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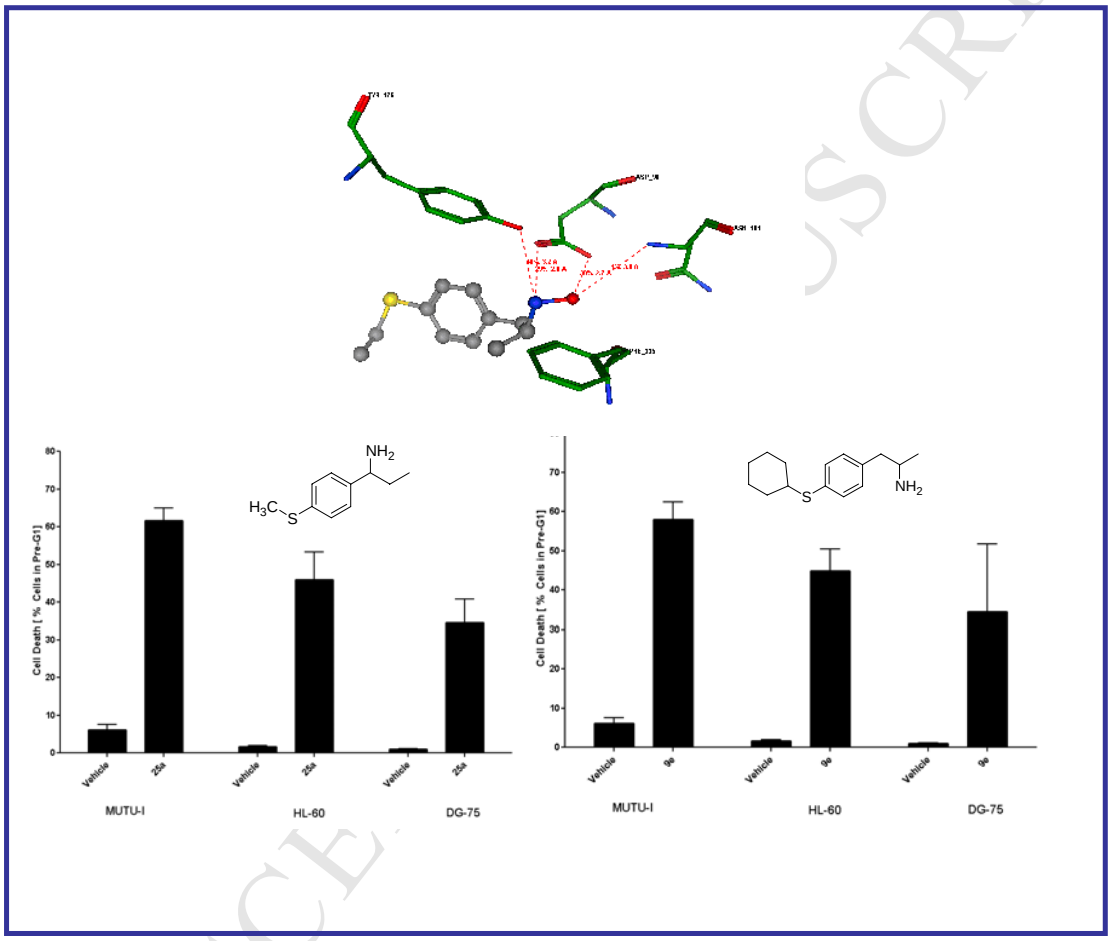

Synthesised structural analogues of 4-MTA inhibit the serotonin transporter but have SERT-independent antiproliferative activity in a number of malignant cell lines.

Synthesis and serotonin transporter activity of Sulphur-Substituted α-Alkyl Phenethylamines as a new class of anticancer agents.

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Abstract

The discovery that some serotonin reuptake transporter (SERT) ligands have the potential to act as pro-apoptotic agents in the treatment of cancer adds greatly to their diverse pharmacological application. 4-Methylthioamphetamine (MTA) is a selective ligand for SERT over other monoamine transporters. In this study, a novel library of structurally diverse 4-MTA analogues were synthesised with or without N-alkyl and /or C-α methyl or ethyl groups so that their potential SERT-dependent antiproliferative activity could be assessed.

Many of the compounds displayed SERT binding activity as well as cytotoxic activity. While there was no direct correlation between these two effects, a number of derivatives displayed anti-tumour effects in lymphoma, leukaemia and breast cancer cell lines, showing further potential to be developed as possible chemotherapeutic agents.

Abbreviations

4-MTA	4-methylthioamphetamine
5-HT	5-hydroxytryptamine
ALEPH-2	1-(2, 5-dimethoxy-4-ethylthiophenyl)-2-aminopropane
AMPH	Amphetamine
BBB	Blood-brain-barrier
BCB	Blood-cerebrospinal fluid barrier
BL	Burkitt's lymphoma
DAT	Dopamine Transporter
EBV	Epstein Barr Virus
FACS	Flourescent-activated cell sorting
HEK	Human Embryonic Kidney Cells
MDMA	3, 4-methylenedioxymethamphetamine
NAT	Noradrenaline Transporter
NR	Neutral Red
PBS	Phosphate buffered saline
PCD	Programmed Cell Death
PI	Propidium Iodide
PMA	Para-methoxyamphetamine (4-methoxyamphetamine)
SERT	Serotonin Transporter
SSRI	Selective Serotonin Reuptake Inhibitors
TCA	Tricyclic Antidepressant

Introduction

The serotonin transporter (SERT) transports 5-hydroxytryptamine (5-HT) from central and enteric nervous system synapses back into pre-synaptic neurons determining the duration and magnitude of 5-HT responses. Alterations in SERT activity, binding site density and polymorphisms of the SERT gene have implicated the transporter in many psychiatric and neurodegenerative disorders such as depression, anxiety, substance abuse, suicide, autism, Alzheimer's and Parkinson's disease [1].

The serotonin transporter is an important pharmacological target that has high affinity in vivo for tricyclic anti-depressants (TCAs) such as imipramine, serotonin-selective reuptake inhibitors (SSRIs), like fluoxetine and nonselective stimulants including cocaine and amphetamines [2]. Apart from its high expression in the nervous system, SERT is expressed in a wide range of specialised non-neuronal cells [3-5] and more interestingly, it has been found in a number of B cell malignancies including diffuse large B cell lymphoma, multiple myeloma and Burkitt's lymphoma [6].

Recently, a new pharmacological application for SERT has been reported, linking it with drug-induced apoptosis in lymphoma [6-8], where is thought to act as a pro-apoptotic target for SERT ligands. It has been implicated in the mechanism of cytotoxicity associated with the amphetamine analogues, fenfluramine and 3,4 methylenedioxymethamphetamine (MDMA) and SERT-targeting antidepressants [6, 9, 10]. The SSRI class of antidepressants including, fluoxetine, paroxetine and citalopram have been reported to induce apoptosis in a range of malignant cell lines [8, 11-14] and the tricyclic antidepressants have been implicated in apoptotic activities over a range of cell lines both neuronal and malignant [12, 15-17]. Some reports have questioned the role

of SERT in such apoptosis [18]. High concentrations of MDMA have been shown to induce SERT-independent cytotoxic effects in a number of cell lines [19] and non-serotonergic effects of fluoxetine have been reported [20].This suggests that (a) molecular target(s) other than SERT may exist on the lymphoma cell with the possibility that some antidepressants preferentially target the proliferating B cell [8].

Because of its growing association with HIV infection, Burkitt's lymphoma (BL) is becoming a more common malignancy in humans [21]. It is a tumour of B cells that accounts for 30-50% of lymphomas in children [22] and remains a serious health problem in those areas where it is endemic: namely the malarial belts of equatorial Africa, north-eastern Brazil, and Papua New Guinea [23]. With combination and CNS chemotherapy, the survival rate of patients with BL is reported to be at least 60%. Patients with bone marrow and CNS involvement have a poor prognosis and adults with the disease, especially those in the advanced stage, do more poorly than affected children with reoccurrence common. The development of novel selective apoptotic drugs and drug targets is therefore imperative to the future of effective anti-cancer drug design.

Amphetamine analogues substituted at the 4-position with an alkylthio group are a pharmacologically important class of compounds which demonstrate potent biochemical activity in the central nervous system. The parent member of the series, 4-MTA (4-methylthioamphetamine) [24] (Figure 1) is a ligand for SERT. It is a potent inhibitor of serotonin reuptake in the rat brain [25], a serotonin releasing agent in rat brain synaptosomes and has been shown to have inhibitory effects on 5-HT-mediated vascular contraction in isolated rat aortas [26] . It is also a selective reversible monoamine oxidase–A (MAO-A) inhibitor [27].

In the present study we report the synthesis of a series of novel 4-MTA analogues, with or without N-alkyl and /or C-α methyl or ethyl groups as well as the synthesis of the structurally related 4-thioethyl series. The aim of this study is to investigate the SERT-binding activity and the SERT-selective cytotoxic activity of these derivatives. We wish to determine if any observed toxicity is related to the ability of each compound to bind to SERT. SERT binding will be tested using a [^{3}H] 5-HT uptake inhibition assay with the specific structural requirements of these sulphur-substituted α-alkyl phenethylamines at the serotonin transporter examined using a recently developed homology model for human SERT [28]. The results obtained from such experiments can then used in molecular modelling studies to rationalise SERT-binding requirements and to determine if there are any structural correlations relating to SERT binding and any observed toxicity. Based on existing data linking SERT as a pro-apoptotic target for SERT-targeting ligands [6-8] in B cell malignancies, the ultimate aim of the present study is to use the 4-MTA scaffold as a starting point to synthesise a number of possible SERT-targeting agents. We wish to evaluate these agents for activity as novel antiproliferative agents. This will not only allow for the development and synthesis of more potent, selective analogues that offer the potential to be used as future anticancer agents but will also help confirm or contradict reports of SERT's potential role as a pro-apoptotic target.

Insert Figure 1

2. Results and Discussion

2.1 Chemistry

The structures of the sulphur-substituted α-alkyl phenethylamines and related compounds investigated in the present study are arranged in six distinct groups, Types I - VI (See Table 1-4; Schemes 1-5):

Type I: Structures based on 4-MTA scaffold with N-alkyl, N, N-dialkyl, N-hydroxyl or N-alkoxy substituents, Compounds **4a-4r**

Type II: Structures based on thiomethyl substituted α-methylphenethylamine scaffold with alkyl, aryl or benzyl type sulphur substituent, Compounds **9a-9e.**

Type III: Structures based on ethylthio-substituted α-methylphenethylamines, Compounds **15a-15d**

Type IV: Structures based on ethylthio-substituted α-ethylphenethylamines, Compounds **15e-15i**.

Type V: Structures based on thiomethyl substituted α-ethylphenethylamine scaffold with N-alkyl, N, N-dialkyl, N-hydroxyl or N-alkoxy substituents, Compounds **19a-19q.**

Type VI: Structures based on 1-phenylpropylamine, Compounds **25a**, **25b**.

Type I compounds: The synthetic routes which were utilised to obtain the sulphur-substituted α-alkyl phenethylamines are illustrated in Schemes 1-4 and are based on modifications of previously described processes [27, 29-33]. 4-MTA, (**4a**) together with the N-substituted analogues **4b-r** are obtained in good yield by a two step synthesis via the nitropropene **2** which was obtained from a modified Henry (Knovenegel) reaction of 4-methylthiobenzaldehyde (**1**) with nitroethane and N,N-dimethylamine and KF, (Scheme 1). The nitropropene **2** was then reduced with Fe iron powder to afford the

propanone **3** which was then treated with sodium cyanoborohydride and the appropriate amine to afford the Type I products **4a-4n.** 4-MTA (**4a**) was also obtained in 80% yield by direct reduction of the nitrostyrene **2** with $LiAlH_4$. N-hydroxy and N-alkoxy compounds **4o-r** were prepared by sodium cyanoborohydride reduction of the corresponding oximes **5a-d** (isolated as syn/anti mixture from the ketone **3** by reaction with the appropriated amines). Oxidation of the 4-MTA with mCPBA afforded the sulfone **4s** [34].

Type II compounds: The required benzaldehydes **7a-e** for Type II compounds **9a-e** were prepared by reaction of 4-fluorobenzaldehyde (**6**) with the appropriate thiol in a sealed tube at 120^{o} in high yield in a modification of the method of Tanaka [35], (Scheme 2). Condensation of the aldehydes **7a-e** with nitroethane afforded the nitrostyrenes **8a-e** which were then reduced with lithium aluminium hydride to afford the desired amines **9a-e**. 4-Ethylthioamphetamine (**9a**) [27] was also obtained by reductive amination of the ketone **14a**, (Scheme 2).

Type III and IV compounds: The synthetic route to the Type III compounds **15a-d** and Type IV compounds **15e-i** is illustrated in Scheme 3 and required the common precursor ketones **12a, b**. Henry condensation of nitroethane and nitropropane with 4-bromobenzaldehyde (**10**) afforded the nitrostyrenes **11a, b** which were then reduced to the corresponding ketones **12a, b**. Quantitative conversion to the 1, 3-dioxolanes **13a, b** was followed by lithiation and reaction with diethyl sulphide. Reductive amination of the ketones **14a, b** with the appropriate amines afforded the required products **15a, b, d-g, i**. The N-hydroxy compounds **15c, h** were obtained via sodium cyanoborohydride reduction (at pH3) of the oximes **16a, b** respectively, (Scheme 3). In the ^{1}HNMR spectrum of **15h**,

the OH and NH protons are initially displayed as two broad singlets at δ5.20 and δ6.25; each integrating for one proton. In subsequent ^{1}HNMR spectra acquired over a period of 480 seconds, a broad signal at δ5.80, integrating for two protons had replaced these two signals. This exchange may be due to trace of acid present and is also observed for compound **15c**.

Type V compounds: Synthesis of the Type V compounds **19a-q** is illustrated in Scheme 4. Ketone **18** was obtained by Henry condensation of 4-methylthiobenzaldehyde (**1**) with nitropropane and subsequent reduction of the nitrostyrene **17.** Reductive amination of the ketone **18** afforded the required products **19a-l** and **19n.** The N-hydroxy derivative **19m,** together with the N-alkoxy compounds **19o-q** were obtained via reduction of the oximes **20a-d** which were obtained from the ketone **18** by condensation reaction with the appropriate amines (Scheme 4).

Type VI compounds: Synthesis of the Type VI compounds **25a** and **25b** was achieved in moderate yield by reductive amination of the ketones **22** and **24** respectively, (Scheme 5). Treatment of the thioether **23** (obtained by alkylation of methythiobenzene with n-butyl iodide and sec-butyl lithium[36, 37]) with propionyl chloride afforded the ketone **24**, while Grignard alkylation of 4-methylthiobenzaldehyde (**1**) provided the alcohol **21** which was oxidised to ketone **22** using PCC.

Insert Schemes 1-5

Insert Tables 1-4

2.2 Inhibition of SERT activity

Amphetamines (AMPHs) are thought to release stores of catecholamines from nerve endings by converting the respective molecular transporters into open channels [38].They are thought to compete with substrate for the transporters, reversing the transport of monoamines by either binding to the transporter as a substrate or binding without being transported. It has been difficult to determine if amphetamines are genuine substrates of SERT, due to their lipophilic nature and to the lack of a crystal structure of human SERT. [39]. The amphetamine analogues, 4-MTA, MDMA, 3,4-methylenedioxyamphetamine (MDA) and para-chloroamphetamine (PCA) have all been previously shown to inhibit SERT activity with IC_{50} values of 74nM (4-MTA) [29] , 425nM (MDMA)[40], 478nM (MDA) [25] and 182nM (PCA) [25] respectively. These amphetamines also inhibit the activity of the noradrenaline transporter (NAT) (2,375nM (4-MTA), 405nM (MDMA), 266nM (MDA) and 207nM (PCA)) and inhibit the activity of the dopamine transporter (DAT) (3,073nM (4-MTA) , 1,442nM (MDMA), 890nM (MDA) and 424nM (PCA))[25, 29, 41]. Para-methoxyamphetamine (PMA) is another common substituted amphetamine that is thought to act in a similar way to MDMA, with evidence of PMA decreasing SERT binding sites in rat forebrains [42] and synpatosomal 5-HT uptake and content [43, 44].

To determine the potential SERT binding activity of these derivatives, the 4-MTA library of compounds synthesised was screened using a [^{3}H] 5-HT uptake inhibition assay with HEK293 (Human Embryonic Kidney) T-REx cells stably expressing rSERT. The percentage reuptake of [^{3}H] 5-HT for the compounds was initially determined at concentrations of 1µM and 100µM. The IC_{50} value for reuptake inhibition of the more

potent compounds (>90% uptake inhibition at 100µM) was then determined from the appropriate sigmoidal dose-dependent curves. The SSRI citalopram was used as a positive control and was found to have an IC_{50} of 3.17nM, while the amphetamines MDMA, MDA and PMA gave inhibition of serotonin reuptake with IC_{50} values of 1.06µM, 0.996µM and 173nM respectively (Tables 1-4). The results obtained for the SERT reuptake experiments for the series of compounds synthesised together with the cLogP values are given in Tables 1-4. Compounds 4-MTA and PMA which are para substituted with electron donating groups $-SCH_3$ and $-OCH_3$ respectively, show considerably more potent SERT activity than 3, 4-methylenedioxy substituted MDA and MDMA.

