1H, J 7.6, 1.3, H-6).

δ_{C} (100MHz, CDCl_3): 11.4 (CH_3 , 13), 22.2 (CH_2 , 12), 46.0 (CH_2 , 4 or 11), 46.4 (CH_2 , 4 or 11), 116.0 (CH Ar, 5), 118.4 (CH Ar, 7), 121.3 (q Ar, 10), 129.4 (CH Ar, 6), 130.6 (CH Ar, 8), 145.3 (q Ar, 9), 181.1 (q, 2).

ν_{max} (ATR)/ cm^{-1} : 3224 (NH), 3063 (NH), 2961, 2930, 2873, 1627 (C=N), 1605, 1546, 1495, 1457, 1376, 1335, 1288, 1264, 1227, 1156, 1101, 1063, 944, 870, 749.

HRMS: (m/z ESI^+): Found: 190.1932 ($\text{M}^+ + \text{H}$, $\text{C}_{11}\text{H}_{16}\text{N}_3$ Requires: 190.1344).

***N*-(Ethoxy)-1,4-dihydroquinazolin-2-amine hydrochloride (**58a**)**



To *N*-(ethylacetyl)-1,4-dihydroquinazolin-2-amine **55a** (275 mg, 1.18 mmol) was added excess 1.25 M HCl/methanol (6.0 eq., 5.66 mL, 7.07 mmol). The resulting solution was stirred at 55 °C for 4 h. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in water (4 mL). It was washed with CH_2Cl_2 (2×3 mL) and then purified by recrystallization (slow diffusion of Et_2O in methanol) to afford **58a** (255 mg, 95%) as a white, crystalline solid. **M.p.** 139-140 °C, clean melt.

δ_{H} (400 MHz, $\text{DMSO}-d_6$): 3.57 (t, 2H, J 7.5, H-12), 3.90 (t, 2H, J 7.5, H-11), 3.90 (broad s, 1H, OH), 4.84 (d, 2H, J 5.2, CH_2 , H-4), 7.34-7.50 (m, 4H, Ar), 8.45 (broad s, 1H, NH, H-3'), 9.99 (broad s, 1H, NH, H-4'), 10.96 (broad s, 1H, NH, H-1').

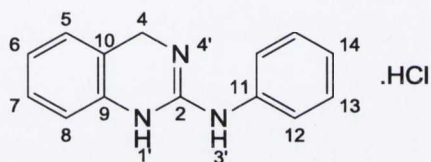
δ_C (100 MHz, DMSO- D_6): 44.0 (CH_2 , 11), 30.9 (CH_2 , 12), 48.8 (CH_2 , 4), 123.6 (CH Ar, 5), 127.8 (CH Ar, 7), 128.8 (CH Ar, 6), 129.1 (q Ar, 10), 129.4 (CH Ar, 8), 131.3 (q Ar, 9), 170.1 (q, 2).

ν_{max} (ATR)/ cm^{-1} : 2793, 2561, 2020, 1634 (C=N), 1580, 1494, 1455, 1343, 1292, 1244, 1127, 1071, 940, 873, 755, 679.

HRMS (m/z ESI $^+$): Found: 192.1143 ($M^+ + H$, $C_{10}H_{14}N_3O$ Requires: 192.1137).

Purity by HPLC: 97.3% (t_R 19.96 min).

N-Phenyl-1,4-dihydroquinazolin-2-amine hydrochloride (**58b**)



Following Method G excess 4 M HCl/dioxane (3.0 eq., 178 μ L, 0.71 mmol) was added to a solution of **55b** (1.0 eq., 53 mg, 0.23 mmol) in CH_3OH (1 mL). The solution was stirred at RT for 1 h. After usual workup and purification using reverse phase chromatography (C-8 silica) using 100% H_2O as mobile phase, **58b** (58 mg, 93%) was obtained as a white, crystalline solid. **M.p.** 122-124 $^{\circ}C$, clean melt.

δ_H (600 MHz, DMSO- D_6): 4.75 (d, 2H, J 5.2, CH_2 , H-4), 7.04 (broad s, 1H, Ar), 7.09 (broad s, 1H, Ar), 7.14 (t, 1H, J 7.3, H-14), 7.22 (t, 1H, J 7.1, Ar), 7.30-7.33 (m, 1H, Ar), 7.34 (t, 2H, J 7.3, H-13), 7.46 (d, 2H, J 7.3, H-12), 8.21 (broad s, 1H, NH, H-3'), 9.84 (broad s, 1H, NH, H-1').

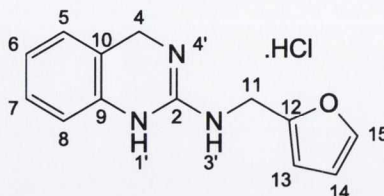
δ_C (150 MHz, DMSO- D_6): 42.9 (CH_2 , 4), 123.7 (q Ar), 123.8 (CH Ar), 124.8 (CH Ar), 128.3 (CH Ar), 128.6 (CH Ar), 129.0 (CH Ar), 129.5 (q Ar), 130.6 (q Ar), 133.1 (CH Ar), 139.7 (CH Ar), 181.5 (q, 2).