Examination of the data from the Type I compounds (Table 1) showed that 4-MTA (**4a**) was the most active compound in the series as a serotonin reuptake inhibitor with IC_{50} 0.207µM, (previously reported SERT reuptake inhibition potency for 4-MTA was hSERT K_i 0.45+0.10µM [45] and whole rat brain synaptosomes determinations IC_{50} 0.074$\pm$0.010µM [25]). It was found to be more potent than MDMA (1.06 µM) and MDA (0.996µM), had a similar activity to PMA (173nM) but was not as potent as the SSRI, citalopram at SERT (3.17nM).

Substantial SERT activity is preserved for small substituents on the primary amine group e.g. compounds **4b**, **4l**, **4o**, **4p** which have IC_{50} values in the range 0.417-0.664µM. A dramatic decrease in the binding capabilities of the N-hydroxy, N-methyl analogue **4n,** (IC_{50} 25.06µM) was observed compared with **4a**. Oxidation of 4-MTA to the sulfone product **4s** resulted in very low activity for SERT reuptake inhibition (100% reuptake at 100mM), possibly due to poor solubility of the product. No clear correlation was

observed between the calculated lipophilicity (cLogP) of the compounds **4a-s** and the SERT reuptake activity (Table 1).

The effect of the nature and size of the sulphur substituent was investigated in the Type II compounds (Table 2). Introduction of the ethyl or benzyl substituent on the sulphur resulted in small decrease in SERT activity (IC_{50} values of 1.403μM and 0.679μM respectively for compounds **9a** and **9c**) compared with 4-MTA (**4a**). Introduction of the t-butyl and aryl sulphur substituents in compounds **9b** and **9e** and basic substituent in compound **9d** resulted in decrease in activity**.**

The effect of N-substitution in the S-ethyl compounds was next examined, (Type III compounds, Table 3). Small substituents such as Me, (**15a**), Et (**15b**) and OH (**15c**) resulted in improved activity over the unsubstituted compound **9a.** In the series of 4-ethylthiosubstituted α-ethylphenethylamines (Type IV compounds), the primary amine compound **15e** was the most potent, with IC_{50} = 1.290μM. Introduction of single N-substituents (Et or OH) or disubstituted (OH and Me) resulted in a small decrease in activity as expected (Table 3).

The effect on SERT reuptake inhibition of the elongation of amphetamine carbon scaffold of 4-MTA to a 4 carbon chain was also examined in Type V compounds (Table 4). Most compounds in the series displayed good SERT activity (IC_{50} values in the range 0.399-2.821μM). The optimum activity for the series was displayed by compounds having a methyl or propargryl nitrogen substituent, e.g. compounds **19b** (IC_{50} = 0.437μM) and **19g** (IC_{50} =0.399μM). The related 1-aminophenethylamines **25a** and **25b** was found to be inactive at SERT with IC_{50}>25μM, indicating that the position of the primary amine in the phenethylamine structure is critical for SERT activity.

The majority of derivatives inhibited the uptake of 5-HT with IC_{50} values in the high nanomolar range, similar to the IC_{50} values of other known amphetamines at SERT. These IC_{50} values were found to be significantly higher than the IC_{50} value of a known inhibitor of SERT (citalopram, 3.17nM). We can therefore hypothesise that these derivatives are most likely acting in the same way as other amphetamines at SERT, by reversing the transport of 5-HT, by either binding as a substrate or binding without being transported. Further experiments, such as 5-HT-release experiments or in vivo studies would be needed to verify such speculations.

2.3 Molecular Modeling Study

2.3.1 Homology Model of hSERT

To date no crystal structure of human SERT (hSERT) exists to avail of in the ligand binding site docking process. A recent homology model of hSERT was constructed by Jorgensen et al [28] using LeuT as a template (which belongs to the same transporter family as SERT) and containing escitalopram as a bound ligand. Human SERT is 92% homologous to the rat protein [46] (rSERT used in SERT inhibition study) and has similar residues involved in ligand and sodium binding. Subsequent investigation of the flexibility of the binding site in complex with the natural substrate (5-HT) was undertaken to determine key protein ligand interactions through MD simulation in a membrane environment [47].

In the present study FlexE [48] was selected as a docking platform to allow these binding site variations to be taken into account. To establish the key interactions that the series of 4-MTA analogues make, it was necessary to initially determine the binding modes for 5-

HT and the related methylenedioxymethamphetamine (MDMA) within the SERT homology model. Figure 2 illustrates the key interactions that are observed for 5-HT, 4-MTA and MDMA within the active site of SERT where the cationic head is paramount to these interactions and residues Ala96, Asp98 and Phe335 are common to the binding mode. If the terminal nitrogen of the ligand is unsubstituted, it will interact (H-bond) with all three residues, Ala96, Asp98, Phe335 whereas, if there is a substituent on the terminal nitrogen, as in MDMA, only interactions with Asp98 and Phe335 occur. Interactions with Ala96, Asp98, Phe335 were also found to be important for the representative 4-MTA derivative, **9a** binding in the 5-HT binding site (Fig.2D). Recent findings by Walline et al from hSERT mutant studies of transmembrane helix III (TMHIII) indicate that key residue Ile172 is involved in the binding interaction of amphetamines, while Tyr 95 and Ile172 have been previously reported as key residues [49-51]. These interactions are identified in our studies of 5-HT, MDMA, 4-MTA, and its related analogue **9a**. 5-HT also makes additional interactions with Thr439 to ensure efficacious binding of the endogenous ligand. The interaction of Thr439 is also present in, MDMA, **9a** and escitalopram binding to SERT but is not present in the 4-MTA model.

Reuptake inhibitors such as escitalopram are thought to inhibit SERT function by binding to the substrate-binding site, where they are thought to interact via the protonated ligand amine of escitalopram and the Asp98 residue on SERT. Several hydrophobic contacts between aromatic parts of the ligand and residues Ile172, Phe341, Gly442 and Tyr176 are also thought to be important for escitalopram binding to SERT [28]. It has also been recently demonstrated by Anderson et al, the importance of a direct contact between Ser438 and aminopropyl groups of SSRIs and tricyclic antidepressants as a critical

determinant for their potency at SERT [52] and it is suggested that an overlap of substrate and inhibitor sites in hSERT exists. This implies that, antidepressants may act by a mechanism that involves direct occlusion of the 5-HT site. In the present study, a similar binding pattern was observed for escitalopram (Fig.2E) as with 5HT, 4-MTA, MDMA and **9a** (Fig.2A-D), identifying key interactions of each ligand with residues Tyr95, Ile172, Asp98 and Phe335 on SERT, conclusive with previous findings. The main difference observed is the presence of an arene-cation bond between escitalopram and Tyr95, an interaction that is absent in the MDMA, 5-HT, 4-MTA and **9a** models (Fig.2). In all cases, it is important to note that the interactions illustrated may vary slightly depending upon the docking routine and subsequent minimisation protocols chosen.

The importance of a direct contact between Ser438 and aminopropyl groups of SSRIs and tricyclic antidepressants as a critical determinant for the potency observed [52] was found in our modelling studies, where the positioning of Ser438 ~4.5Å from the escitalopram aminopropyl group, effectively shapes the 5-HT binding site. This interaction is also present in the 4-MTA, MDMA, **9a** and 5-HT models supporting the theory that antidepressants may act by a mechanism that involves direct occlusion of the 5-HT site. These results therefore appear to be consistent with the ability of **9a** and the other novel 4-MTA derivatives binding to SERT in the 5-HT binding site in a similar way to MDMA, 5-HT and 4-MTA.

Insert Figure 2

2.3.2 Structure Activity Relationship - SERT Binding and Inhibition

A detailed molecular modeling study was undertaken to rationalise the observed SERT binding activity for the 4-MTA type compounds synthesised. An initial examination of the docked structure of compound **4a** (4-MTA) with the internal vDW surface of the cavity mapped shows that there is ample space in the SERT ligand binding site to accommodate the N-alkyl substituent of these ligands (Figure 2). Chemical analogues in the Type I series with increasing chain length substitutions close to the cationic head of 4-MTA generate steric hindrance and in turn prevent interaction other than salt bridge interaction with additional residues such as Phe335 and Ala96. This translates to a sequential decrease in SERT activity observed as the size of the alkyl or alkoxy nitrogen substituent increases. The introduction of both hydroxyl and methyl substituents rather than the cationic primary amine in **4a** neutralises any positive charge at pH7.4 and consequently may interfere with the ability of **4m** to form a salt bridge with Asp98 (Figure 3A). As a result a weaker H-bond is formed to Asp98 through the oxygen of the hydroxylamine **4m** when compared with interactions proposed for primary amine **4a** (Figure 3A) and SERT reuptake inhibition is reduced. This result underlines the fact that a positive charge at this position is a crucial determinant of SERT reuptake. The effect could also be explained by steric effects of the nitrogen substituents which prevent the cationic nitrogen from interacting with Asp98.

The 2D depiction of the docked complex **9a (**4-ethylthioamphetamine**,** Type II) in the SERT binding site is illustrated in Figure 2, where the contact with Phe335 and Asp98 are clearly evident. The decrease in activity observed on introduction of larger S substituents, as in compounds **9b**, **9d** and **9e** is possibly due to increased solvent exposure

correlating with substituent size. The docked structure of the S-ethylsubstituted compound **15c** (Type III) in the SERT binding site is illustrated in Figure 3B showing the key interactions of the hydroxyl with Asn101 and Asp98 and the nitrogen with Asp98 and Tyr176. Many of the compounds of Type V with α-ethyl substitution retain SERT binding activity. The docked structure of compound **19b** in the SERT binding site is illustrated in Figure 3C, which illustrating that the contacts of the nitrogen with Phe335 and Asp98 are preserved for this scaffold structure.

Insert Figure 3

2.4 Biological Activity

2.4.1 Cytotoxicity in HEK293 and hSERT overexpressing cell lines

4-MTA has previously been shown to be toxic to rat hypothalamic cultures [53] and to CYP2D6 expressing cells [54]. MDMA and its related metabolites, including MDA have been found to be toxic to numerous cell lines [19, 55-59] while there is little information on the in vitro cellular toxicity of PMA or PCA. Metabolic pathways for 4-MTA have been demonstrated in vivo in mice [60] and in vitro in primary hepatocytes [61]. In humans, metabolism of 4-MTA could involve the following: oxidative deamination to a ketone metabolite, that can be further reduced to the corresponding alcohol or suffer degradation of the side chain into the 4-methylthiobenzoic acid metabolite , ring hydroxylation to a phenolic structure or β-hydroxylation of the side chain to 4-methylthioephedrine, oxidation of thioether [54]. The metabolism of the new derivatives reported in the present study is most likely the same.

To assess the potential SERT dependent toxic effects of 4-MTA and related analogues, the cytotoxicity of the series was determined in HEK cells previously shown to stably express the human monoamine transporter, hSERT (Supplemental Figure 1) using an in vitro cytotoxicity assay. The Neutral Red (NR) assay is a simple, accurate reproducible system where viable cells take up the NR dye with any change in the amount of the dye incorporated by the cells indicating the degree of cytotoxicity caused by the test material. The cytotoxic potentials of a library of 4-MTA derivatives on HEK293 and HEK293hSERT cells are presented in Tables 1-4 and Figure 4. It was found that MDMA, MDA and PMA did not show any selective cytotoxic effects toward SERT expressing cells.

Insert Figure 4

2.4.1.1 Selective toxicity of 4-MTA derivatives 4a-r, (Type I) and derivatives 19a-q, (Type V) on hSERT overexpressing HEK cell lines in vitro.

All of the Type I and Type V derivatives displayed similar low micromolar cytotoxic potencies implying that neither show any obvious selective cytotoxic activity toward SERT (based on an unpaired T-Test with $P<0.05$ representing a significant difference) (Table 1 and 5, Figure 4). Compounds **4n**, **4s, 19b** and **19d** displayed the least cytotoxic effect on both cell lines with EC_{50} values in the high micromolar range. The N-hydroxy, N-methyl analogue **4n,** (IC_{50} 25.06µM) showed decreased SERT binding activity compared with **4a** and oxidation of 4-MTA to the sulphone product **4s** resulted in very low activity for SERT reuptake inhibition However, derivative **19b** showed optimum SERT binding activity (IC_{50} = 0.437µM) for the Type V series and **19d** also had good

SERT inhibitory activity (IC_{50} = 0.621μM) compared to 4-MTA (IC_{50} = 0.207μM). These results imply that there is no correlation between the SERT binding and inhibitory activities of these derivatives with their cytotoxic activities.

Statistical analysis of mean values for each concentration group revealed that at 1μM, 4-MTA (**4a**) and compound **19h** showed a selective cytotoxic effect to SERT expressing cells ($P<0.05$) (data not shown). Although 4-MTA is highly selective for serotonin uptake inhibition compared to dopamine (ratio of IC_{50} value for serotonin versus dopamine: $3.07x10^3$) [25] and noradrenaline (ratio IC_{50} value for serotonin versus noradrenaline: $2.37x10^3$) [25], the cytotoxic effect of 4-MTA on HEK cells overexpressing the dopamine transporter (DAT) and noradrenaline transporter (NAT) proteins was also determined and found to be very similar indicating that the cytotoxicity observed for the compounds cannot be directly related to SERT activity. (For **4a** pEC_{50} values for HEK cells, 4.73±0.14; SERT expressing HEK cells, 4.65±0.15; DAT expressing HEK cells, 4.64±0.14; NET expressing HEK cells, 4.46±0.14). It therefore appears that SERT expression alone may not be sufficient to confer 4-MTA-toxicity to HEK cell lines. To the best of our knowledge this study is the first report comparing the cytotoxicities of a range of different 4-MTA derivatives on a human serotonin transporter overexpressing cell line hSERT HEK293.

2.4.1.2 Lack of selective toxicity of 4-MTA derivatives 9a-d (Type II), 15a-d (Type III) and 15e-i (Type IV) on hSERT overexpressing HEK cell lines in vitro.

All of the Type II and Type IV derivatives displayed similar ($P>0.05$) low micromolar cytotoxic potencies implying that there is no obvious selective cytotoxic activity toward

SERT. Compounds, **15f** and **15i** had less of a general cytotoxic effect on both cell lines with EC_{50} values in the high micromolar range (Tables 2 and 3) despite their moderate SERT inhibitory activity with IC_{50} values of 1.804μM and 2.003μM respectively, again further proving the lack of correlation between cytotoxic effect and SERT inhibitory activity.

2.4.1.3 Selective cytotoxicity toward SERT expressing Burkitt's lymphoma

The DG-75 cell line is a B-lymphocyte, chemoresistant Burkitt's lymphoma cell line derived from a metastatic pleural effusion (lung) of a sporadic case of EBV (Epstein Barr Virus) negative Burkitt's lymphoma [62] that has been shown to express SERT (Supplementary Figure 1). The SHSY-5Y cell line is a neuroblastoma cell line established from a metastatic bone tumour [63] that was chosen to use in the screen as it has been shown not to express SERT [64]. In this study using the neutral red assay it was found that a number of 4-MTA derivatives in particular the Type IIa derivatives showed potent cytotoxic activity toward the chemoresistant SERT expressing BL cell line DG-75 (Table's 1-5) and to the SHSY-5Y cell line with approximate pEC_{50} values of 5 correlating to an EC_{50} range of between 1μM and 5μM. These Type II derivatives showed a small decrease in SERT activity (IC_{50} values of 1.403μM and 0.679μM respectively for compounds compound **9a** and **9c**) compared with 4-MTA (**4a**). The introduction of the *t*-butyl and aryl sulphur substituents in compounds **9b** and **9e** and basic substituent in compound **9d** resulted in decrease in activity**.** The lack of potent SERT binding by **9e** and the result that compound **9e** was found to have the most potent cytotoxic effect on both the DG-75 and SHSY-5Y as well as on the other cell lines (with no evidence of selective

activity toward the HEK293hSERT) (Table 2) cell line implies that SERT is most likely not involved in such cytotoxicity. MDMA and PMA were also found to more cytotoxic to the DG-75 cell line, compared to MDA and 4-MTA, whereas 4-MTA was the only one of these controls that showed cytotoxicity to the SHSY-5Y neuroblastoma cell line.

2.4.2 Antiproliferative Activity

To assess if the observed cytotoxicity of these compounds was due to an antiproliferative effect, compound **9e** was chosen as a representative example using the Alamar Blue assay for antiproliferative activity in a range of malignant cell lines. **9e** was found to have potent antiproliferative activity in two BL cell lines (MUTU-I and DG-75) (Fig.5A). After 24h (MUTU-I) and 72h (DG-75), only 10% of cells were found to be viable upon treatment with 50μM of **9e**, implying 90% of the cells ceased to grow (Fig. 5A). Derivative **9e** was also found to have a potent antiproliferative effect in three breast carcinoma cell lines (MCF-7, MDA-MB231, 4TI) (Figs. 5A and 5B) having a potent effect (~90% antiproliferative activity) in the 4TIs and MDA-MB321 cells at 50μM and leaving only 40% of MCF-7 cells viable after 24h treatments (Fig.5B). The microtubule targeting agent, Paclitaxel (Taxol) was used as positive control for antiproliferative activity as it has been shown to have effects in BL cell lines with IC_{50}'s of between 10 and 40nM [65, 66]. In other cell lines IC_{50} values for taxol have ranged from 4 to 35ng/ml with cytotoxic effects greater on proliferating cells over quiescent cells [67].

Insert Figure 5

2.4.3 Apoptotic Activity

Designing drugs that can induce programmed cell death (PCD), namely apoptosis, of a cancer cell, whilst ignoring the 'normal cells' of the body is imperative to the future development of safe effective anticancer agents. A drug that can induce PCD in vitro in malignant cell lines has the potential to become an anticancer agent. In order to determine if derivative **9e** induces apoptosis in Burkitt's lymphoma propidium iodide (PI) FACS (Fluorescent-activated cell sorting) analysis was carried out. PI was chosen as the preferred method to detect apoptosis to allow rapid FACS analysis to occur allowing any pro-apoptotic and cell cycle effects to be easily identified. Annexin V staining was avoided, as phosphatidyl serine exposure can result from multiple activation pathways in the cell and is not the best indicator of apoptosis to use in B cell-derived cell lines from its cross reactivity with the B cell receptor [68]. PI FACS analysis was carried out initially using 50μM concentration of compounds **9e** and **25a** (as this also had a potent effect in the DG-75 cell line from the NR assay) in two BL cell lines and in the human promoeolytic leukaemia cell line (HL-60). It was found that both **9e** and **25a** showed strong pro-apoptotic activity (40-65% cell death) in all of the cell lines analysed (Fig.5C). 10μM Taxol was used as a positive control for apoptosis as it has been previously shown to induce apoptosis in HL-60 cells [69] .Further characterisation of these effects using sigmoidal-dose concentration curves (data not shown) revealed EC_{50} values for the pro-apoptotic effects of both derivative **9e** and **25a** in the low micromolar range (e.g. for MUTU-c179 cell line, EC_{50} values of 14.21μM and 21.02μM respectively) (Table 5).

Insert Table 5

2.4.4 Selective Anticancer Activity

In order to assess the selectivity of these two derivatives towards proliferating malignant cells over the 'normal' cells of the body, the effects of **9e** and **25a** were assessed in peripheral blood mononuclear cells (PBMCs). After 24h, it was found that at 10μM, derivatives **9e** and **25a** had little effect on the viability of PBMCs compared to the effect of 10μM **9e** on a range of malignant cells lines (Figs. 5 and 6).At 50μM, derivative **9e** reduced the viability of these PBMCs to approximately 50% (compared to <10% in the DG-75, MUTU-I, and breast cancer derived cell lines Fig.5). Derivative **25a** had less of an effect in the PBMCs at 50μM (Fig.6B) compared to its pro-apoptotic effect in the MUTU-I, HL-60 and DG-75 cell lines. Such results are consistent with these agents selectively targeting cells of malignant origin over 'normal cells' of the body.

To assess the anticancer therapeutic potential of these derivatives, a number of issues require discussion. The use of 4-MTA derivatives as anticancer agents may pose the question of these agents having CNS activity or toxicity. Both derivatives **9e** and **25a** have very little SERT inhibitory activity ((IC_{50}'s of 14.33μM and >25μM respectively compared to 4-MTA (IC_{50} 0.207μM) implying possible differential pharmacological activity to 4-MTA and hence a lack of CNS activity. Further safety-toxicology studies on these derivatives will assess such concerns. Alternatively, most anticancer agents have limited brain penetration resulting in a greater need for new pharmacological approaches to enhance delivery into the brain. Despite aggressive therapy, the majority of primary and metastatic brain tumour patients have a poor prognosis with brief survival periods as before systemically administered drugs can distribute into the CNS, they must cross two membrane barriers, the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier

(BCB)[70]. Assessing the effects of these agents against primary and metastatic brain tumour derived cell lines is also worth further future exploration.

A comparison of the potency of these agents to induce apoptosis to other effective anticancer agents would place them in middle range between anti-metabolites and receptor targeted agents. Although derivatives **9e** and **25a** appear to have a similar effect at 10μM to the positive controls used in this study, the EC_{50} values of the lead 4-MTA derivatives places them agents behind the efficacy of other known chemotherapeutic agents (14-20μM for the 4-MTA derivatives compared to 10-40nM range of the other agents). As these derivatives are of amphetamine origin, they may be having an indirect SERT mediated effect, whereby these agents may release 5-HT from intracellular stores, initiating a variety of secondary effects, which could in turn induce PCD in the malignant cell lines. As these derivatives were found from an initial screen, with little information regarding their suspected target-based mechanism of action, extensive further investigations are required to develop more potent analogues and to identify their mechanism of action.

Insert Figure 6

3. Conclusion

The original rationale behind this study was to design and synthesise a series of potentially potent novel SERT ligands based on the structure of 4-MTA, working on the hypothesis that SERT could act as a pro-apoptotic target in Burkitt's lymphoma. In the present work, a series of 4-MTA analogues, with or without N-alkyl and /or C-α methyl or ethyl groups were synthesised and tested for SERT binding. It was found that the

majority of these novel compounds were found to inhibit the reuptake of serotonin at concentrations below 1μM, implying they behave in a similar way to 4-MTA and other amphetamines. The structure-activity relationship molecular modelling study of the SERT inhibition for these compounds revealed similar specific molecular binding requirements for 4-MTA and related analogues at the 5-HT binding site, again implying these derivatives behave in similar way to 4-MTA and MDMA.

No SERT-dependent cytotoxic effect was found for any of the compounds despite the ability of these 4-MTA derivatives to reduce the viability of both HEK293 cells and HEK cells overexpressing hSERT.Despite this lack of correlation between SERT expression and selective cytotoxicity, it was found that a number of the Type IIa compounds showed strong cytotoxic effects (EC_{50} values of less than 10μM) toward the chemoresistant Burkitt's lymphoma cell line DG-75, which expresses SERT. These derivatives also had a potent effect on the neuroblastoma cell line SHSY-5Y which does not express SERT [64] further eliminating a pro-apoptotic role for SERT.

Derivative **9e** was subsequently investigated for antiproliferative activity and was found to show potent activity in two Burkitt's lymphoma and three breast cancer cell lines having an effect at 10μM and 50μM. **9e** and **25a** were further found to induce apoptosis in two BL cell lines and in leukemia cell line with EC_{50} values for such an effect in the low micromolar range (Table 5). These derivatives were also shown to have little effect on peripheral blood mononuclear cells implying the potential selectivity of these agents for cells of malignant origin.

Using the 4-MTA scaffold as a base to design new SERT targeting ligands could be disputed as 4-MTA is a known MDMA-like drug of abuse and is classified as a Schedule

1 controlled substance [71]. There have been six reported fatalities associated with 4-MTA in the UK and Netherlands together with one death and seven non-fatal cases of 4-MTA intoxication in Belgium [72] . However, such fatalities are thought to be associated with large doses (21.3-9800mg/kg) [25, 72], the use of other drugs and to interindividual differences in metabolism [54, 55]. In vivo, reported fatal 4-MTA intoxications consist of blood concentrations of 4-MTA between 8.27 and 29.6μM [73-76] whereas in tissue such as brain or liver concentrations have been found as high as 170-202μM [74, 77, 78]. Assuming the derivatives used in this study behave in a similar way to 4-MTA at the concentrations (EC_{50}s in the low micromolar range) used in this study, they could be compared to the in vivo toxic concentrations (25-480μM) [74, 75] mentioned in the reported fatalities. However as MTA is a relatively new amphetamine derivative and the number of reported poisonings is limited, the pathology findings and the mechanism of death due to this product have not yet been fully evaluated. Further safety-toxicology studies on 4-MTA derivatives will assess such concerns. In addition, both derivatives **9e** and **25a** have very little SERT inhibitory activity ((IC_{50}'s of 14.33μM and >25μM respectively compared to 4-MTA (IC_{50} 0.207μM) implying possible differential pharmacological activity to 4-MTA.

Burkitt's lymphoma remains a serious health problem in areas where it is endemic [23]. In certain regions of equatorial Africa and other tropical locations between latitudes 10° South and 10° North, incidence is 100 per million children [79]. Despite its incidence in developing countries it is also rapidly increasing in developed countries. Burkitt's and Burkitt's-like/atypical Burkitt's lymphomas make up the largest group of HIV-associated non-Hodgkin lymphomas, comprising up to 35–50% of these neoplasms [80]with the

relative risk of non-Hodgkin lymphoma increased 60–200 fold in HIV-infected patients [81]. The need for the development of selective, potent economical alternatives in the treatment of Burkitt's lymphoma is worthy of further exploration and interest. The pro-apoptotic abilities of derivatives **9e** and **25a** provide sufficient justification for the further development of more potent selective 4-MTA related analogues as anticancer agents and to search for their possible target-based mechanism of action.

4. Experimental

4.1. Materials and methods: chemistry

Uncorrected melting points were measured on a Gallenkamp apparatus. Infra-red (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrometer. ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded at 27°C on a Brucker DPX 400 spectrometer (400.13MHz, ^{1}H; 100.61MHz, ^{13}C; 376.47MHz, ^{19}F) in either $CDCl_3$ (internal standard tetramethylsilane (TMS)) or CD_3OD. For $CDCl_3$, ^{1}H-NMR spectra were assigned relative to the TMS peak at 0.00 δ and ^{13}C-NMR spectra were assigned relative to the middle $CDCl_3$ triplet at 77.00 ppm. For CD_3OD, ^{1}H and ^{13}C-NMR spectra were assigned relative to the center peaks of the CD_3OD multiplets at 3.30 δ and 49.00 ppm respectively. ^{19}F-NMR spectra were not calibrated. Coupling constants are reported in Hertz. For ^{1}H-NMR assignments, chemical shifts are reported: shift value (number of protons, description of absorption, coupling constant(s) where applicable). Electrospray ionisation mass spectrometry (ESI-MS) was performed in the positive ion mode on a liquid chromatography time-of-flight mass spectrometer (Micromass LCT, Waters Ltd., Manchester, UK). The samples were introduced into the ion source by an LC system

(Waters Alliance 2795, Waters Corporation, USA) in acetonitrile: water (60:40 %v/v) at 200 μL/min. The capillary voltage of the mass spectrometer was at 3 kV. The sample cone (de-clustering) voltage was set at 40V. For exact mass determination, the instrument was externally calibrated for the mass range m/z 100 to m/z 1000. A lock (reference) mass (m/z 556.2771) was used. Mass measurement accuracies of < ±5 ppm were obtained. Low resolution mass spectra (LRMS) were acquired on a Hewlett-Packard 5973 MSD GC-MS system in electron impact (EI) mode. Elemental analyses were performed on an Exetor Analytical CE4400 CHN analyser in the microanalysis laboratory, Department of Chemistry, University College Dublin. R_f values are quoted for thin layer chromatography on silica gel Merck F-254 plates, unless otherwise stated. Flash column chromatography was carried out on Merck Kieselgel 60 (particle size 0.040-0.063mm), Aldrich aluminium oxide, (activated, neutral, Brockmann I, 50 mesh) or Aldrich aluminium oxide, (activated, acidic, Brockmann I, 50 mesh). Compounds **3**[31], **4s**[34], **17** [82] were prepared according to the literature methods.

4.1.2 General method A: reductive amination procedure

To a stirred mixture of 1-(4-methylthiophenyl)-2-alkanones **3** or **18** (11.45 mmol) in dry methanol (50 mL) was added either ammonium acetate (primary amines) or an appropriate alkylamine HCl salt (80 mmol) and sodium cyanoborohydride (15.92 mmol, 1.00 g) and the reaction was stirred at 20°C for 72 h. The pH of the reaction was occasionally adjusted to pH 5-6 by the addition of 4 M methanolic HCl as determined by damp universal pH paper. Excess hydride was decomposed by the addition of 10% aq. HCl (150 mL) and resulting aqueous phase washed with dichloromethane (3 x 50mL). The aqueous phase was basified with 15% aq. NaOH solution and extracted with

dichloromethane (3 x 50mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and volatiles removed *in vacuo* leaving the product as an oil.

4.1.2.1. 2-Amino-1-(4-methylthiophenyl) propane (4-MTA) (4a) was prepared from **3** and ammonium acetate according to the general method A. Colourless oil (85%) (lit.[29] b.p. 108-118°C/0.6mmHg). IRν_{max} (film) 3359, 3285, 1584, 1093 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.10 (3H, d, J = 6.5Hz, CHC*H*$_3$), 1.25 (2H, br s, N*H*$_2$), 2.46 (3H, s, SC*H*$_3$), 2.48 (1H, dd, J = 8.0 Hz, J = 13.3Hz, ArC*H*$_2$CH), 2.67 (1H, dd, J = 5.0 Hz, J = 13.3 Hz, ArC*H*$_2$CH), 3.13 (1H, m, ArCH$_2$C*H*), 7.10 (2H, d, J = 8.0 Hz, Ar*H*), 7.21 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 23.4, 46.0, 48.3, 127.0, 129.6, 135.7, 136.7; MS *m/z* 181 (M^+). **HCl salt**: Colourless solid. mp 187-189 ºC (ethanol/hexane) (lit. [27, 29]190-191°C).

4.1.2.2. 2-*N*-Methylamino-1-(4-methylthiophenyl) propane (4b) was prepared from **3** and methylamine HCl according to the general method A. Colourless oil (49%). IRν_{max} (film) 3318, 2789, 1600, 1094 cm^{-1}. ^1H NMR ppm ($CDCl_3$) 1.04 (3H, d, J = 6.0 Hz, CHC*H*$_3$), 1.35 (1H, br s, N*H*), 2.39 (3H, s, NC*H*$_3$), 2.47 (3H, s, SC*H*$_3$), 2.57 (1H, dd, J = 6.5 Hz, 13.3 Hz, ArC*H*$_2$CH), 2.67 (1H, dd, J = 7.0 Hz, 13.0 Hz, ArC*H*$_2$CH), 2.76 (1H, m, ArCH$_2$C*H*), 7.11 (2H, d, J = 8.6 Hz, Ar*H*), 7.20 (2H, d, J = 8.6 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 19.6, 33.9, 42.8, 56.2, 127.0, 129.7, 135.7, 136.5. MS *m/z* 195 (M^+); **HCl salt**. Colourless solid., mp 161-162 ºC (ethanol/hexane)[27]. Anal. Calculated for $C_{11}H_{18}ClNS$: C, 57.00; H, 7.83; N, 6.04; S, 13.83. Found: C, 56.81; H, 7.79; N,6.05; S, 13.43%.

4.1.2.3. 2-*N*-Ethylamino-1-(4-methylthiophenyl)propane (4c) was prepared from **3** and ethylamine HCl according to the general method A. Colourless oil (35%).

IRν_{max} (film) 3306, 1600, 1093 cm^{-1}. ^{1}H NMR ppm ($CDCl_3$) 1.04 (3H, d, J = 6.0 Hz, CHC*H*$_3$), 1.06 (3H, t, J = 7.0 Hz, NCH$_2$C*H*$_3$), 1.26 (1H, br s, N*H*), 2.47 (3H, s, SC*H*$_3$), 2.53-2.75 (4H, m, CHC*H*$_3$, NC*H*$_2$CH$_3$), 2.88 (1H, m, ArCH$_2$C*H*), 7.10 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 15.3, 16.1, 20.1, 41.4, 43.0, 54.45, 126.9, 129.7, 135.6, 136.5. **HCl salt**. Colourless solid. mp 176-179 ºC (ethanol/hexane)[27]. Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.63; H, 8.20; N, 5.70; S, 13.04. Found: C, 58.48; H, 8.10; N, 5.82; S, 12.64%.

4.1.2.4. 2-*N*-(Isopropyl)amino-1-(4-methylthiophenyl)propane (4d) was prepared from **3** and *n*-propylamine HCl according to the general method A. Colourless oil (36%). IRν_{max} (film) 3304, 1599, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.97 (3H, d, J = 6.0 Hz, NCH(C*H*$_3$)$_2$), 1.00 (3H, d, J = 6.0 Hz, NCH(C*H*$_3$)$_2$), 1.04 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 1.18 (1H, br s, N*H*), 2.46 (3H, s, SC*H*$_3$), 2.51 (1H, dd, J = 7.0 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.70 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.92 (1H, m, NC*H*(CH$_3$)$_2$), 2.98 (1H, m, ArCH$_2$C*H*), 7.09 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 15.9, 20.4, 22.7, 23.5, 43.0, 45.1, 51.0, 126.7, 129.6, 135.5, 136.4. **HCl salt**. Colourless solid. M.p. 147-148ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{22}ClNS$: C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 59.89; H, 8.41; N, 5.57; S, 12.17%.

4.1.2.5. 2-*N*-Cyclopropylamino-1-(4-methylthiophenyl)propane (4e) was prepared from **3** and cyclopropylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: hexane/ethyl acetate 60/40). Pale amber oil (21%). IRν_{max} (film) 3313, 1599, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.26-0.51 (4H, m, NCH(C*H*$_2$)$_2$), 1.09 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 1.43 (1H, br s, N*H*),

2.06 (1H, m, NC*H*$(CH_2)_2$), 2.47 (3H, s, SC*H*$_3$), 2.55 (1H, dd, J = 6.5 Hz, 13.6 Hz, ArC*H*$_2$CH), 2.75 (1H, dd, J = 7.0 Hz, 13.6 Hz, ArC*H*$_2$CH), 3.01 (1H, m, ArCH_2C*H*), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 5.8, 7.0, 16.1, 20.4, 28.6, 43.0, 55.3, 127.0, 129.7, 135.7, 136.6. **HCl salt**. Colourless solid. M.p. 137-138ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{20}ClNS$: C, 60.56; H, 7.82; N, 5.43; S, 12.44. Found: C, 60.86; H, 7.59; N, 5.26; S, 12.21%.