ν_{max} (ATR)/ cm^{-1} : 3221 (NH), 2810, 2554, 1980, 1744, 1594 (C=N), 1534, 1494, 1452, 1380, 1343, 1313, 1265, 1121, 1093, 1029, 939, 835, 748, 694.

HRMS (m/z ESI $^+$): Found: 224.1194 ($M^+ + H$, $C_{14}H_{14}N_3$ Requires: 224.1188).

Purity by HPLC: 96.4% (*t*R 28.55 min).

***N*-(2-Furanylmethyl)-1,4-dihydroquinazolin-2-amine hydrochloride (**58c**)**



Following Method G excess 4 M HCl/dioxane (3.0 eq., 2.44 mL, 9.77 mmol) was added to a solution of **55c** (1.0 eq., 740 mg, 3.26 mmol) in CH₂Cl₂ (33 mL) at 0 °C under argon. The solution was stirred at RT for 1 h, when a white precipitate was observed. After usual workup and purification using reverse phase chromatography (C-8 silica) using 100% H₂O as mobile phase, **58c** (256 mg, 30%) was obtained as a tan, crystalline solid in poor yield due to decomposition of product in acid. **M.p.** 50-52 °C, clean melt.

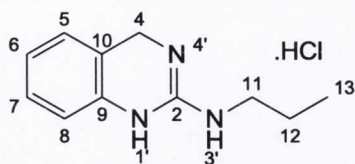
δ_H (400 MHz, DMSO-D₆): 4.66 (s, 2H, CH₂, H-4), 4.84 (d, 2H, J 4.6, H-11), 6.29 (broad s, 1 H, H-13), 6.40 (s, 1H, H-14), 7.37 (m, 1H, Ar), 7.38-7.40 (m, 2H, Ar), 7.47 (s, 1H, Ar), 7.59 (s, 1H, H-15), 8.31 (broad s, 1H, NH), 8.39 (broad s, 1H, NH).

δ_C (100 MHz, DMSO-D₆): 38.4 (CH₂, 11), 41.3 (CH₂, 4), 108.8 (CH, Ar), 111.1 (CH, Ar), 116.0 (CH, Ar), 124.8 (CH, Ar), 126.7 (CH, Ar), 129.0 (CH, Ar), 133.8 (q, Ar), 140.3 (q, Ar), 143.6 (CH, Ar), 152.4 (q, Ar), 174.2 (q, 2).

ν_{max} (ATR)/cm⁻¹: 2834, 2574, 1623 (C=N), 1590, 1560, 1496, 1456, 1299, 1226, 1070, 1011, 939, 883, 753.

HRMS (*m/z* ESI⁺): Found: 228.1146 (M⁺ + H. C₁₃H₁₄N₃O Requires: 228.1137).

Purity by HPLC: 97.6% (*t*R 27.95 min).

***N*-(*n*-Propyl)-1,4-dihydroquinazolin-2-amine hydrochloride (58d)**

Following Method G excess 1.25 M HCl/CH₃OH (1.1 eq., 930 μ L, 1.16 mmol) was added to a solution of **55d** (1.0 eq., 200 mg, 1.06 mmol) in CH₃OH (5 mL). The solution was stirred at RT for 1 h. After usual workup and purification using reverse phase chromatography (C-8 silica) using 100% H₂O as mobile phase, followed by recrystallisation from CH₃OH, **58d** (214 mg, 90%) was obtained as a white, crystalline solid. **M.p.** 80-82 °C, clean melt.

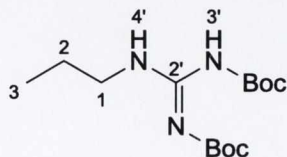
δ_{H} (400 MHz, DMSO-D₆): 0.92 (t, 3H, J 7.1, CH₃, H-13), 1.54 (m, 2H, CH₂, H-12), 3.27 (m, 2H, CH₂, H-11), 4.46 (s, 2H, CH₂, H-4), 7.02-7.09 (m, 2H, H-7 and H-8), 7.19 (d, 1H, J 7.2, H-5), 7.24 (t, 1H, J 7.2, H-6), 8.32 (broad s, 1H, NH, H-3'), 8.79 (broad s, 1H, NH, H-4'), 10.82 (broad s, 1H, H-1').

δ_{C} (100 MHz, DMSO-D₆): 11.0 (CH₃, 13), 21.9 (CH₂, 12), 40.8 (CH₂, 11), 42.7 (CH₂, 4), 123.9 (q Ar), 126.2 (CH Ar), 128.4 (CH Ar), 133.5 (CH Ar), 145.5 (q Ar), 151.8 (CH Ar), 166.9 (q, 2).