4.1.2.6. 2-*N*-Allylamino-1-(4-methylthiophenyl)propane (4f) was prepared from **3** and allylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: dichloromethane/methanol 90/10). Amber oil (23%). IRν_{max} (film) 3310, 1600, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.06 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.37 (1H, br s, N*H*), 2.46 (3H, s, SC*H*$_3$), 2.58 (1H, dd, J = 7.0 Hz, 13.3 Hz, ArC*H*$_2$CH), 2.77 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.95 (1H, m, ArCH_2C*H*), 3.23 (1H, dd, J = 6.0 Hz, 14.0 Hz, NC*H*$_2$$CHCH_2$), 3.34 (1H, dd, J = 5.5 Hz, 14.0 Hz, NC*H*$_2$$CHCH_2$), 5.08 (1H, d, J = 10.5 Hz, NCH_2CHC*H*$_2$), 5.15 (1H, d, J = 17.0 Hz, NCH_2CHC*H*$_2$), 5.90 (1H, m, NCH_2C*H*CH_2), 7.10 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.0, 19.6, 42.5, 49.4, 53.7, 116.2, 126.9, 129.7, 135.8, 136.0, 136.1. **HCl salt**. Colourless solid. mp 170-172 ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{20}ClNS$: C, 60.56; H, 7.82; N, 5.38; S, 12.44. Found: C, 60.16; H, 7.74; N, 5.45; S, 13.23%.

4.1.2.7. 2-*N*-Propargylamino-1-(4-methylthiophenyl)propane (4g) was prepared from **3** and propargylamine HCl according to the general method A. Colourless oil (31%). IRν_{max} (film) 3291, 2104, 1600 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 1.42 (1H, br s, N*H*), 2.18 (1H, m, NCH_2CC*H*), 2.47 (3H, s, SC*H*$_3$), 2.59

(1H, dd, J = 6.5 Hz, 13.6 Hz, ArC*H*$_2$CH), 2.66 (1H, dd, J = 7.0 Hz, 13.6 Hz, ArC*H*$_2$CH), 3.13 (1H, m, ArCH$_2$C*H*), 3.40 (1H, dd, J = 2.0 Hz, 17.3 Hz, NC*H*$_2$CCH), 3.47 (1H, dd, J = 2.5 Hz, 17.3 Hz, NC*H*$_2$CCH), 7.12 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.0, 19.4, 35.4, 42.7, 52.3, 71.2, 81.8, 126.8, 129.7, 135.8, 135.9. **HCl salt**. Colourless solid. mp 173-177 ºC (dec.) (ethanol/hexane). Anal. Calculated for $C_{13}H_{18}ClNS$: C, 61.04; H, 7.09; N, 5.48; S, 12.54. Found: C, 60.37; H, 7.05; N, 5.66; S, 12.56%.

4.1.2.8. 2-*N*-(2-Hydroxyethyl)amino-1-(4-methylthiophenyl)propane (4h) was prepared from **3** and 2-hydroxyethylamine HCl according to the general method A. Colourless oil (68%). IRν_{max} (film) 3286, 3121, 1597, 1060 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.0 Hz, CHC*H*$_3$), 2.36 (2H, br s, N*H*, NCH$_2$CH$_2$O*H*), 2.46 (3H, s, SC*H*$_3$), 2.56 (1H, dd, J = 7.0 Hz, 13.3 Hz, ArC*H*$_2$CH), 2.68-2.82 (3H, m, ArC*H*$_2$CH, NC*H*$_2$CH$_2$OH), 2.88 (1H, m, ArCH$_2$C*H*), 3.59 (2H, m, NCH$_2$C*H*$_2$OH), 7.09 (2H, d, J = 8.5 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.0, 20.1, 42.9, 48.4, 54.3, 61.1, 126.9, 129.7, 135.8, 136.2. **HCl salt**. HRMS Calculated for $C_{12}H_{20}NOS$: (M^++1) 226.1266; Found: 226.1265.

4.1.2.9. 2-*N*-(2-Methoxyethyl)amino-1-(4-methylthiophenyl)propane (4i) was prepared from **3** and 2-methoxyethylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: dichloromethane/methanol 90/10). Colourless oil (28%). IRν_{max} (film) 3322, 1600, 1494 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.39 (1H, br s, N*H*), 2.55 (1H, dd, J = 7.0 Hz, 13.6 Hz, ArC*H*$_2$CH), 2.47 (3H, s, SC*H*$_3$), 2.74-2.86 (3H, m, ArC*H*$_2$CH, NC*H*$_2$CH$_2$O), 2.91 (1H, m, ArCH$_2$C*H*), 3.31 (3H, s, OC*H*$_3$), 3.48 (2H, t, J =

5.5 Hz, NCH_2CH_2O), 7.11 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 19.7, 42.6, 46.5, 54.6, 58.6, 71.8, 126.9, 129.7, 135.7, 136.2. **HCl salt**. Colourless solid. mp 156-158 ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{22}ClNOS$: C, 56.61; H, 8.04; N, 5.08; S, 11.62. Found: C, 56.63; H, 8.09; N, 5.04; S, 11.25%.

4.1.2.10. 2-*N*-(2-Ethanethiol)amino-1-(4-methylthiophenyl)propane (4j) was prepared from **3** and 2-ethanethiolamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: diethylether/methanol 50/50). Pale amber oil (27%). IRν_{max} (film) 3302, 2848, 1599, 1093 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.0 Hz, $CHCH_3$), 1.57 (1H, br s, N*H*), 2.46 (3H, s, SCH_3), 2.57 (1H, dd, J = 6.5 Hz, 13.0 Hz, $ArCH_2CH$), 2.66-2.96 (7H, m, $ArCH_2CH$, $ArCH_2CH$, NCH_2CH_2SH), 7.10 (2H, d, J = 8.5 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.0, 20.1, 38.8, 42.9, 45.2, 54.1, 126.8, 129.6, 135.7, 136.09. HRMS Calculated for $C_{12}H_{18}NS_2$: (M^+) 240.0881, found: 240.0875.

4.1.2.11. 2-*N*-(*n*-Propyl)amino-1-(4-methylthiophenyl)propane (4k) was prepared from **3** and *n*-propylamine HCl according to the general method A. Colourless oil (26%). IRν_{max} (film) 3312, 1600, 1092 cm^{-1}. ^1H NMR ppm ($CDCl_3$) 0.86 (3H, t, J = 7.5 Hz, $NCH_2CH_2CH_3$), 1.04 (3H, d, J = 6.0 Hz, $CHCH_3$), 1.37-1.54 (3H, m, N*H*, $NCH_2CH_2CH_3$), 2.46 (3H, s, SCH_3), 2.50 (1H, m, $NCH_2CH_2CH_3$), 2.55 (1H, dd, J = 7.0 Hz, 13.3 Hz, $ArCH_2CH$), 2.62 (1H, m, $NCH_2CH_2CH_3$), 2.69 (1H, dd, J = 7.0 Hz, 13.3 Hz, $ArCH_2CH$), 2.85 (1H, m, $ArCH_2CH$), 7.10 (2H, d, J = 8.5 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 11.6, 16.0, 20.0, 23.2, 42.9, 49.1, 54.3, 126.8, 129.6, 135.6, 136.4. **HCl salt**. Colourless solid. mp 160-162 ºC (dec.) (ethanol/hexane). Anal.

Calculated for $C_{13}H_{22}ClNS$: C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 60.35; H, 8.61; N, 5.08; S, 12.38%.

4.1.2.12. 2-*N,N*-dimethylamino-1-(4-methylthiophenyl)propane (4l) was prepared from **3** and dimethylamine HCl according to the general method A. Colourless oil (48%). IRν_{max} (film) 3073, 1600, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.91 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.31 (6H, s, N(C*H*$_3$)$_2$), 2.34 (1H, m, ArC*H*$_2$CH), 2.45 (3H, s, SC*H*$_3$), 2.74 (1H, m, ArCH$_2$C*H*), 2.91 (1H, dd, J = 4.5 Hz, 13.0 Hz, ArC*H*$_2$CH), 7.09 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 13.7, 16.2, 38.5, 40.6, 61.2, 126.9, 129.6, 135.2, 137.6. **HCl salt**. Colourless solid. mp 164-166 ºC (ethanol/hexane)[27]. Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.63; H, 8.20; N, 5.70; S, 13.04. Found: C, 58.35; H, 8.09; N, 5.66; S, 13.37%.

4.1.2.13. 2-(*N*-Hydroxy-*N*-methylamino-1-(4-methylthiophenyl)propane (4m) was prepared from **3** and *N*-hydroxy-*N*-methylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: diethylether). Colourless oil (20%). IRν_{max} (film) 3220, 1600, 1494 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.92 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.33-2.38 (1H, m, ArC*H*$_2$CH), 2.38 (3H, s, SC*H*$_3$), 2.60 (3H, s, NC*H*$_3$), 2.80-2.88 (1H, m, ArCH$_2$C*H*), 3.07 (1H, dd, J = 4.0 Hz, 13.3 Hz, ArC*H*$_2$CH), 6.36 (1H, br s, NO*H*), 7.02 (2H, d, J = 8.5 Hz, Ar*H*), 7.12 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.2, 30.3, 38.7, 44.0, 65.0, 127.0, 129.8, 135.6, 136.8. **HCl salt**. Colourless solid. mp 115-116 ºC (ethanol/hexane). Anal. Calculated for $C_{11}H_{18}ClNOS$: C, 53.32; H, 7.32; N, 5.65; S, 12.94. Found: C, 53.06; H, 7.24; N, 5.72; S, 12.65%.

4.1.2.14. 2-(*N*-Methoxy-*N*-methylamino-1-(4-methylthiophenyl)propane (4n) was prepared from **3** and *N*-methoxy-*N*-methylamine HCl according to the general method A. Colourless oil (73%). IRν_{max} (film) 3060, 2781, 1599, 1045 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.95 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.41 (1H, dd, J = 9.5 Hz, 13.1 Hz, ArC*H*$_2$CH), 2.46 (3H, s, SC*H*$_3$), 2.61 (3H, s, NC*H*$_3$), 2.87 (1H, m, ArCH$_2$C*H*), 3.10 (1H, dd, J = 4.0 Hz, 13.1 Hz, ArC*H*$_2$CH), 3.54 (3H, s, OC*H*$_3$), 7.11 (2H, d, J = 8.5 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.2, 38.9, 40.8, 60.1, 64.1, 127.0, 129.8, 135.4, 137.2. **HCl salt**. Colourless solid. mp 135-137 ºC (ethanol/hexane). Anal. Calculated for $C_{12}H_{20}ClNOS$: C, 55.05; H, 7.70; N, 5.35; S, 12.25. Found: C, 54.87; H, 7.67; N, 5.28; S, 11.93%.

4.1.3 General method B: HCl salt formation procedure

10 mmol of amine was dissolved in propan-2-ol (IPA) (3 mL). Concentrated HCl (15 drops) was added and the resulting solution diluted with diethylether until turbid. The mixture was allowed to stand at room temperature until crystal formation had occurred and resulting solids isolated by gravity or vacuum filtration. The crude HCl salt was recrystallised from a mixture of ethanol and hexane.

4.1.4. General method C: Synthesis of imines 5a-d, 20a-d.

A solution of the appropriate ketone **3** or **18** (8.32 mmol) and amine HCl salt (29.93 mmol) in pyridine (7.5 mL) and ethanol (7.5 mL) was refluxed for 2 h. After cooling, the mixture was acidified with dilute HCl and extracted with dichloromethane (3 x 50 mL). The extracts were combined, dried over anhydrous Na_2SO_4 and solvent removed *in vacuo* to provide the required imines as pale amber oils requiring no further purification. Note:

The imines were isolated as mixtures of *syn*/*anti* isomers (as determined by ^{1}H-NMR, major isomer is indicated by "*").

4.1.4.1. 1-(4-Methylthiophenyl)-2-propanone oxime (**5a**) was prepared from (**3**) (2.50 g, 12.87 mmol scale) according to general method C and chromatographed on silica gel (eluent : diethylether/hexane : 35/65), providing a 25/75 mixture of *syn*/*anti* isomers. Colourless solid (85%). mp. 57-59 ºC (ethanol/water). R_f 0.75 (diethylether/hexane : 80/20). IRν_{max} (KBr) 3260 (OH), 1668 (C=N) cm^{-1}. ***Anti*-(5a)**. ^{1}H NMR δ ($CDCl_3$) 1.81 (2.25H, s, H-3'), 2.46 (3H, s, SCH_3), 3.45 (1.5H, s, H-1'), 7.14, 7.20 (3H, 2d, J=8.0Hz, J=8.6Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.0, 19.6, 41.5, 127.0, 129.4, 133.6, 136.7, 157.4. ***Syn*-(5a)**. ^{1}H NMR δ ($CDCl_3$) 1.80 (0.75H, s, H-3'), SCH_3 signal overlapping with *anti*-(**5a**), 3.70 (0.5H, s, H-1'), 7.15, 7.19 (1H, 2d, J=8.5Hz, 9.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.0, 19.6, 34.2, 127.1, 129.6, 133.4, 136.3, 156.7. *m/z* 195, M^+ (*syn*/*anti* isomer mixture), Anal. Calculated for $C_{10}H_{13}NOS$: C, 61.50; H, 6.71; N, 7.17. Found: C, 61.40; H, 6.71; N, 7.08%.

4.1.4.2. 2-*N*-Methoxyimino-1-(4-methylthiophenyl)propane (5b) was prepared from **3** and methoxylamine HCl according to general method C as a 70/30 mixture of isomers. Pale amber oil (97%). IRν_{max} (film) 2898, 2814, 1636, 1598, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.72*, 1.77 (3H, 2s, CC*H*$_3$), 2.47 (3H, s, SC*H*$_3$), 3.42*, 3.62 (2H, 2s, ArC*H*$_2$C), 3.87, 3.88* (3H, 2s, OC*H*$_3$), 7.11, 7.14* (2H, 2d, J = 8.0 Hz, Ar*H*), 7.19, 7.21* (2H, 2d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 13.5*, 15.9*, 16.0, 19.6, 34.8, 41.5*, 61.1, 61.2*, 126.9*, 127.0, 129.4*, 129.5, 133.5, 133.8*, 136.2, 136.6*, 155.8, 156.3*. HRMS Calculated for $C_{11}H_{15}NOS$ (M^+)208.0796; Found 208.0790.

4.1.4.3. 2-*N*-Ethoxyimino-1-(4-methylthiophenyl)propane (5c) was prepared from **3** and ethoxylamine HCl according to general method C as a 70/30 mixture of isomers. Pale amber oil (93%). IRν_{max} (film) 2878, 1638, 1597, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.27*, 1.28 (3H, 2t, J = 7.0 Hz, $NOCH_2CH_3$), 1.73*, 1.77 (3H, 2s, CCH_3), 2.46 (3H, s, SCH_3), 3.42*, 3.63 (2H, 2s, $ArCH_2C$), 4.12, 4.13* (2H, 2q, J = 7.0 Hz, $NOCH_2CH_3$), 7.12, 7.14* (2H, 2d, J = 8.0 Hz, Ar*H*), 7.19, 7.20* (2H, 2d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 13.6*, 14.6*, 14.6, 16.0*, 16.0, 19.6, 34.9, 41.6*, 68.8, 68.8*, 127.0*, 127.0, 129.3*, 129.5, 133.8, 134.0*, 136.1, 136.5*, 155.3, 155.9*. *m/z*. HRMS Calculated for $C_{12}H_{18}NOS$: (M^++1) 224.1109: Found: 224.1120.

4.1.4.4. 2-(*N*-(*O*-Allyl))imino-1-(4-methylthiophenyl)propane (5d) was prepared from **3** and allyloxyamine HCl according to general method C as a 70/30 mixture of isomers. Pale amber oil (92%). IRν_{max} (film) 2862, 1644, 1598, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.75*, 1.78 (3H, 2s, CCH_3), 2.46 (3H, s, SCH_3), 3.42*, 3.65 (2H, 2s, $ArCH_2C$), 4.58-4.61 (2H, m, $NOCH_2CHCH_2$), 5.20*, 5.21 (1H, 2d, J = 10.3 Hz, J = 10.6 Hz, $NOCH_2CHCH_2$), 5.29, 5.30* (1H, 2d, J = 17.6 Hz, J = 17.1 Hz, $NOCH_2CHCH_2$), 5.97-6.07 (1H, m, $NOCH_2CHCH_2$), 7.13, 7.14* (2H, 2d, J = 8.0 Hz, J = 8.5Hz, Ar*H*), 7.19, 7.20* (2H, 2d, J = 8.0 Hz, J = 8.0Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 13.7*, 15.9*, 16.0, 19.6, 34.9, 41.5*, 74.2*, 74.2, 116.9*, 126.9*, 117.0, 127.0, 129.3*, 129.5, 133.5, 133.8*, 134.5*, 134.5, 136.2, 136.6*, 156.0, 156.6*. HRMS Calculated for $C_{13}H_{18}NOS$: (M^++1) 236.1109: Found: 236.1104. .

4.1.5. General method D: Reduction of imines: Preparation of 4o-r.

To a stirred mixture of an appropriate imine **5a-d, 20a-d** (4.78 mmol) in dry methanol (40 mL) there was added sodium cyanoborohydride (15.92 mmol, 1.00 g) and the

reaction was stirred at 20^{o} for 72 h. The pH of the reaction was occasionally adjusted to pH 3 by the addition of 4 M methanolic HCl as determined by damp universal pH paper. Excess hydride was decomposed by the addition of 10% aq. HCl (150 mL) and resulting aqueous phase washed with dichloromethane (3 x 50mL). The aqueous phase basified with 15% aq. NaOH solution and extracted with dichloromethane (3 x 50mL). The organic phases were combined, dried over anhydrous Na_2SO_4, and volatiles removed *in vacuo* leaving the product as an oil.

4.1.5.1. 2-*N*-Hydroxyamino-1-(4-methylthiophenyl)propane (4o) was prepared from the oxime **5a** according to the general method D. Colourless solid (68%). mp. 73-74 ºC. IRν_{max} (film) 3243, 3126, 1493, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.08 (3H, d, J = 6.0 Hz, CHC*H*$_3$), 2.47 (3H, s, SC*H*$_3$), 2.58 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.82 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 3.17 (1H, m, ArCH$_2$C*H*), 5.15 (1H, br s, N*H*), 6.82 (1H, br s, NO*H*), 7.12 (2H, d, J = 8.0 Hz, Ar*H*), 7.21 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 17.4, 39.3, 58.3, 127.0, 129.8, 135.5, 136.0 Anal. Calculated for $C_{10}H_{10}NOS$: C, 60.88; H, 7.66; N, 7.10. Found: C, 60.89; H, 7.57; N, 7.05%.**HCl salt**. Colourless solid. mp. 124-126 ºC (ethanol/hexane). Anal. Calculated for $C_{10}H_{16}ClNOS$: C, 51.38; H, 6.90; N, 5.99; S, 13.72. Found: C, 51.39; H, 6.83; N, 6.10; S, 13.30%..

4.1.5.2. 2-*N*-Methoxyamino-1-(4-methylthiophenyl)propane (4p) was prepared from **5b** according to the general method D. Colourless oil (81%). IRν_{max} (film) 3239, 2808, 1598, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.07 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.47 (3H, s, SC*H*$_3$), 2.57 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.79 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 3.20 (1H, m, ArCH$_2$C*H*), 5.47 (1H, br s, N*H*), 7.13 (2H, d, J = 8.0 Hz, Ar*H*), 7.21 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 15.9, 17.6, 39.5, 57.1, 62.3,

126.8, 129.7, 135.5, 135.8. *m/z*. **HCl salt**. Colourless solid. mp. 106-107 ºC (ethanol/hexane). Anal. Calculated for $C_{11}H_{18}ClNOS$: C, 53.32; H, 7.32; N, 5.65; S, 12.94. Found: C, 53.13; H, 7.16; N, 5.66; S, 12.99%.

4.1.5.3. 2-*N*-Ethoxyamino-1-(4-methylthiophenyl)propane (4q) was prepared from imine **(5c)** according to the general method D. Colourless oil (93%). IRν_{max} (film) 3238, 2866, 1599, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 1.16 (3H, t, J = 7.0 Hz, NOCH$_2$C*H*$_3$), 2.46 (3H, s, SC*H*$_3$), 2.55 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.78 (1H, dd, J = 7.0 Hz, 13.5 Hz, ArC*H*$_2$CH), 3.19 (1H, m, ArCH$_2$C*H*), 3.73 (2H, q, J = 7.0 Hz, NOC*H*$_2$CH$_3$), 5.34 (1H, s, N*H*), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 14.1, 16.1, 17.7, 39.7, 57.2, 69.7, 127.0, 139.7, 135.8, 135.8. HRMS Calculated for $C_{12}H_{20}NOS$: (M^++1) 226.1266; found: 226.1266.

4.1.5.4. 2-(*N*-(*O*-Allyl))amino-1-(4-methylthiophenyl)propane (4r) was prepared from imine **(5d)** according to the general method D. Colourless oil (89%). IRν_{max} (film) 3243, 2856, 1599, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.06 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 2.46 (3H, s, SC*H*$_3$), 2.56 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 2.80 (1H, dd, J = 6.5 Hz, 13.5 Hz, ArC*H*$_2$CH), 3.22 (1H, m, ArCH$_2$C*H*), 4.20 (2H, d, J = 6.0 Hz, NOC*H*$_2$CHCH$_2$), 5.17 (1H, d, J = 10.6 Hz, NOCH$_2$CHC*H*$_2$), 5.26 (1H, dd, J = 1.5 Hz, J = 17.3 Hz, NOCH$_2$CHC*H*$_2$), 5.43 (1H, s, N*H*), 5.93 (1H, m, NOCH$_2$C*H*CH$_2$), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 17.7, 39.6, 57.3, 75.5, 117.3, 127.0, 129.8, 134.4, 135.8, 135.9. HRMS Calculated for $C_{13}H_{20}NOS$: (M^++1) 238.1266; Found: 238.1261.

4.1.6. General method E: synthesis of Nitrostyrenes 8b-e

The appropriate aldehyde **7a-e** (6.340 mmol) was dissolved in absolute ethanol (20 mL). Nitroethane (0.456 mL, 6.340 mmol) was added followed by N,N-dimethylamine HCl or cyclohexylamine (8 drops) and potassium fluoride(1mmol). The solution was heated at reflux for 18 hours the concentrated and the residue was chromatographed on silica gel (eluent , 4:1 diethyl ether/hexane).

4.1.6.1. 1-tert-Butylsulfanyl-4-(2-nitro-propenyl)-benzene (8b)

The product was obtained from **7b** according to the general method E as a yellow solid was purified on silica gel using a 4:1 hexane/diethyl ether mobile phase. The solid isolated was recrystallised from hot ethanol to give the title compound as yellow needles (75%) mp 60-62 °C . IRυ_{max} (KBr) cm^{-1}: 1511, 1318 (conj-NO_2). ^{1}H NMR ($CDCl_3$) δ: 1.30 (s, 9H, C(C*H*$_3$)$_3$), 2.45 (s, 3H, C*H*$_3$), 7.37-7.39 (d, J=8.0 Hz, Ar*H*), 7.58-7.60 (d, J=8.0 Hz, Ar*H*), 8.05 (s, 1H, C*H*=C). ^{13}C NMR ($CDCl_3$) δ: 13.7, 30.8, 46.5, 129.7, 132.3, 132.5, 135.3, 137.2, 147.8. Anal: Calculated for $C_{13}H_{17}NO_2S$: C, 62.12; H, 6.82; N, 5.57. Found C, 62.05; H, 6.78; N, 5.51%.

4.1.6.2. 1-Benzylsulfanyl-4-(2-nitropropenyl)-benzene (8c)

The product was obtained from **7c** according to the general method E in 23% yield, yellow needles, (72%), mp 84 °C (lit. 74-75°C). IRυ_{max} (KBr) cm^{-1}: 1518, 1316 (NO_2). ^{1}H NMR ($CDCl_3$) δ: 2.47 (s, 3H, C*H*$_3$), 4.22 (s, 2H, SC*H*$_2$), 7.28-7.39 (m, 10H, SCH_2C_6*H*$_5$ + / Ar*H*), 8.06 (s, 1H, C*H*=C). ^{13}C NMR ($CDCl_3$) δ: 14.1, 37.6, 127.4, 128.7, 128.7, 128.7, 129.2, 130.4, 133.0, 135.0, 136.4, 140.2, 147.1. Anal: Calculated for $C_{16}H_{15}NO_2S$: C, 67.34; H, 5.30; N, 4.91. Found C, 67.71; H, 5.14; N, 4.68%.

4.1.6.3. 1-(2-Dimethylaminoethylsulfanyl)-4-(2-nitropropenyl)-benzene (8d)

The product was obtained from **7d** according to the general method E as a yellow oil, (73%), which was used in the subsequent reaction without further purification. IRυ_{max} (KBr) cm^{-1}: 1523, 1311 (conj-NO_2). ^{1}H NMR ($CDCl_3$) δ: 2.28 (s, 6H, N(CH_3)$_2$, 2.44 (s, 3H, CH_3), 2.58-2.62 (t, J=7.0 Hz, 2H, NCH_2), 3.07-3.11(t, J=7.0 Hz, 2H, SCH_2), 7.28-7.36 (m, 4H, C_6H_4), 8.02 (s, 1H, C*H*=C). ^{13}C NMR ($CDCl_3$) δ: 14.0, 30.2, 45.1, 57.94, 127.1, 128.9, 130.4, 132.9, 140.4, 146.8. MS m/z 267, (M^++1).

4.1.6.4. 1-Phenylsulfanyl-4-(2-nitro-propenyl)-benzene (8e)

The product was obtained from **7e** according to the general method E as a yellow oil (46%), which was used in the subsequent reaction without further purification**.** IRυ_{max} (KBr) cm^{-1}: 1584, 1300 (NO_2). ^{1}H NMR ($CDCl_3$) δ: 2.47 (s, 3H, CH_3), 7.27-7.29 (m, 2H, Ar*H*), 7.34-7.36 (m, 2H, Ar*H*), 7.41-7.44 (m, 3H, Ar*H*), 7.50-7.52 (m, 2H, Ar*H*), 8.05 (s, 1H, C*H*=C). ^{13}C NMR ($CDCl_3$) ppm: 14.1, 128.5, 129.6, 129.8, 130.6, 132.6, 132.9, 133.3, 140.8.147.2.

4.1.7. General method F: Reduction of nitrostyrenes 8b-e

To a stirred suspension of $LAlH_4$ (0.655 g, 17.26 mmol) in dry THF(20 mL) was added very slowly the appropriate nitrostyrene (3.45 mmol) in of dry THF(10 mL) under a nitrogen atmosphere. The suspension was heated to reflux for 12 h and then quenched by the careful dropwise addition at 0ºC of 10 mL of 20:1 methanol/H_2O followed by the addition of 15% NaOH(50 mL). The inorganic salts were removed by filtration and the filtrate was extracted with ethyl acetate (3 x 75 mL). The organic layer was washed with 10% HCl (4 x 50 mL), this acidic extract was basified with 15% NaOH to pH 8 and extracted with ethyl acetate (3 x 100 mL). The solvent was removed *in vacuo* to leave a

yellow oil that was further purified on a short silica column using a 100% methanol eluent.

4.1.7.1. 2-(4-tert-Butylsulfanyl-phenyl)-1-methyl-ethylamine (9b)

The product was obtained from **8b** according to the general method F as a pale yellow oil, (58%). IRυ_{max} (KBr-HCl salt) cm^{-1}: 2956 (N-H), 1505, 797 (ArCH). ^{1}H NMR ($CDCl_3$) δ: 1.06-1.08 (d, J=6.0 Hz, 3H, CHC*H*$_3$), 1.23 (s, 9H, C(C*H*$_3$)$_3$), 1.39 (s, 2H, N*H*$_2$), 2.47-2.68 (2xdd, J_{gem}=13 Hz, J_{vic}=8.0 Hz (C*H*$_2$CH), J_{gem}=13 Hz, J_{vic}=5.5 Hz (C*H*$_2$CH), 2H, C*H*$_2$CH), 3.09-3.17 (m, 1H, CH$_2$C*H*), 7.10-7.12 (d, J=8.0 Hz, 2H, Ar*H*), 7.41-7.43 (d, J=8.0 Hz, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$) δ: 23.3, 30.7, 45.4, 46.0, 48.1, 129.1, 129.9, 137.3, 140.2. HRMS Calculated for $C_{13}H_{22}NS$: (M^++1) 224.1473, found 224.1479.

4.1.7.2. 1-(4-Benzylsulfanyl-phenyl)-propylamine (9c)

The product was obtained from **8c** according to the general method F as a pale yellow oil,[29] (71%)**.** IRυ_{max} (KBr-HCl salt) cm^{-1}: 2936 (N-H). ^{1}H NMR ($CDCl_3$) δ: 1.12 (d, J=6.2 Hz, 3H, CHC*H*$_3$), 1.58 (s, 2H, N*H*$_2$), 2.47-2.52 (dd, 1H, J_{gem}=13.3 Hz, J_{vic}=8.0 Hz (CH$_2$CH), 3.11-3.19 (m, 1H, CH$_2$C*H*), 4.11 (s, 2H, SC*H*$_2$), 7.08-7.10 (d, J=8.1 Hz, 2H, Ar*H*), 7.25-7.32 (m, 7H, Ar*H*). ^{13}C NMR ($CDCl_3$) δ: 23.3, 39.3, 46.0, 48.3, 127.0, 128.3, 128.7, 129.9, 130.2, 133.6, 137.4, 138.0. HRMS Calculated for $C_{16}H_{19}NS$: (M^++1) 257.1238, found 257.1242

4.1.7.3. 2-[4-(2-Dimethylamino-ethylsulfanyl)-phenyl]-1-methyl-ethylamine (9d)

The product was obtained from **8d** according to the general method F as a pale yellow oil, (52%). IRυ_{max} (film) cm^{-1}: 3438 (N-H). ^{1}H NMR ($CDCl_3$) δ: 1.10-1.12 (d, J=6.5 Hz,

3H, CHC*H*$_3$), 1.26 (s, 2H, N*H*$_2$), 2.26 (s, 6H, N(C*H*$_3$)$_2$), 2.45-2.51 (dd, J_{gem}=13.0 Hz, J_{vic}=8.0 Hz, 1H, (C*H*$_2$CH)), 2.53-2.57 (t, J=7.0 Hz, 2H, (NC*H*$_2$)), 2.64-2.69 (dd, J_{gem}=13.0 Hz, J_{vic}=5.5 Hz, 1H, (C*H*$_2$CH)), 2.98-3.03 (t, J=7.0 Hz, 2H, (SC*H*$_2$)), 3.09-3.18 (m, 1H, CH$_2$C*H*), 7.10-7.12 (d, J=8.0 Hz, 2H, Ar*H*), 7.28-7.30 (d, J=8.0 Hz, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$) δ: 23.5, 31.7, 45.2, 46.0, 48.3, 58.5, 129.2, 129.7, 133.7, 137.6. HRMS Calculated for $C_{13}H_{22}N_2S$: (M^++1) 239.1582, found 239.1580.

4.1.7.4. 1-Methyl-2-(4-phenylsulfanyl-phenyl)-ethylamine (9e)

The product was obtained from **8e** according to the general method H as a pale yellow oil, (47%). IRυ$_{max}$ (KBr-HCl salt) cm^{-1}: 2905 (N-H) . ^{1}H NMR ($CDCl_3$) δ: 1.13-1.15 (d, J=6.5 Hz, 3H, CHC*H*$_3$), 1.95 (s, 2H, N*H*$_2$), 2.52-2.73 (2dd, J_{gem}=13.5 Hz, J_{vic}=7.5 Hz (C*H*$_2$CH), J_{gem}=13.5 Hz, J_{vic}=5.5 Hz (C*H*$_2$CH), 2H, C*H*$_2$CH), 3.14-3.20 (m, 1H, CH$_2$C*H*), 7.14-7.16 (d, J=8.0 Hz, 2H, Ar*H*), 7.25-7.32 (m, 7H, Ar*H*). ^{13}C NMR ($CDCl_3$) δ: 23.2, 45.8, 48.2, 126.7, 129.0, 130.0, 130.4, 131.4, 132.8, 136.1, 138.7. HRMS Calculated for $C_{15}H_{17}NS$: (M^++1) 244.1160, found 244.1172.

4.1.8. General preparation G: 1-(4-bromophenyl)-2-(1,3-dioxyl)alkanes (13a-b)

A mixture of the appropriate 1-(4-bromophenyl)-2-alkanone **12a-b** (92.67 mmol) and ethylene glycol (832 mmol, 67mL) in toluene (350 mL) was vigorously stirred. To this mixture was added *p*-TSA (2.50 mmol, 0.50 g) and the reaction was refluxed with a Dean-Stark trap for 18h. After cooling, the reaction phases were separated. The toluene phase was washed with satd. aq. $NaHCO_3$ (3x75 mL). The ethylene glycol phase was diluted with water (450 mL) and extracted with toluene (3x50 mL). All toluene phases were combined and washed with satd. aq. $NaHCO_3$ (3x 50mL). The organic phases were

dried over anhydrous Na_2SO_4, and concentrated *in vacuo*., providing the product as a colourless oil.

4.1.8.1. 1-(4-Bromophenyl)-2-(1,3-dioxyl)propane (**13a**) was prepared from (**12a**) (19.75 g, 92.67 mmol scale) according to the general method G. Colourless oil (100%). IRν_{max} (film) 1480 (ArH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.30 (3H, s, H-3'), 2.87 (2H, s, H-1'), 3.71 (2H, m, $OCH_2C\mathit{H}_2O$), 3.87 (2H, m, $OC\mathit{H}_2CH_2O$), 7.14, 7.39 (4H, 2d, J=8.5Hz, 8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 24.3, 44.7, 64.8, 109.3, 120.4, 130.9, 132.2, 135.8. *m/z* 257 (M^+, 1%). HRMS Calculated for $C_{11}H_{13}BrO_2$: (M^+) 256.00989, found: 256.00958.

4.1.8.2. 1-(4-Bromophenyl)-2-(1,3-dioxyl)butane (**13b**) was prepared from (**12b**) (23.30 g, 102.60 mmol scale) according to the general method G. Colourless oil (100%). IRν_{max} (film) 1481 (ArH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.62 (2H, q, $J_{3',4'}$=7.5Hz, H-3'), 2.84 (2H, s, H-1'), 3.65 (2H, m, $OCH_2C\mathit{H}_2O$), 3.85 (2H, m, $OC\mathit{H}_2CH_2O$), 7.15, 7.38 (4H, 2d, J=8.0Hz, 8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 7.8, 30.7, 42.7, 65.2, 120.2, 130.8, 132.2, 135.8. *m/z* 271 (M^++1, 1%). HRMS Calculated for $C_{12}H_{15}BrO_2$: (M^+) 270.02554, found: 270.02389.

4.1.9. General preparation H: 1-(4-ethylthiophenyl)-2-alkanones (14a-b)

A solution of the appropriate 1-(4-bromophenyl)-2-(1,3-dioxyl)alkane (**13a-b)** (33.38 mmol) in dry THF (60 ml) was stirred and cooled to –78 ºC under nitrogen. After 30min at –78 ºC, *n*-BuLi (2.5M in hexanes, 36.00 mmol, 14.40 mL) was added and the reaction was stirred for a further 1h at -78 ºC. Diethyl disulphide (36.00 mmol, 4.40 g, 4.13 ml) was then added and the reaction was stirred for a further 1h at -78 ºC, then at 20 ºC overnight. The solution was then diluted with water (250 mL) and extracted with

dichloromethane (3x50 mL). The organic phases were combined and volatiles removed *in vacuo* leaving an oil which was dissolved in a mixture of ethanol (100 mL) and 20% aq. HCl (50 mL) and refluxed for 2h. After cooling the reaction was diluted with water (250 mL) and extracted with dichloromethane (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and volatiles removed *in vacuo* leaving an oil. This was purified by flash chromatography on silica gel followed by vacuum distillation.