ν_{max} (ATR)/cm⁻¹: 3233 (NH), 2872, 2558, 1992, 1618 (C=N), 1555, 1496, 1456, 1382, 1333, 1291, 1213, 1179, 1064, 935, 837, 750.

HRMS (m/z ESI⁺): Found: 190.1347 (M⁺ + H. C₁₁H₁₆N₃ Requires: 190.1344).

Purity by HPLC: 96.7% (t_{R} 26.23 min).

1-(*n*-Propyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59a)

Following Method H Copper(II) chloride (1.05 eq., 96 mg, 0.71 mmol) was added to a solution of *n*-propylamine (1.0 eq., 40 mg, 0.68 mmol), **8** (1.05 eq., 196 mg, 0.71 mmol) and triethylamine (3.5 eq., 330 μ L, 2.37 mmol) in CH_2Cl_2 (3 mL) at 0 $^\circ\text{C}$. Stirring at RT was continued for 3 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59a** (182 mg, 89%) as a white, crystalline solid. **M.p.** 64-65 $^\circ\text{C}$, clean melt.

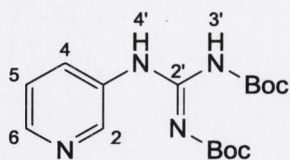
δ_{H} (400 MHz, CDCl_3): 0.96 (t, 3, J 7.4, CH_3 , H-3), 1.50 (s, 9H, CH_3 , Boc), 1.50 (s, 9H, CH_3 , Boc), 1.60 (app. s, 2H, J 7.4, CH_2 , H-2), 3.39 (m, 2H, CH_2 , H-1), 8.33 (broad s, 1H, NH, H-4'), 11.52 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 11.4 (CH_3 , 3), 22.2 (CH_2 , 2), 28.0 (CH_3 , Boc), 28.3 (CH_3 , Boc), 42.6 (CH_2 , 1), 79.2 (q ^tBu , Boc), 83.0 (q ^tBu , Boc), 153.3 (q CO, Boc), 156.2 (q, 2'), 163.7 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3332 (NH), 3125 (NH), 2978, 2931, 1736 (C=O), 1647, 1624 (C=N), 1572, 1475, 1451, 1413, 1397, 1360, 1345, 1307, 1268, 1249, 1222, 1053, 1026, 887, 859, 806, 749, 724.

HRMS (m/z ESI $^+$): Found: 324.1896 ($\text{M}^+ + \text{Na}$. $\text{C}_{14}\text{H}_{27}\text{N}_3\text{O}_4\text{Na}$ Requires: 324.1899).

1-(Pyridin-3-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**59b**)



Following Method H Copper(II) chloride (1.05 eq., 38 mg, 0.28 mmol) was added to a solution of 3-aminopyridine (1.0 eq., 25 mg, 0.27 mmol), **8** (1.05 eq., 77 mg, 0.28 mmol) and triethylamine (3.5 eq., 130 μ L, 0.93 mmol) in CH_2Cl_2 (3 mL) at 0 $^\circ\text{C}$. Stirring at RT was continued for 3 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59b** (81 mg, 89%) as a white, crystalline solid. **M.p.** 138-140 $^\circ\text{C}$, clean melt.

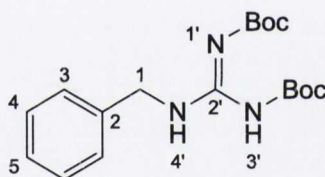
δ_{H} (400 MHz, CDCl_3): 1.50 (s, 9H, CH_3 , Boc), 1.54 (s, 9H, CH_3 , Boc), 7.28 (dd, 1H, J 8.3, 4.1, H-5), 8.21 (dd, 1H, J 8.3, 1.5, H-4), 8.34 (d, 1H, J 4.1, H-6), 8.67 (d, 1H, J 1.5, H-2), 10.41 (broad s, 1H, NH, H-3'), 11.61 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 27.6 (CH_3 , Boc), 27.6 (CH_3 , Boc), 79.6 (q ^tBu , Boc), 83.7 (q ^tBu , Boc), 123.1 (CH Ar, 5), 129.2 (CH Ar, 4), 133.4 (q Ar, 3), 142.7 (CH Ar, 2), 145.0 (CH Ar, 6), 152.8 (q CO, Boc), 153.3 (q, 2'), 162.8 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3146 (NH), 2981, 1725 (C=O), 1636 (C=N), 1587, 1561, 1477, 1396, 1367, 1329, 1280, 1252, 1226, 1151, 1132, 1101, 1057, 1029, 942, 883, 845, 801, 773, 746, 719, 702, 656.

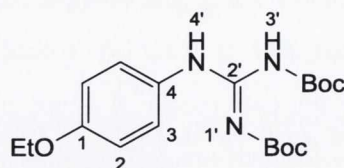
HRMS (m/z ESI^+): Found: 337.1870 ($\text{M}^+ + \text{H}$, $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_4$ Requires: 337.1876).