4.1.9.1. 1-(4-Ethylthiophenyl)-2-propanone (**14a**) was prepared from (**13a**) according to the general method H and chromatographed on silica gel (eluent : hexane/diethylether : 60/40). Amber oil (71%). IRν_{max} (film) 1713 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.14 (3H, s, H-3'), 2.93 (2H, q, J=7.6Hz, SCH_2CH_3), 3.65 (2H, s, H-1'), 7.11, 7.29 (4H, 2d, J=8.5Hz, 8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.3, 27.6, 29.1, 50.3, 129.3, 129.8, 131.7, 135.4, 205.9. *m/z* 194 (M^+, 59%). HRMS Calculated for $C_{11}H_{14}OS$: (M^+) 194.07654; found: 194.07631.

4.1.9.2. 1-(4-Ethylthiophenyl)-2-butanone (**14b**) was prepared from (**13b**) according to the general method H. Colourless oil (68%). IRν_{max} (film) 1712 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.03 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.46 (2H, q, $J_{3',4'}$=7.5Hz, H-3'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 3.64 (2H, s, H-1'), 7.12, 7.28 (4H, 2d, J=8.6Hz, 8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 7.7, 14.3, 27.7, 35.2, 49.1, 129.3, 129.8, 132.0, 135.2, 208.5. *m/z* 208 (M^+). HRMS Calculated for $C_{12}H_{16}OS$: (M^+) 208.09219; found: 208.09247.

4.1.2.15. 2-Amino-1-(4-ethylthiophenyl)propane (**4-ETA**) (**9a**) was prepared from ketone (**14a**) (1.50 g, 7.80 mmol scale) and ammonium acetate according to the

general method A. Colourless oil (75%). IRν_{max} (film) 3357, 3282 (NH_2) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.10 (3H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 1.33 (2H, br s, NH_2), 2.49 (1H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=8.0Hz, H-1'), 2.66 (1H, dd, J_{gem}=13.0Hz, $J_{1',2'}$=5.5Hz, H-1'), 2.91 (2H, q, J=7.5Hz, SCH_2CH_3), 3.14 (1H, m, H-2'), 7.10, 7.27 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.3 (SCH_2CH_3), 23.4, 27.9, 46.1, 48.3, 129.5, 129.6, 133.9, 137.5. *m/z* 195 (M^+). **HCl salt**. Colourless solid. mp 176-178 ºC (ethanol/hexane)[27]. IRν_{max} (KBr) 2515 (NH^+) cm^{-1}. Anal. Calculated for $C_{11}H_{18}ClNS$: C, 57.00; H, 7.83; N, 6.04; S, 13.83. Found: C, 56.82; H, 7.73; N, 6.05; S, 13.52%.

4.1.2.16 2-*N*-Methylamino-1-(4-ethylthiophenyl)propane (**15a**) was prepared from ketone (**14a**) (1.50 g, 7.80 mmol scale) and methylamine HCl according to the general method A. Colourless oil (34%). IRν_{max} (film) 3321 (NH), 2788 (NCH_3) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.04 (3H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.28 (1H, br s, NH), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.39 (3H, s, NCH_3), 2.57 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.68 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=7.0Hz, H-1'), 2.77 (1H, m, H-2'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 7.10, 7.27 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.4, 19.6, 27.9, 33.9, 42.9, 56.24, 129.4, 129.7, 133.9, 137.3. *m/z* 209 (M^+). **HCl salt**. Colourless solid. mp 130-132 ºC (ethanol/hexane). IRν_{max} (KBr) 2465 (NH^+) cm^{-1}. Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.63; H, 8.20; N, 5.70; S, 13.04. Found: C, 58.57; H, 8.14; N, 5.79; S, 13.11%.

4.1.2.17. 2-*N*-Ethylamino-1-(4-ethylthiophenyl)propane (**15b**) was prepared from (**14a**) (1.50 g, 7.80 mmol scale) and ethylamine HCl according to the general method A. Colourless oil (17%). IRν_{max} (film) 3307 (NH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.04 (3H, d,

$J_{3',2'}$=6.0Hz, H-3'), 1.06 (3H, t, J=7.0Hz, NCH_2CH_3), 1.28 (1H, br s, NH), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.55 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.57-2.74 (3H, m, NCH_2CH_3, H-1'), 2.89 (1H, m, H-2'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 7.10, 7.27 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.4, 15.4, 20.2, 28.0, 41.4, 43.1, 54.4, 129.5, 129.7, 133.9, 137.4. *m/z* 223 (M^+). **HCl salt**. Colourless solid. mp 164-166 ºC (ethanol/hexane). IRν_{max} (KBr) 2481, 2369 (NH^+)cm^{-1}. Anal. Calculated for $C_{13}H_{22}ClNS$: C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 59.84; H, 8.45; N, 5.36; S, 11.99%.

4.1.2.18. 2-(*N*-Methyl-*N*-hydroxy)amino-1-(4-ethylthiophenyl)propane (**15d**) was prepared from (**14a**) (1.50 g, 7.80 mmol scale) and N-methylhydroxylamine HCl according to the general method A. Colourless oil (35%). IRν_{max} (film) 3206 (OH), 2792 (NCH_3) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.99 (3H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.45 (1H, dd, J_{gem}=13.0Hz, $J_{1',2'}$=9.5Hz, H-1'), 2.68 (3H, s, NCH_3), 2.91 (2H, q, J=7.5Hz, SCH_2CH_3), 2.92 (1H, m, H-2'), 3.13 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=4.5Hz, H-1'), 6.76 (1H, br s, OH), 7.10, 7.26 (2H, 2s, J=8.0Hz, J=8.6Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.4, 23.4, 28.0, 38.9, 44.2, 65.0, 129.6, 129.8, 133.7, 137.8. *m/z* 225 (M^+). **HCl salt**. Colourless solid. mp. 111-112 ºC (ethanol/hexane). IRν_{max} (KBr) 2546, 2509 (NH^+) cm^{-1}. Anal. Calculated for $C_{12}H_{20}ClNOS$: C, 55.05; H, 7.70; N, 5.35; S, 12.25. Found: C, 54.86; H, 7.58; N, 5.21; S, 12.46%.

4.1.2.19. 2-Amino-1-(4-ethylthiophenyl)butane (**15e**) was prepared from (**14b**) (1.50 g, 7.20 mmol scale) and ammonium acetate according to the general method A. Colourless oil (73%). IRν_{max} (film) 3367, 3293 (NH_2) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.97 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.16 (2H, br s, NH_2), 1.30 (3H, t, J=7.3Hz, SCH_2CH_3), 1.35

(1H, m, H-3'), 1.50 (1H, m, H-3'), 2.42 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=8.7Hz, H-1'), 2.76 (1H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=4.5Hz, H-1'), 2.88 (1H, m, H-2'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 7.11, 7.27 (2H, 2s, J=8.5Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 10.5, 14.4, 28.0, 30.3, 43.7, 54.1, 129.5, 129.7, 133.9, 137.6. MS *m/z* 209 (M^+). **HCl salt**. Colourless solid. mp 130-132 ºC (ethanol/hexane). IRν_{max} (KBr) 2617, 2526 (NH^+) cm^{-1}. Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.65; H, 8.20; N, 5.70; S, 13.04. Found: C, 58.67; H, 8.14; N, 5.64; S, 12.70%.

4.1.2.20. 2-*N*-Methylamino-1-(4-ethylthiophenyl)butane (**15f**) was prepared from (**14b**) (1.50 g, 7.20 mmol scale) and methylamine HCl according to the general method A. Colourless oil (27%). IRν_{max} (film) 3333 (NH), 2789 (NCH_3) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.18 (1H, br s, NH), 1.30 (3H, t, J=7.3Hz, SCH_2CH_3), 1.35-1.52 (2H, m, H-3'), 2.37 (3H, s, NCH_3), 2.53-2.71 (3H, m, H-1', H-2'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 7.11, 7.26 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 9.7, 14.4, 25.5, 28.0, 33.7, 39.3, 62.0, 129.5, 129.7, 133.8, 137.6. MS *m/z* 223 (M^+). **HCl salt**. Colourless solid. mp. 137-139 ºC (ethanol/hexane). IRν_{max} (KBr) 2463 (NH^+) cm^{-1}. Anal. Calculated for $C_{13}H_{22}ClNS$: C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 59.90; H, 8.49; N, 5.30; S, 11.94%.

4.1.2.21. 2-*N*-Ethylamino-1-(4-ethylthiophenyl)butane (**15g**) was prepared from (**14b**) (1.50 g, 7.20 mmol scale) and ethylamine HCl according to the general method A. Colourless oil (28%). IRν_{max} (film) 3317 (NH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.92 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.02 (1H, br s, NH), 1.03 (3H, t, J=7.0Hz, NCH_2CH_3), 1.30 (3H, t, J=7.3Hz, SCH_2CH_3), 1.37-1.46 (2H, m, H-3'), 2.52-2.73 (5H, m, H-1', H-2', NCH_2CH_3), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 7.11, 7.26 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5,

H-6). ^{13}C NMR ppm ($CDCl_3$) 9.9, 14.4, 15.4, 26.2, 28.0, 39.8, 41.3, 60.3, 129.5, 129.7, 133.7, 137.7. MS *m/z* 237 (M^+). **HCl salt**. Colourless solid. mp 144-146 ºC (ethanol/hexane). IRν_{max} (KBr) 2476, 2378 (NH^+) cm^{-1}. Anal. Calculated for $C_{14}H_{24}ClNS$: C, 61.40; H, 8.83; N, 5.11. Found: C, 59.79; H, 8.53; N, 5.45%.

4.1.2.22. 2-(*N*-Methyl-*N*-hydroxy)amino-1-(4-ethylthiophenyl)butane (**15i**) was prepared from (**14b**) (1.50 g, 7.20 mmol scale) and *N*-methylhydroxylamine HCl according to the general method A. Colourless oil (62%). IRν_{max} (film) 3226 (OH), 2784 (NCH_3) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.90 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.32 (3H, t, J=7.5Hz, SCH_2CH_3), 1.43-1.57 (2H, m, H-3'), 2.55 (1H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=9.0Hz, H-1'), 2.69 (3H, s, NCH_3), 2.80 (1H, m, H-2'), 2.94 (2H, q, J=7.5Hz, SCH_2CH_3), 3.12 (1H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=4.5Hz, H-1'), 7.14 (1H, br s, OH), 7.15, 7.29 (2H, 2s, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 10.5, 14.4, 22.6, 28.1, 34.8, 43.3, 70.8, 129.6, 129.7, 133.5, 138.4. *m/z* 225 (M^+). **HCl salt**. Colourless solid. mp 87-90 ºC (ethanol/hexane). IRν_{max} (KBr) 2597, 2524 (NH^+) cm^{-1}. Anal. Calculated for $C_{13}H_{22}ClNOS$: C, 56.61; H, 8.04; N, 5.08; S, 11.62. Found: C, 56.87; H, 8.03; N, 5.07; S, 11.33%.

4.1.4.5. 1-(4-Ethylthiophenyl)-2-propanone oxime (**16a**) was prepared from (**14a**) (2.50 g, 13.00 mmol scale) according to the general method C and chromatographed on silica gel (eluent : hexane/diethylether : 65/35), providing a 30/70 mixture of *syn*/*anti* isomers. Pale amber solid (90%). mp 57-60 ºC (hexane). IRν_{max} (KBr) 3250 (OH) cm^{-1}. ***Anti*-(608)** ^{1}H NMR δ ($CDCl_3$) 1.80 (2.1H, s, H-3'), 1.30 (3H, t, J=7.3Hz, SCH_2CH_3), 2.91 (2H, q, J=7.6Hz, SCH_2CH_3), 3.46 (1.4H, s, H-1'), 7.14, 7.27 (2.8H, 2s, H-2, H-3, H-5, H-6), 7.27 (1.4H, s, ArH). ^{13}C NMR ppm ($CDCl_3$) 14.0, 27.8,

41.6, 129.4, 129.5, 134.4, 134.9, 157.4. *m/z* 209 (M^+). ***Syn*-(16a)** ^{1}H NMR δ ($CDCl_3$) 1.81 (0.9H, s, H-3'), (SCH_2CH_3 signals overlap with *anti*-(**16a**)), 3.70 (0.6H, s, H-1'), 7.15, 7.26 (1.2H, 2s, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 13.1, 27.9, 34.2, 129.5, 129.6, 134.2, 134.5, 156.6. *m/z* 209 (M^+). Anal. (*syn*/*anti* isomer mixture) Calculated for $C_{11}H_{15}NOS$: C, 63.12; H, 7.22; N, 6.69. Found: C, 63.41; H, 7.27; N, 6.69%.

4.1.4.6. 1-(4-Ethylthiophenyl)-2-butanone oxime (**16b**) was prepared from (**14b**) (2.50 g, 12.00 mmol scale) according to the general method C and chromatographed on silica gel (eluent : hexane/diethylether : 70/30), providing a 45/55 mixture of *syn*/*anti* isomers. Colourless solid (86%). IRν_{max} (film) 3246 (OH) cm^{-1}. ***Anti*-(16b)** ^{1}H NMR δ ($CDCl_3$) 1.05 (1.65H, t, $J_{4',3'}$=7.0Hz, H-4'), 1.30 (1.65H, t, J=7.3Hz, SCH_2CH_3), 2.18 (1.65H, q, $J_{3',4'}$=7.5Hz, H-3'), 2.90 (2H, q, J=7.5Hz, SCH_2CH_3), 3.47 (1.3H, s, H-1'), 7.16, 7.12 (2.20H, 2d, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6), 9.29 (0.55H, s, OH). ^{13}C NMR ppm ($CDCl_3$) 10.0, 14.3, 20.5, 27.9, 39.5, 129.5*, 134.3, 134.7, 160.2. MS *m/z* 223 (M^+). ***Syn*-(16b)** ^{1}H NMR δ ($CDCl_3$) 0.99 (1.35H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.29 (1.35H, t, J=7.3Hz, SCH_2CH_3), 2.31 (1.35H, q, $J_{3',4'}$=7.7Hz, H-3'), (SCH_2CH_3 signal overlaps with *anti*-(**16b**)), 3.70 (0.7H, s, H-1') 7.15, 7.27 (1.80H, 2d, J=8.5Hz, J=8.0Hz, H-2, H-3, H-5, H-6), 9.20 (1H, s, OH). ^{13}C NMR ppm ($CDCl_3$) 10.5, 14.3, 20.5, 27.0, 33.0, 129.5, 134.4, 134.5,161.6. MS *m/z* 223 (M^+). Anal. (*syn*/*anti* isomer mixture) Calculated for $C_{12}H_{17}NOS$: C, 64.53; H, 7.67; N, 6.27. Found: C, 64.75; H, 7.73; N, 6.33%.

4.1.5.5. 2-*N*-Hydroxyamino-1-(4-ethylthiophenyl)propane (**15c**) was prepared from imine (**16a**) (0.80 g, 3.82 mmol scale) according to the general method D and chromatographed on silica gel (eluent : hexane/diethylether : 60/40). Colourless solid (86%). mp 78-79 ºC (ethylacetate/hexane). IRν_{max} (KBr) 3254, 3120 (NH, OH) cm^{-1}.

^{1}H NMR δ ($CDCl_3$) 1.08 (3H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.30 (3H, t, J=7.5Hz, SCH_2CH_3), 2.59 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.92 (2H, q, J=7.5Hz, SCH_2CH_3), 2.83 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.8Hz, H-1'), 3.17 (1H, m, H-2'), 5.18, 6.87 (2H, 2br s, OH, NH), 7.11, 7.27 (4H, 2d, J=8.0Hz, J=7.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 14.8, 24.2, 28.1, 37.2, 64.3, 129.6, 129.8, 134.2, 136.9. *m/z* 211 (M^+). Anal. Calculated for $C_{11}H_{17}NOS$: C, 62.52; H, 8.11; N, 6.63. Found: C, 62.22; H, 7.82; N, 6.54%. **HCl salt**. Colourless solid. mp 100-101 ºC (ethanol/hexane). IRν$_{max}$ (KBr) 2542 (NH^+) cm^{-1} .Anal. Calculated for $C_{11}H_{18}ClNOS$: C, 53.22; H, 7.32; N, 5.65; S, 12.94. Found: C, 53.27; H, 7.17; N, 5.60 S, 13.09%.

4.1.5.6. 2-*N*-Hydroxyamino-1-(4-ethylthiophenyl)butane (**15h**) was prepared from imine (**16b**) (0.80 g, 3.58 mmol scale) according to the general method D and chromatographed on silica gel (eluent : hexane/diethylether : 65/35). Colourless oil (73%). IRν$_{max}$ (film) 3249 (NH, OH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.96 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.30 (3H, t, J=7.0Hz, SCH_2CH_3), 1.45 (1H, m, H-3'), 1.56 (1H, m, H-3'), 2.71 (1H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=6.0Hz, H-1'), 2.76 (1H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=7.5Hz, H-1'), 2.92 (2H, q, J=7.0Hz, SCH_2CH_3), 2.90 (1H, m, H-2'), 5.24, 6.23 (2H, 2br s, OH, NH), 7.13, 7.27 (4H, 2d, J=8.5Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 10.4, 14.4, 24.1, 28.0, 36.9, 64.2, 129.6, 129.8, 134.1, 136.7. MS *m/z* 225 (M^+). **HCl salt**. Colourless solid. mp 100-101 ºC (ethanol/hexane). IRν$_{max}$ (KBr) 2519 (NH^+) cm^{-1}. Anal. Calculated for $C_{12}H_{20}ClNOS$; C, 55.05; H, 7.70; N, 5.35; S, 12.25. Found: C, 55.30; H, 7.73; N, 5.35; S, 12.11%.