1-(Benzyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**59c**)



Following Method H Copper(II) chloride (1.05 eq., 132 mg, 0.98 mmol) was added to a solution of benzylamine (1.0 eq., 100 mg, 0.93 mmol), **8** (1.05 eq., 271 mg, 0.98 mmol) and triethylamine (3.5 eq., 455 μL , 3.27 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 3 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59c** (269 mg, 83%) as a white powder. **M.p.** 124-125 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, CH_3 , Boc), 1.53 (s, 9H, CH_3 , Boc), 4.64 (d, 2H, J 3.4, H-1), 7.33 (m, 5H, H-3, H-4 and H-5), 8.61 (broad s, 1H, NH, H-4'), 11.60 (broad s, 1H, NH, H-3').²³⁵

1-(4-Ethoxyphenyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59d)

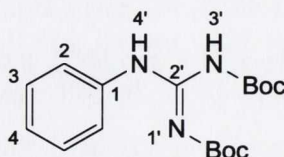
Following Method H Copper(II) chloride (1.05 eq., 51 mg, 0.38 mmol) was added to a solution of 4-ethoxyaniline (1.0 eq., 50 mg, 0.36 mmol), **8** (1.05 eq., 106 mg, 0.38 mmol) and triethylamine (3.5 eq., 178 μ L, 1.28 mmol) in CH_2Cl_2 (4 mL) at 0 °C. Stirring at RT was continued for 3 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59d** (105 mg, 76%) as a white solid. **M.p.** 118–120 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.42 (t, 3H, J 6.9, CH_3 , OEt), 1.51 (s, 9H, CH_3 , Boc), 1.55 (s, 9H, CH_3 , Boc), 4.03 (q, 2H, J 6.9, CH_2 , OEt), 6.87 (d, 2H, J 8.8, H-2), 7.49 (d, 2H, J 8.8, H-3), 10.21 (broad s, 1H, NH, H-3'), 11.67 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 14.4 (CH_3 , OEt), 27.6 (CH_3 , Boc), 27.8 (CH_3 , Boc), 63.2 (CH_2 , OEt), 79.0 (q ^tBu , Boc), 83.1 (q ^tBu , Boc), 114.2 (CH Ar, 2), 123.4 (CH Ar, 3), 129.2 (q Ar, 4), 152.9 (q Ar, 1), 153.2 (q, 2'), 155.7 (q CO, Boc), 163.2 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3280 (NH), 3165 (NH), 2933, 1716 (C=O), 1630 (C=N), 1605, 1573, 1511, 1390, 1345, 1227, 1155, 1117, 1057.

HRMS (m/z ESI^+): Found: 380.2181 ($\text{M}^+ + \text{H}$. $\text{C}_{19}\text{H}_{30}\text{N}_3\text{O}_5$ Requires: 380.2185).

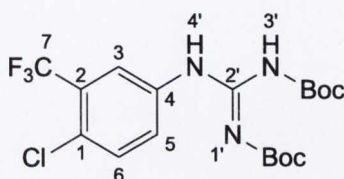
1-(Phenyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59e)

Following Method H Copper(II) chloride (1.05 eq., 152 mg, 1.13 mmol) was added to a solution of aniline (1.0 eq., 100 mg, 1.07 mmol), **8** (1.05 eq., 312 mg, 1.13 mmol) and triethylamine (3.5 eq., 524 μ L, 3.76 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography

using gradient elution (hexane:EtOAc) yielded **59e** (261 mg, 73%) as a white, crystalline solid. **M.p.** 118-120 °C, clean melt, lit. 119-121 °C.¹⁵⁷

δ_{H} (400 MHz, CDCl_3): 1.53 (s, 9H, CH_3 , Boc), 1.56 (s, 9H, CH_3 , Boc), 7.14 (t, 1H, J 7.1, H-4), 7.35 (app. t, 2H, J 7.1, H-3), 7.63 (d, 2H, J 7.1, H-2), 10.37 (broad s, 1H, NH, H-3'), 11.68 (broad s, 1H, NH, H-4').

1-[(4-Chloro-3-trifluoromethyl)phenyl]-2,3-di(*tert*-butoxycarbonyl)guanidine (**59f**)



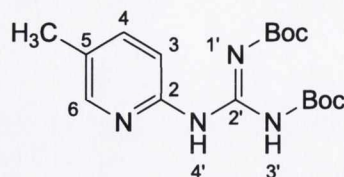
Following Method H Copper(II) chloride (1.05 eq., 72 mg, 0.54 mmol) was added to a solution of 4-chloro-3-trifluoromethylaniline (1.0 eq., 100 mg, 0.51 mmol), **8** (1.05 eq., 148 mg, 0.54 mmol) and triethylamine (3.5 eq., 249 μL , 1.79 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59f** (160 mg, 72%) as a white, crystalline solid. **M.p.** 130-132 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.52 (s, 9H, CH_3 , Boc), 1.56 (s, 9H, CH_3 , Boc), 7.47 (d, 1H, J 8.9, H-5), 7.91-7.93 (m, 2H, H-3 and H-6), 10.51 (broad s, 1H, NH, H-3'), 11.61 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 28.1 (CH_3 , Boc), 28.1 (CH_3 , Boc), 80.1 (q ^1Bu , Boc), 84.3 (q ^1Bu , Boc), 120.9 (CH Ar, q, ^3J 5.4, 3), 122.55 (q CF_3 , q, ^1J 273.2, 7), 126.2 (CH Ar, 6), 127.3 (q Ar, 1), 128.6 (q Ar, q, ^2J 31.3, 2), 131.9 (CH Ar, 5), 135.8 (q Ar, 4), 153.3 (q CO, Boc), 153.4 (q, 2'), 163.1 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3131 (NH), 2985, 1716 (C=O), 1641 (C=N), 1568, 1486, 1456, 1393, 1373, 1343, 1320, 1296, 1252, 1235, 1142, 1130, 1104 (CF_3), 1060, 1032, 978, 880, 866, 835, 802, 765, 721, 700, 669.

HRMS (m/z ESI^+): Found: 460.1227 ($\text{M}^+ + \text{Na}$. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4\text{F}_3^{35}\text{ClNa}$ Requires: 460.1227).

1-(5-Methylpyridin-2-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59g)

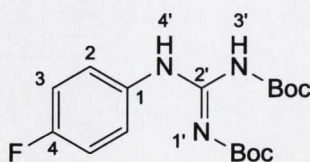
Following Method H Copper(II) chloride (1.05 eq., 131 mg, 0.97 mmol) was added to a solution of 2-amino-5-methylpyridine (1.0 eq., 100 mg, 0.93 mmol), **8** (1.05 eq., 268 mg, 0.97 mmol) and triethylamine (3.5 eq., 451 μ L, 3.24 mmol) in CH_2Cl_2 (9 mL) at 0 $^\circ\text{C}$. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59g** (217 mg, 67%) as a white powder. **M.p.** 137-139 $^\circ\text{C}$, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.48 (s, 9H, CH_3 , Boc), 1.49 (s, 9H, CH_3 , Boc), 2.23 (s, 3H, CH_3), 7.45 (dd, 1H, J 8.4, 1.9, H-4), 8.07 (d, 1H, J 1.9, H-6), 8.20 (broad s, 1H, H-3), 10.78 (broad s, 1H, NH, H-3'), 11.52 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 17.8 (CH_3), 28.0 (CH_3 , Boc), 28.1 (CH_3 , Boc), 79.7 (q, Boc), 83.7 (q, Boc), 115.6 (CH Ar, 3), 129.1 (q Ar, 5), 138.6 (CH Ar, 4), 147.9 (CH Ar, 6), 148.4 (q Ar, 2), 152.7 (q CO, Boc), 153.0 (q, 2'), 163.3 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3244 (NH), 2978, 1720 (C=O), 1632 (C=N), 1585, 1560, 1475, 1454, 1404, 1374, 1324, 1305, 1289, 1268, 1252, 1230, 1151, 1136, 1107, 1058, 1025, 882, 856, 838, 803, 758, 749, 709.

HRMS (m/z ESI^+): Found: 351.2036 ($\text{M}^+ + \text{H}$. $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_4$ Requires: 351.2032).

1-(4-Fluorophenyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59h)

Following Method H Copper(II) chloride (1.05 eq., 70 mg, 0.52 mmol) was added to a solution of 4-fluoroaniline (1.0 eq., 55 mg, 0.50 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)thiourea **8** (1.05 eq., 144 mg, 0.52 mmol) and triethylamine (3.5 eq.,

241 μL , 1.73 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59h** (108 mg, 62%) as a yellow/white, crystalline solid. **M.p.** 124-126 °C, clean melt.

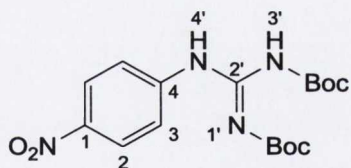
δ_{H} (400 MHz, CDCl_3): 1.52 (s, 9H, CH_3 , Boc), 1.56 (s, 9H, CH_3 , Boc), 7.00-7.07 (m, 2H, H-3), 7.55-7.60 (m, 2H, H-2), 10.31 (broad s, 1H, NH, H-3'), 11.66 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 28.1 (CH_3 , Boc), 28.2 (CH_3 , Boc), 79.7 (q ^tBu , Boc), 83.8 (q ^tBu , Boc), 115.6 (d, ^2J 22.0, CH Ar, 3), 124.0 (d, ^3J 8.0, CH Ar, 2), 132.8 (d, ^4J 2.5, q Ar, 1), 153.3 (q CO, Boc), 153.7 (q, 2'), 159.8 (d, ^1J 244.5, q Ar, 4), 163.5 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3271 (NH), 2987, 1716 (C=O), 1619 (C=N), 1585, 1510, 1494, 1456, 1390, 1367, 1302, 1275, 1243, 1164, 1153, 1109, 1094, 1058, 1031, 1012, 918, 886, 852, 829, 797, 749, 674.