4.1.10. 1-(4-Methylthiophenyl)-2-butanone (**18**)

A suspension of iron powder (57.3 mmol, 3.20 g) in glacial acetic acid (15 mL) was heated on a steam bath for 20 min, stirring occasionally. To this mixture, a solution of the nitrostyrene **17**[82] (3.19 g, 14.30 mmol) in glacial acetic acid (15mL) was added over 20 min, while stirring the reaction occasionally. The reaction was heated for a further 2 h when the mixture became a grey-white colour. The reaction was allowed to cool to ambient temperature and added to a mixture of ice/water (120 mL). The product was extracted with dichloromethane (4x100 mL) and organic extracts washed with 15% aq. NaOH (3x100 mL). The organic layer was then dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and the resulting residue vacuum distilled, producing the desired ketone as a colourless solid (96%). mp 38-39ºC; bp 116 °C/0.25mm Hg[83]. IRν_{max} (KBr) 1711 (C=O) cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.02 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 2.26 (2H, q, $J_{3',4'}$=7.3Hz, H-3'), 2.46 (3H, s, SCH_3), 3.63 (2H, s, H-1'), 7.12, 7.22 (4H, 2d, J=8.5, J=8.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 7.73, 15.95, 35.18, 49.11, 127.05, 129.81, 131.29, 137.04, 208.70. MS *m/z* 194 (M^+). Anal. Calculated for $C_{11}H_{14}OS$; C, 68.00; H, 7.26. Found: C, 68.24; H, 7.40%.

4.1.2.23. 2-Amino-1-(4-methylthiophenyl)butane (19a) was prepared from **18** and ammonium acetate according to the general method A. Colourless solid (53%). mp 109-110 ºC. IRν_{max} (film) 3360, 3292, 1597, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.97 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.17 (2H, br s, NH_2), 1.36 (1H, m, $CHCH_2CH_3$), 1.52 (1H, m, $CHCH_2CH_3$), 2.41 (1H, dd, J = 8.5 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.47 (3H, s, SCH_3), 2.75 (1H, dd, J = 4.5 Hz, J = 13.5 Hz, $ArCH_2CH$), 2.88 (1H, m, $ArCH_2CH$), 7.12 (2H, d, J = 8.0 Hz, Ar*H*), 7.21 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.5, 16.2, 30.3, 43.6, 54.1, 127.1, 129.7, 135.7, 136.8. MS *m/z* 195 (M^+). **HCl salt**. Colourless solid. mp

154-155 ºC (ethanol/hexane)[27]. Anal. Calculated for $C_{11}H_{18}ClNS$: C, 57.00; H, 7.83; N, 6.04; S, 13.83. Found: C, 56.90; H, 7.69; N, 5.78; S, 12.88%.

4.1.2.24. 2-*N*-Methylamino-1-(4-methylthiophenyl)butane (19b) was prepared from **18** and methylamine HCl according to the general method A. Colourless oil (77%). IRν_{max} (film) 3331, 2788, 1599, 1091 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.36-1.52 (2H, m, $CHCH_2CH_3$), 1.55 (1H, br s, N*H*), 2.37 (3H, s, NCH_3), 2.47 (3H, s, SCH_3), 2.53-2.72 (3H, m, $ArCH_2CH$), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.75, 16.14, 25.48, 33.69, 39.23, 62.05, 127.04, 129.74, 135.67, 136.70. MS *m/z* 209 (M^++1). **HCl salt**. Colourless solid. mp 128-129 ºC (ethanol/hexane). Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.53; H, 8.20; N, 5.70; S, 13.04. Found: C, 58.50; H, 8.22; N, 5.78; S, 12.75%.

4.1.2.25. 2-*N*-Ethylamino-1-(4-methylthiophenyl)butane (19c) was prepared from **18** and ethylamine HCl according to the general method A. Colourless oil (28%). IRν_{max} (film) 3266, 2872, 1602 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.92 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.04 (3H, t, J = 7.0 Hz, NCH_2CH_3), 1.26 (1H, br s, N*H*), 1.37-1.49 (2H, m, $CHCH_2CH_3$), 2.47 (3H, s, SCH_3), 2.53-2.70 (5H, m, NCH_2CH_3, $ArCH_2CH$), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.8, 15.4, 16.1, 26.1, 39.7, 41.3, 60.3, 127.0, 129.7, 135.5, 136.8. **HCl salt**. Colourless solid. mp 130-132 ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{22}ClNS$: C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 59.79; H, 8.53; N, 5.45; S, 12.28%.

4.1.2.26. 2-*N*-(*n*-Propyl)amino-1-(4-methylthiophenyl)butane (19d) was prepared from **18** and *n*-propylamine HCl according to the general method A. Colourless oil (26%). IRν_{max} (film) 3323, 1600, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.92 (3H, t, J = 7.5

Hz, $CHCH_2CH_3$), 1.04 (3H, t, J = 7.0 Hz, NCH_2CH_3), 1.26 (1H, br s, N*H*), 1.37-1.49 (2H, m, $CHCH_2CH_3$), 2.47 (3H, s, SCH_3), 2.53-2.70 (5H, m, NCH_2CH_3, $ArCH_2CH$), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.8, 15.4, 16.1, 26.1, 39.7, 41.3, 60.3, 127.0, 129.7, 135.5, 136.8. **HCl salt**. Colourless solid. mp 134-135 ºC (ethanol/hexane). Anal. Calculated for $C_{14}H_{24}ClNS$: C, 61.40; H, 8.83; N, 5.11; S, 11.71. Found: C, 60.90; H, 8.69; N, 5.00; S, 12.30%.

4.1.2.27. 2-*N*-(Isopropyl)amino-1-(4-methylthiophenyl)butane (19e) was prepared from **18** and isopropylamine HCl according to the general method A. Colourless oil (15%). IRν_{max} (film) 3323, 1600, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.91 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 0.94 (3H, d, J = 6.5 Hz, $NCH(CH_3)_2$), 1.02 (3H, d, J = 6.5 Hz, $NCH(CH_3)_2$), 1.11 (1H, br s, N*H*), 1.28-1.43 (2H, m, $CHCH_2CH_3$), 2.47 (3H, s, SCH_3), 2.59 (1H, dd, J = 7.5 Hz, J = 13.8 Hz, $ArCH_2CH$), 2.65 (1H, dd, J = 7.0 Hz, J = 13.8 Hz, $ArCH_2CH$), 2.73 (1H, m, $ArCH_2CH$), 2.85 (1H, m, $NCH(CH_3)_2$), 7.10 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.8, 16.1, 23.1, 23.5, 26.6, 40.1, 45.6, 57.2, 126.8, 129.7, 135.4, 136.7. **HCl salt**. Colourless crystals, mp 150-151 ºC (ethanol/hexane). Anal. Calculated for $C_{14}H_{24}ClNS$: C, 61.40; H, 8.83; N, 5.11; S, 11.71. Found: C, 61.28; H, 8.93; N, 5.08; S, 12.04%.

4.1.2.28. 2-*N*-Cyclopropylamino-1-(4-methylthiophenyl)butane (19f) was prepared from **18** and cyclopropylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: diethylether). Colourless oil (30%). IRν_{max} (film) 3290, 1493, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.26 (2H, m, $NCH(CH_2)_2$), 0.42 (2H, m, $NCH(CH_2)_2$), 0.92 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.38-1.57 (2H, m, $CHCH_2CH_3$), 1.63 (1H, br s, N*H*), 2.03 (1H, m, $NCH(CH_2)_2$), 2.47 (3H, s, SCH_3),

2.68 (2H, d, J = 7.0 Hz, ArC*H*$_2$CH), 2.80 (1H, m, ArCH$_2$C*H*), 7.12 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 6.1, 7.0, 9.9, 16.1, 26.2, 28.6, 39.8, 61.0, 126.9, 129.7, 135.5, 136.9. **HCl salt**. Colourless solid. mp 136-137 ºC (ethanol/hexane). Anal. Calculated for $C_{14}H_{22}ClNS$: C, 61.85; H, 8.16; N, 5.15; S, 11.80. Found: C, 61.87; H, 8.11; N, 5.31; S, 12.19%.

4.1.2.29. 2-*N*-Propargylamino-1-(4-methylthiophenyl)butane (19g) was prepared from **18** and propargylamine HCl according to the general method A. Colourless oil (26%). IRν_{max} (film) 3290, 1599, 1091 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, J = 7.5 Hz, CHCH$_2$C*H*$_3$), 1.40-1.48 (2H, m, CHC*H*$_2$CH$_3$), 1.49 (1H, br s, N*H*), 2.16 (1H, m, NCH$_2$CC*H*), 2.47 (3H, s, SC*H*$_3$), 2.60 (1H, dd, J = 7.5 Hz, J = 13.6 Hz, ArC*H*$_2$CH), 2.70 (1H, dd, J = 6.0 Hz, J = 13.6 Hz, ArC*H*$_2$CH), 2.93 (1H, m, ArCH$_2$C*H*), 3.40 (2H, d, J = 2.5 Hz, NC*H*$_2$CCH), 7.13 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.5, 16.1, 25.6, 35.6, 39.4, 58.2, 71.1, 82.1, 127.0, 129.7, 135.8, 136.2. **HCl salt**. Orange solid. mp 111-113 ºC (dec.) (ethanol/hexane). Anal. Calculated for $C_{14}H_{20}ClNS$: C, 62.32; H, 7.47; N, 5.19; S, 11.88. Found: C, 61.23; H, 7.35; N, 5.53; S, 12.33%.

4.1.2.30. 2-*N*-(2-Methoxyethyl)amino-1-(4-methylthiophenyl)butane (19h) was prepared from **18** and 2-methoxyethylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: diethylether/methanol 90/10). Pale amber oil (39%). IRν_{max} (film) 3327, 1599, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, J = 7.5 Hz, CHCH$_2$C*H*$_3$), 1.37-1.45 (2H, m, CHC*H*$_2$CH$_3$), 1.74 (1H, br s, N*H*), 2.47 (3H, s, SC*H*$_3$), 2.63-2.69 (2H, m, NC*H*$_2$CH$_2$O), 2.70-2.81 (3H, m, ArC*H*$_2$C*H*), 3.29 (3H, s, OC*H*$_3$), 3.44 (2H, t, J = 5.5 Hz, NCH$_2$C*H*$_2$O), 7.12 (2H, d, J =

8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.8, 16.2, 26.0, 39.7, 46.5, 58.6, 60.4, 72.1, 127.0, 129.7, 135.6, 136.7. **HCl salt**. Colourless solid. mp112-114 ºC (ethanol/hexane). Anal. Calculated for $C_{14}H_{24}ClNOS$: C, 58.01; H, 8.35; N, 4.83; S, 11.06. Found: C, 57.98; H, 8.20; N, 4.75; S, 10.69%.

4.1.2.31. 2-*N*-Allylamino-1-(4-methylthiophenyl)butane (19i) was prepared from **18** and allylamine HCl according to the general method A. Colourless oil (33%). IRν_{max} (film) 3342, 1641, 1493, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.37-1.48 (2H, m, $CHCH_2CH_3$), 1.56 (1H, br s, N*H*), 2.47 (3H, s, SCH_3), 2.61-2.76 (3H, m, $ArCH_2CH$), 3.20 (1H, dd, J = 6.0 Hz, 14.0 Hz, NCH_2CHCH_2), 3.27 (1H, dd, J = 5.5 Hz, 14.4 Hz, NCH_2CHCH_2), 5.05 (1H, dd, J = 1.5 Hz, J = 10.3 Hz, NCH_2CHCH_2), 5.11 (1H, d, J = 1.5 Hz, J = 17.0 Hz, NCH_2CHCH_2), 5.83 (1H, m, NCH_2CHCH_2), 7.11 (2H, d, J = 8.5 Hz, Ar*H*), 7.20 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.8, 16.1, 25.9, 39.5, 49.6, 59.5, 115.7, 126.9, 129.8, 135.6, 136.6, 136.9. **HCl salt**. Colourless solid. mp 127-128 ºC (ethanol/hexane). Anal. Calculated for $C_{14}H_{22}ClNS$: C, 61.85; H, 8.16; N, 5.15; S, 11.80. Found: C, 61.81; H, 8.12; N, 5.20; S, 11.73%.

4.1.2.32. 2-(*N*-2-Hydroxyethyl)amino-1-(4-methylthiophenyl)butane (19j) was prepared from **18** and 2-hydroxyethylamine HCl according to the general method A. Colourless oil (43%). IRν_{max} (film) 3304, 2873, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.40-1.47 (2H, m, $CHCH_2CH_3$), 2.14 (2H, br s, , N*H*, NCH_2CH_2OH), 2.46 (3H, s, SCH_3), 2.58-2.78 (5H, m, $ArCH_2CH$, $ArCH_2CH$, NCH_2CH_2OH), 3.54 (2H, m, NCH_2CH_2OH), 7.10 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 9.9, 16.1, 26.3, 39.8, 48.2, 60.0, 61.1, 126.9,

129.6, 135.7, 136.5. HRMS Calculated for $C_{13}H_{22}NOS$: (M^+) 240.1422, Found 240.1432.

4.1.2.33. 2-*N,N*-Dimethylamino-1-(4-methylthiophenyl)butane (19k) was prepared from **18** and dimethylamine HCl according to the general method A. Colourless oil (16%). IRν_{max} (film) 3072, 2817, 1600, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.84 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.28-1.47 (2H, m, $CHCH_2CH_3$), 2.30 (6H, s, $N(CH_3)_2$), 2.32 (1H, dd, J = 9.0 Hz, J = 13.3 Hz, $ArCH_2CH$), 2.46 (3H, s, SCH_3), 2.50 (1H, m, $ArCH_2CH$), 2.86 (1H, dd, J = 4.5 Hz, J = 13.3 Hz, $ArCH_2CH$), 7.09 (2H, d, J = 8.5 Hz, Ar*H*), 7.19 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 11.4, 16.3, 23.0, 34.0, 40.5, 67.7, 127.1, 129.6, 138.4, 139.4. **HCl salt**. Colourless solid. mp 150-153 ºC (ethanol/hexane). Anal. Calculated for $C_{13}H_{22}ClNS$; C, 60.09; H, 8.53; N, 5.39; S, 12.34. Found: C, 59.57; H, 8.42; N, 5.26; S, 12.00%.

4.1.2.34. 2-(*N*-Methoxy-*N*-methyl)amino-1-(4-methylthiophenyl)butane (19l) was prepared from **18** and *N*-methoxy-*N*-methylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: hexane/diethylether 60/40). Colourless oil (68%). IRν_{max} (film) 3073, 1600, 1493, 1096 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.88 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.37-1.49 (2H, m, $CHCH_2CH_3$), 2.46 (3H, s, SCH_3), 2.49 (1H, dd, J = 8.5 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.60 (3H, s, NCH_3), 2.72 (1H, m, $ArCH_2CH$), 3.01 (1H, dd, J = 4.5 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.50 (3H, s, OCH_3), 7.12 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.7, 16.2, 22.7, 34.6, 40.1, 59.7, 69.5, 127.0, 129.7, 135.2, 137.9. **HCl salt**. Colourless solid. mp 115-116 ºC (ethanol/hexane). Anal.

Calculated for $C_{13}H_{22}ClNOS$: C, 56.61; H, 8.04; N, 5.08; S, 11.62. Found: C, 56.91; H, 8.03; N, 5.05; S, 11.56%.

4.1.2.35. 2-(*N*-Hydroxy-*N*-methylamino-1-(4-methylthiophenyl)butane (19n) was prepared from **18** and *N*-hydroxy-*N*-methylamine HCl according to the general method A. The resulting residue was purified by flash chromatography (eluent: hexane/diethylether 60/40). Colourless oil (51%). IRν_{max} (film) 3090, 1599, 1493 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.87 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.38-1.55 (2H, m, $CHCH_2CH_3$), 2.46 (3H, s, SCH_3), 2.51 (1H, dd, J = 9.0 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.66 (3H, s, NCH_3), 2.78 (1H, m, $ArCH_2CH$), 3.11 (1H, dd, J = 4.5 Hz, J = 13.6 Hz, $ArCH_2CH$), 7.11 (2H, d, J = 8.0 Hz, Ar*H*), 7.19 (2H, d, J = 8.0 Hz, Ar*H*), 7.66 (1H, s, NO*H*). ^{13}C NMR ppm ($CDCl_3$) 10.5, 16.2, 22.6, 34.3, 43.1, 70.8, 127.0, 129.6, 135.3, 137.5. **HCl salt**. Colourless solid. mp 115-116 ºC (ethanol/hexane) Anal. Calculated for $C_{12}H_{20}ClNOS$; C, 55.05; H, 7.70; N, 5.35; S, 12.25. Found: C, 54.98; H, 7.67; N, 5.42; S, 13.37%.