HRMS (m/z ESI^+): Found: 376.1654 ($\text{M}^+ + \text{Na}$. $\text{C}_{17}\text{H}_{24}\text{N}_3\text{O}_4\text{FNa}$ Requires: 376.1649).

1-(4-Nitrophenyl)-2,3-di(*tert*-butoxycarbonyl)guanidine (**59i**)

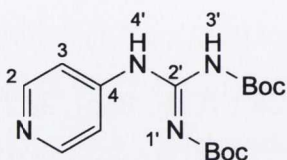


Following Method H Copper(II) chloride (1.05 eq., 102 mg, 0.76 mmol) was added to a solution of 4-nitroaniline (1.0 eq., 100 mg, 0.72 mmol), **8** (1.05 eq., 210 mg, 0.76 mmol) and triethylamine (3.5 eq., 353 μL , 2.52 mmol) in dimethylformamide (4 mL) at 0 °C. Stirring at 60 °C was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59i** (113 mg, 41%) as a white solid. **M.p.** 187-189 °C, decomposition.²³⁵

δ_{H} (400 MHz, CDCl_3): 1.55 (s, 18H, CH_3 , Boc), 7.87 (d, 2H, J 8.4, H-3), 8.23 (d, 2H, J 8.4, H-2), 10.79 (broad s, 1H, NH, H-3'), 11.62 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 27.6 (CH_3 , Boc), 27.6 (CH_3 , Boc), 80.0 (q ^tBu , Boc), 84.1 (q ^tBu , Boc), 120.8 (CH Ar, 3), 124.4 (CH Ar, 2), 142.5 (q Ar, 4), 143.1 (q Ar, 1), 152.7 (q, 2'), 152.8 (q CO, Boc), 162.5 (q CO, Boc).

1-(Pyridin-4-yl)-2,3-di(*tert*-butoxycarbonyl)guanidine (59j**)**



Following Method H Copper(II) chloride (1.05 eq., 75 mg, 0.56 mmol) was added to a solution of 4-aminopyridine (1.0 eq., 50 mg, 0.53 mmol), **8** (1.05 eq., 154 mg, 0.56 mmol) and triethylamine (3.5 eq., 259 μL , 1.86 mmol) in dimethylformamide (3 mL) at 0 °C. Stirring at 60 °C was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59j** (27 mg, 26%) as a white solid. **M.p.** 106-107 °C, clean melt.

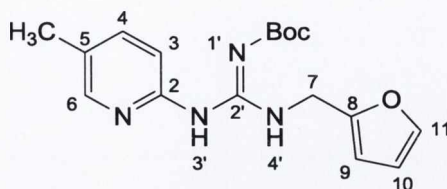
δ_{H} (400 MHz, CDCl_3): 1.52 (s, 9H, CH_3 , Boc), 1.55 (s, 9H, CH_3 , Boc), 7.73 (broad s, 2H, H-3), 8.69 (broad s, 2H, H-2), 10.58 (broad s, 1H, NH, H-3'), 11.59 (broad s, 1H, NH, H-4').

δ_{C} (100 MHz, CDCl_3): 27.8 (CH_3 , Boc), 27.9 (CH_3 , Boc), 80.2 (q ^tBu , Boc), 83.1 (q ^tBu , Boc), 144.0 (Ar), 148.8 (Ar), 148.8 (Ar), 150.0 (Ar), 150.7 (Ar), 153.0 (q CO, Boc), 153.1 (q, 2'), 162.8 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3222 (NH), 2979, 2936, 1788, 1719, 1710 (C=O), 1636 (C=N), 1590, 1484, 1455, 1413, 1394, 1369, 1321, 1297, 1271, 1251, 1209, 1134, 1111, 1058, 1030, 1005, 980, 871, 809, 773, 753, 741, 652.

HRMS (m/z ESI^+): Found: 337.1874 ($\text{M}^+ + \text{H}$. $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_4$ Requires: 337.1876).

1-(5-Methylpyridin-2-yl)-2-(*tert*-butoxycarbonyl)-3-(2-furanylmethyl)guanidine (59k)



Following Method H Copper(II) chloride (1.05 eq., 65 mg, 0.49 mmol) was added to a solution of 2-amino-5-methylpyridine (1.0 eq., 50 mg, 0.46 mmol), thiourea **48c** (1.05 eq., 124 mg, 0.49 mmol) and triethylamine (3.5 eq., 226 μ L, 1.62 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59k** (63 mg, 41%) as a white solid. **M.p.** 121-122 °C, clean melt.

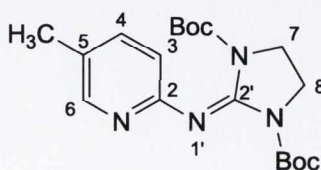
δ_{H} (400 MHz, CDCl_3): 1.56 (s, 9H, CH_3 , Boc), 2.28 (s, 3H, CH_3), 4.75 (d, 2H, J 4.8, CH_2 , H-7), 6.30 (d, 1H, J 3.1, H-9), 6.35 (s, 1H, H-10), 6.78 (d, 1H, J 8.2, H-3), 7.40 (s, 1H, H-11), 7.47 (d, 1H, J 8.2, H-4), 8.01 (s, 1H, H-6), 10.57 (broad s, 1H, NH, H-4'), 12.02 (broad s, 1H, NH, H-3').

δ_{C} (100 MHz, CDCl_3): 17.2 (CH_3), 28.0 (CH_3 , Boc), 37.7 (CH_2 , 7), 78.3 (q ^tBu , Boc), 106.6 (CH Ar, 9), 109.9 (CH Ar, 10), 112.3 (CH Ar, 3), 126.7 (q Ar, 5), 139.0 (CH Ar, 4), 141.6 (CH Ar, 11), 145.1 (CH Ar, 6), 150.3 (q Ar, 2), 151.1 (q Ar, 8), 157.0 (q, 2'), 163.7 (q CO, Boc).

ν_{max} (ATR)/ cm^{-1} : 3005 (NH), 2963, 2981, 2929, 1640 (C=O), 1620 (C=N), 1599, 1566, 1506, 1493, 1482, 1427, 1386, 1348, 1336, 1299, 1262, 1252, 1236, 1214, 1167, 1153, 1122, 1085, 1059, 1036, 976, 964, 801, 772, 739, 698, 670.

HRMS (m/z ESI^+): Found: 331.1764 ($\text{M}^+ + \text{H}$, $\text{C}_{17}\text{H}_{23}\text{N}_4\text{O}_3$ Requires: 331.1770).

1-(5-Methylpyridin-2'-yl)-2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidine (59l)



Following Method H Copper(II) chloride (1.05 eq., 131 mg, 0.97 mmol) was added to a solution of 2-amino-5-methylpyridine (1.0 eq., 100 mg, 0.93 mmol), di-Boc-imidazolidine-2-thione **41** (1.05 eq., 294 mg, 0.97 mmol) and triethylamine (3.5 eq., 451 μ L, 3.24 mmol) in CH_2Cl_2 (5 mL) at 0 °C. Stirring at RT was continued for 16 h. Usual workup and purification by silica gel chromatography using gradient elution (hexane:EtOAc) yielded **59I** (256 mg, 74%) as a white solid. **M.p.** 89-91 °C, clean melt.

δ_{H} (400 MHz, CDCl_3): 1.56 (s, 18H, CH_3 , Boc), 2.28 (s, 3H, CH_3), 3.83 (s, 4H, H-7 and H-8), 7.47 (dd, 1H, J 7.6, H-4), 7.91 (broad s, 1H, H-3), 8.10 (s, 1H, H-6).

δ_{C} (100 MHz, CDCl_3): 17.6 (CH_3), 28.1 (CH_3 , Boc), 45.2 (CH_2 , 7 and 8), 82.4 (q ^tBu , Boc), 112.6 (CH Ar, 3), 126.6 (q Ar, 5), 138.4 (CH Ar, 4), 147.6 (CH Ar, 6), 149.8 (q CO, Boc), 150.3 (q Ar, 2), 152.3 (q, 2').

ν_{max} (ATR)/ cm^{-1} : 3265, 2980, 2933, 2885, 1804, 1697 (C=O), 1647 (C=N), 1602, 1577, 1543, 1480, 1467, 1365, 1330, 1307, 1288, 1253, 1232, 1149, 1115, 1026, 879, 851, 840, 767, 748, 740, 701, 684.

HRMS (m/z ESI^+): Found: 377.2186 ($\text{M}^+ + \text{H}$. $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}_4$ Requires: 377.2189).

5.2. Computational Methods

5.2.1. Pyridin-2-ylguanidine Derivatives – Conformational Control Induced by Intramolecular Hydrogen-Bonding Interactions

Geometries were fully optimised at the B3LYP theoretical level with the 6-31+G(d,p) basis set as implemented in the Gaussian09 program.¹⁸¹ Harmonic frequency calculations verified the nature of the stationary points as either minima (all real frequencies) or transition states (one imaginary frequency). The scanning of the rotation was performed using the IRC type calculation implemented in the Gaussian09 program at the same level.

5.2.2. Cation- π vs. π - π interactions – Complexes of Pyridin-2-ylguanidinium Derivatives and Aromatic Systems

Systems were optimised using the Gaussian09 package¹⁸¹ at the M05-2X²³⁶ and M06-2X²³⁷ computational levels with the 6-31+G(d,p)²³⁸ and the 6-311++G(d,p)²³⁹ basis sets. Frequency calculations were performed at both computational levels to confirm that the resulting optimised structures are energetic minima. Effects of water solvation were included by means of the SCRF-PCM approach implemented in the Gaussian-09 package including dispersing, repulsing and cavitating energy terms of the solvent in the optimisation. The interaction energy of the complexes was calculated as the difference between the supermolecule's energy and the sum of the energies of the isolated monomers in their minimum energy configuration. Considering that all calculations were performed using the PCM (water solvent) approach the interaction energy cannot be corrected from the inherent basis set superposition error (BSSE). Where gas phase interaction energies were calculated (for complexes of guanidinium with aromatic systems) they were corrected from the inherent BSSE using the full counterpoise method.²⁴⁰

The electron density of the complexes was analysed within the AIM theory¹⁶⁰ using the AIMAll²⁴¹ program. The Natural Bond Orbital (NBO) method¹⁶¹ was used to analyse the interaction of the occupied and unoccupied orbitals as these interactions are of utmost importance in non-covalent complexes. The theoretical NICS values were calculated using the GIAO method on the optimised geometries.^{242,243}

5.2.3. A Structural Study of *N,N'*-Bis-aryl-acylguanidines

Geometries were fully optimised at the B3LYP theoretical level with the 6-31+G(d,p) basis set as implemented in the Gaussian09 program.¹⁸¹ Harmonic frequency calculations verified the nature of the stationary points as minima (all real frequencies). NMR calculations were performed using the GIAO protocol.^{242,243} at the same level of theory. Chemical shifts were taken with reference to the tetramethylsilane standard. Where chemically equivalent signals gave different GIAO values these were reported as obtained, though this occurs as GIAO is a single point calculation on the optimised structure.

5.3. Pharmacological Methods

5.3.1. Preparation of Membranes

Neural membranes (P2 fractions) were prepared from the PFC of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Post-mortem human brain samples of each subject (~1 g) were homogenised using a Teflon-glass grinder (10 up-and-down strokes at 1500 rpm) in 30 volumes of homogenization buffer (1 mM MgCl₂ and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1,000 g (4 °C), and the supernatant was centrifuged again for 10 min at 40,000 g (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and re-centrifuged in similar conditions. Aliquots of 1 mg protein were stored at -70 °C until assay. Protein concentration was measured according to the Bradford method²⁴⁴ using bovine serum albumin as standard and was similar in the different brain samples.

5.3.2. [³H]RX821002 Binding Assays

Specific [³H]RX821002 binding was measured in 0.55 mL-aliquots (50 mM Tris HCl, pH 7.5) of the neural membranes which were incubated with [³H]RX821002 (1 nM) for 30 min at 25 °C in either the absence or presence of the competing compounds (10⁻¹² to 10⁻³ M, 10 concentrations). Incubations were terminated by diluting the samples with 5 mL of ice-cold Tris incubation buffer (4 °C). Membrane bound [³H]RX821002 was separated by vacuum filtration through Whatman GF/C glass fibre filters. Then the filters were rinsed twice with 5 mL of incubation buffer and transferred to minivials containing 3 mL of OptiPhase “HiSafe” II cocktail and counted for radioactivity by liquid scintillation spectrometry. Specific binding was determined and plotted as a function of the compound concentration. Nonspecific binding was determined in the presence of adrenaline (10⁻⁵ M). Analysis of competition experiments to obtain the inhibition constant (K_i) were performed by non-linear regression using the Graph Pad Prism 5 program. All experiments were analysed assuming a one-site model of radioligand binding, K_i values were normalised to pK_i values.

5.3.3. [³⁵S]GTP γ S Binding Assays

The incubation buffer for measuring [³⁵S]GTP γ S binding to brain membranes contained, in a total volume of 500 μ L, 1 mM ethylene glycol tetraacetic acid (EGTA), 3 mM MgCl₂, 100 mM NaCl, 50 mM GDP, 50 mM Tris-HCl at pH 7.4, and 0.5 nM [³⁵S]GTP γ S. Protein aliquots were thawed and re-suspended in the same buffer. The incubation was started by addition of the membrane suspension (40 μ g of membrane proteins) to the previous mixture and was performed at 30 °C for 120 min with shaking. To evaluate the influence of the compounds on [³⁵S]GTP γ S binding, 8 concentrations (10⁻¹⁰ to 10⁻³ M) of each compound were added to the assay. Incubations were terminated by adding 3 mL of ice-cold re-suspension buffer followed by rapid filtration through Whatman GF/C filters pre-soaked in the same buffer. The filters were rinsed twice with 3 mL of ice-cold re-suspension buffer, transferred to vials containing 5 mL of OptiPhase HiSafe II cocktail (Wallac, UK), and the radioactivity trapped was determined by liquid scintillation spectrometry (Packard 2200CA). The [³⁵S]GTP γ S bound was about 7-14% of the total [³⁵S]GTP γ S added. Nonspecific binding of the radioligand was defined as the remaining [³⁵S]GTP γ S binding in the presence of 10 μ M unlabelled GTP γ S.²⁴⁵

Chapter 6 – References

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