4.1.4.5. 1-(4-Methylthiophenyl)-2-butanone oxime (**20a**) was prepared from **18** (2.50g, 12.87mmol scale) according to the general method C without the need for flash chromatography, generating a 60/40 mixture of *syn*/*anti* isomers. Colourless solid (86%). mp 77-79 ºC (ethanol/water). R_f 0.36 (dichloromethane/hexane : 80/20). IRν_{max} (KBr) 3220 (OH), 1669 (C=N) cm^{-1}. ***Syn*-(20a)**. ^{1}H NMR δ ($CDCl_3$) 1.04 (1.8H, t, $J_{4',3'}$=7.5Hz, H-4'), 2.18 (1.2H, q, $J_{3',4'}$=7.5Hz, H-3'), 2.45 (3H, s, SCH_3), 3.70 (1.2H, s, H-1'), 7.13-7.21 (4H, m, H-2, H-3, H-5, H-6), OH signal not visible. ^{13}C NMR ppm ($CDCl_3$) 10.6, 16.0, 27.0, 32.9, 127.1, 128.5, 133.6, 136.1, 160.4. ***Anti*-(20a)**. ^{1}H NMR δ ($CDCl_3$) 0.99 (1.2H, t, $J_{4',3'}$=7.5Hz, H-4'), 2.31 (0.8H, q, $J_{3',4'}$=7.7Hz, H-3'), SCH_3 signal overlapping with *syn*-(**20a**), 3.46 (0.8H, s, H-1'), H-2, H-3, H-5, H-6 signals overlapping with *syn*-

(**20a**), OH signal not visible. ^{13}C NMR ppm ($CDCl_3$) 10.0, 16.0, 20.4, 39.4, 127.0, 129.5, 133.6, 136.6, 161.7. *m/z* (*syn*/*anti* isomer mixture) 209 (M^+, 69%). Anal. Calculated for $C_{11}H_{15}NOS$: C, 63.12; H, 7.22; N, 6.69. Found: C, 63.46; H, 7.11; N,6.50 %.

4.1.4.6. 2-*N*-Methoxyimino-1-(4-methylthiophenyl)butane (20b) was prepared from **18** and methoxylamine HCl according to the general method C as a 60/40 mixture of isomers. Pale amber oil (96%). IRν_{max} (film) 2897, 2814, 1598, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93, 1.03* (3H, 2t, J = 7.5 Hz, J = 7.5Hz, CCH_2CH_3), 2.13*, 2.20 (2H, 2q, J = 7.5 Hz, J = 7.5Hz, CCH_2CH_3), 2.45*, 2.46 (3H, 2s, SCH_3), 3.42, 3.63* (2H, 2s, $ArCH_2C$), 3.87, 3.88* (3H, 2s, OCH_3), 7.11*, 7.16 (2H, 2d, J = 8.0 Hz, J = 8.0 Hz, Ar*H*), 7.18, 7.20* (2H, 2d, J = 8.0 Hz, J = 8.0Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.2, 11.0*, 15.9, 16.0*, 20.7, 26.9*, 33.1*, 39.4, 61.1*, 61.2, 126.8, 127.0*, 129.4*, 129.4, 133.7, 133.9*, 136.0*, 136.5, 159.6*, 160.9. HRMS Calculated for $C_{12}H_{18}NOS$: (M^++1) 224.1109: Found: 224.1105.

4.1.4.7. 2-*N*-Ethoxyimino-1-(4-methylthiophenyl)butane (20c) was prepared from **18** and ethoxylamine HCl according to the general method C as a 60/40 mixture of isomers. Pale amber oil (95%). IRν_{max} (film) 2876, 1630, 1493, 1091 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93, 1.03* (3H, 2t, J = 7.5 Hz, J = 7.5 Hz, CCH_2CH_3), 1.27 (3H, t, J = 7.0 Hz, $NOCH_2CH_3$), 2.14*, 2.20 (2H, q, J = 7.5 Hz, J = 7.5 Hz, CCH_2CH_3), 2.45, 2.46* (3H, 2s, SCH_3), 3.42, 3.64* (2H, 2s, $ArCH_2C$), 4.11, 4.12* (2H, 2q, J = 7.0 Hz, $NOCH_2CH_3$), 7.12*, 7.15 (2H, 2d, J = 8.0 Hz, J = 8.5 Hz, Ar*H*), 7.18*, 7.20 (2H, d, J = 8.0 Hz, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.2, 11.0*, 14.6, 14.6*, 16.0, 16.1*, 20.9, 27.0*, 33.2*, 39.5, 68.7, 68.8*, 126.9, 127.0*, 129.4, 129.4*, 134.0*, 134.1, 136.0*, 136.4, 159.2*, 160.5. HRMS Calculated for $C_{13}H_{20}NOS$: (M^++1) 238.1266: Found: 238.1267.

4.1.4.8. 2-(*N*-(*O*-Allyl))imino-1-(4-methylthiophenyl)butane (20d) was prepared from **18** and allyloxyamine HCl according to the general method C as a 60/40 mixture of isomers. Pale amber oil (91%). IRν_{max} (film) 2875, 1640, 1493, 1093 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.94, 1.03* (3H, 2t, J = 7.5 Hz, J = 7.5 Hz, CCH_2CH_3), 2.14*, 2.23 (2H, 2q, J = 7.5 Hz, J = 7.5 Hz, $CHCH_2CH_3$), 2.45*, 2.46 (3H, 2s, SCH_3), 3.43, 3.67* (2H, 2s, $ArCH_2C$), 4.59 (2H, d, J = 5.5 Hz, $NOCH_2CHCH_2$), 5.20 (1H, m, $NOCH_2CHCH_2$), 5.30 (1H, m, $NOCH_2CHCH_2$), 6.01 (1H, m, $NOCH_2CHCH_2$), 7.13*, 7.16 (2H, 2d, J = 8.5 Hz, J = 8.5 Hz, Ar*H*), 7.18*, 7.20 (2H, d, J = 8.0 Hz, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.2, 11.04*, 16.0*, 16.0, 21.0, 26.9*, 33.3*, 39.4, 74.2*, 74.2, 116.7, 116.9*, 126.9, 127.0*, 129.4*, 129.4, 133.8*, 133.9, 134.5, 134.6*, 136.0*, 136.5, 159.9*, 161.2. HRMS Calculated for $C_{14}H_{20}NOS$: (M^++1) 250.1266: Found: 250.1260.

4.1.5.7. 2-*N*-Hydroxyamino-1-(4-methylthiophenyl)butane (19m) was prepared from oxime **20a** according to the general method D. Colourless solid (30%). mp 50-51 ºC (Hexane). IRν_{max} (film) 3253, 2863, 1493, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.95 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.38-1.62 (2H, m, $CHCH_2CH_3$), 2.47 (3H, s, SCH_3), 2.70 (1H, dd, J = 6.0 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.76 (1H, dd, J = 7.0 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.92 (1H, m, $ArCH_2CH$), 5.27 (1H, s, N*H*), 6.52 (1H, s, NO*H*), 7.12 (2H, d, J = 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.5 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 10.4, 16.1, 24.0, 36.8, 64.2, 127.1, 129.8, 135.8, 135.9. **HCl salt**. Colourless crystals. mp 114-115 ºC (ethanol/hexane). Anal. Calculated for $C_{11}H_{18}ClNOS$: C, 53.22; H, 7.32; N, 5.65; S, 12.94. Found: C, 53.18; H, 7.11; N, 5.69; S, 12.88%.

4.1.5.8. 2-*N*-Methoxyamino-1-(4-methylthiophenyl)butane (19o) was prepared from imine **(20b)** according to the general method D. Colourless oil (87%). IRν_{max}

(film) 3241, 2806, 1600, 1095 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.97 (3H, t, J = 7.5 Hz, $CHCH_2CH_3$), 1.38-1.59 (2H, m, $CHCH_2CH_3$), 2.46 (3H, s, SCH_3), 2.67 (1H, dd, J = 6.0 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.71 (1H, dd, J = 7.0 Hz, J = 13.6 Hz, $ArCH_2CH$), 2.93 (1H, m, $ArCH_2CH$), 3.52 (3H, s, OCH_3), 5.50 (1H, br s, NH), 7.13 (2H, d, J = 8.5 Hz, ArH), 7.20 (2H, d, J = 8.5 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 10.4, 16.1, 24.3, 37.1, 62.2, 63.2, 127.0, 129.8, 135.8, 136.0. HRMS Calculated for $C_{12}H_{20}NOS$: (M^++1) 226.1266: Found: 226.1274.

4.1.5.9. 2-*N*-Ethoxyamino-1-(4-methylthiophenyl)propane (19p) was prepared from imine **(20c)** according to the general method D. Colourless oil (93%). IRν_{max} (film) 3238, 2866, 1599, 1092 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 6.5 Hz, $CHCH_3$), 1.16 (3H, t, J = 7.0 Hz, $NOCH_2CH_3$), 2.46 (3H, s, SCH_3), 2.55 (1H, dd, J = 6.5 Hz, 13.5 Hz, $ArCH_2CH$), 2.78 (1H, dd, J = 7.0 Hz, 13.5 Hz, $ArCH_2CH$), 3.19 (1H, m, $ArCH_2CH$), 3.73 (2H, q, J = 7.0 Hz, $NOCH_2CH_3$), 5.34 (1H, s, NH), 7.11 (2H, d, J = 8.0 Hz, ArH), 7.20 (2H, d, J = 8.0 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 14.1, 16.1, 17.7, 39.7, 57.2, 69.7, 127.0, 139.7, 135.8, 135.8. HRMS Calculated for $C_{13}H_{22}NOS$: (M^++1) 240.1422, Found: 240.1430.

4.1.5.10. 2-(*N*-(*O*-Allyl))amino-1-(4-methylthiophenyl)propane (19q) was prepared from imine **(20d)** according to the general method D. Colourless oil (89%). IRν_{max} (film) 3243, 2856, 1599, 1094 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.06 (3H, d, J = 6.5 Hz, $CHCH_3$), 2.46 (3H, s, SCH_3), 2.56 (1H, dd, J = 6.5 Hz, 13.5 Hz, $ArCH_2CH$), 2.80 (1H, dd, J = 6.5 Hz, 13.5 Hz, $ArCH_2CH$), 3.22 (1H, m, $ArCH_2CH$), 4.20 (2H, d, J = 6.0 Hz, $NOCH_2CHCH_2$), 5.17 (1H, d, J = 10.6 Hz, $NOCH_2CHCH_2$), 5.26 (1H, dd, J = 1.5 Hz, J = 17.3 Hz, $NOCH_2CHCH_2$), 5.43 (1H, s, NH), 5.93 (1H, m, $NOCH_2CHCH_2$), 7.11 (2H, d, J

= 8.0 Hz, Ar*H*), 7.20 (2H, d, J = 8.0 Hz, Ar*H*). ^{13}C NMR ppm ($CDCl_3$) 16.1, 17.7, 39.6, 57.3, 75.5, 117.3, 127.0, 129.8, 134.4, 135.8, 135.9. HRMS Calculated for $C_{14}H_{22}NOS$: (M^++1) 252.1422: Found: 252.1411.

4.1.11. 1-(4-Methylsulfanyl-phenyl)-propylamine 25a

(i) A solution of 4-methylthiobenzaldehyde (10g) in dry diethylether (20mL) was added dropwise to a solution of ethylmagnesium bromide (prepared by the dropwise addition of bromoethane (7.30 mL) to stirred Mg turnings (2.588 g) in 75 ml of dry diethylether under N_2 atmosphere), and the mixture was subsequently refluxed for three hours. The reaction was quenched by the addition of saturated ammonium chloride (200 mL). The organic phase was separated and washed with 10% HCl (3 x 75 mL). The solvent was dried over anhydrous sodium sulphate and removed under vacuum to give 1-(4-methylsulfanylphenyl)-propan-1-ol **21** a clear yellow oil (29%) which was used without further purification in the next reaction. m/z 183 (M^++1); ^{1}H NMR ($CDCl_3$) δ: 0.91-0.94 (t, J=7.5 Hz, 3H, CH_2CH_3), 1.65-1.90 (m, 2H, CH_2CH_3), 2.50 (s, 3H, SCH_3), 4.56-4.60 (t, J=7.0 Hz, 1H, *CH*OH), 7.25-7.30 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ: 10.21, 30.11, 33.18, 72.80, 126.21, 127.41, 133.42, 143.39. (ii) To a solution of **21** (18.5mmol) in dry DCM (50 mL) and added in one portion a suspension of pyridinium chlorochromate (33.20 g) in dry dichloromethane (50 mL). The reaction mixture was heated to ensure reflux for three hours. The solvent was decanted from the black gum which was triturated with diethylether (5x 100 mL). The ethereal portions combined with DCM extract and solvent was removed under vacuum leaving a dark brown oil which was purified by flash column chromatography (65:35;hexane:diethylether) to afford the product **22** as colourless solid (ethanol/hexane, 90%) which was used without further purification in the

next reaction. m/z: 180, (M^+); IRυ_{max} film: 1672, 1096 cm^{-1}, 792 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.22-1.26 (t, J=7.0 Hz, 3H, CH_2CH_3), 1.65-1.90 (m, 2H, CH_2CH_3), 2.53 (s, 3H, SCH_3), 7.26-7.29 (d, J=8.0 Hz, 2H, ArH), 7.88-7.90 (d, J=8.0 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ: 8.28, 14.74, 31.53, 124.93, 128.36, 133.17, 145.44, 199.85. (iii) To a solution of **24** (0.435g, 2.414 mmol) in dry DCM (50 mL) under a nitrogen atmosphere, was added hexamethyldisilazane (1.273mL, 6.035 mmol) and $TiCl_4$ (0.265 mL, 2.414 mmol) dropwise and the reaction mixture was allowed to stir for 20h after which time $NaCNBH_3$ (0.80g) dissolved in freshly dried methanol was added dropwise. The reaction mixture was poured slowly over crushed ice. 200 ml of 15% NaOH was added and the mixture was extracted with DCM (5 x 100 mL). The solvent was dried over anhydrous sodium sulphate and removed under vacuum resulting in a residue that was purified by flash chromatography over silica gel (50:50 methanol:diethylether) to afford the product 1-(4-methylsulfanyl-phenyl)-propylamine **25a** as a waxy solid (46%). ^{1}H NMR ($CDCl_3$) δ: 0.81 (t, J = 8Hz, 3H, CH_2CH_3), 1.80-2.10 (m, 2H, CH_2CH_3), 2.10 (bs, 2H, NH_2), 4.26(s, 3H, SCH_3), 4.00-4.01(1H, m, $CHCH_2$), 7.21(d, J = 6Hz, 2H, H-2,H-6), 7.39 (d, J = 6Hz, 2H, H-3, H-5), ^{13}C NMR ($CDCl_3$) δ: 9.85, 14.84, 27.03, 56.82, 125.94, 127.75, 132.26, 139.21.HRMS (EI) Found 165.0736; $C_{10}H_{13}S$ requires 165.0738.

4.1.12. 1-(4-Pentylsulfanyl-phenyl)propylamine 25b

(i) A solution of thioanisole (0.497 g, 4.00 mmol) in dry THF (20 mL) was cooled to –57ºC and sec-BuLi (5.714 mL (1.4M in C_6H_{12}), 4.10mmol) was added dropwise. The solution was stirred for 1.5 hours after which, iodobutane (0.478 mL, 4.00 mmol) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3.5 hours before being poured slowly onto crushed ice and extracted with DCM (4 x

100 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered and removed *in vacuo*. The resulting oil was purified by flash chromatography on silica gel (95:5 hexane:diethyl ether) to afford the product **23** as an oil (76%), which was used without further purification in the next reaction. m/z 180 (M^+); IRυ_{max} (film) cm^{-1}: 1514, 729. ^{1}H NMR ($CDCl_3$) δ: 0.92-0.96 (t, J=7.0 Hz, 3H, CH_3CH_2), 1.31-1.49 (m, 4H, $CH_3C_2CH_2$) 1.66-1.73 (m, 2H, CH_2CH_2S), 2.94-2.97 (t, J=7.5 Hz, 2H, CH_2SAr), 7.17-7.21 (m, 1H, ArH), 7.28-7.38 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ: 13.9, 22.2, 28.7, 30.9, 33.4, 125.5, 128.7, 128.7,137.0. (ii) Pentylsulfanylbenzene **23** (0.415 g, 2.31 mmol) was dissolved in dry DCM (30 mL) and the solution was cooled to 0ºC in an ice bath. $AlCl_3$ (0.401 g, 3.00 mmol) was added, followed by the slow dropwise addition of propionyl chloride (0.361 ml, 4.16 mmol). After the addition of the acid chloride the reaction was allowed to warm to ambient temperature and stirred for a further 3 hours. The reaction mixture was diluted with DCM (200 mL) and poured slowly into 100 ml of ice-water. The organic phase was washed with water (6 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed under vacuum. The solid formed was recrystallised from hexane/ether to yield 1-(4-pentylsulfanyl-phenyl)-propan-1-one **23** as colourless needles (94%), which was used without further purification in the next reaction. IRυ_{max} film: 2931, 1677, 1592, 822 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.90-0.94 (t, J=7.0 Hz, 3H, $COCH_2CH_3$), 1.21-1.24 (t, J=7.5 Hz, 3H, $CH_3C_4H_8$), 1.32-1.48 (m, 4H, $CH_3CH_2CH_2$) 1.68-1.75 (m, 2H, CH_2CH_2S), 2.94-3.01 (m, 4H, CH_2SAr, $COCH_2$), 7.29-7.31 (d, J=8.5 Hz, 2H, ArH), 7.86-7.89 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ: 8.25, 13.89, 22.18, 28.38, 30.99, 31.50, 31.87, 126.19, 128.34, 133.36, 144.53, 199.81. (iii) To a solution of **24** (0.270g, 1.139 mmol) in dry DCM (50 mL) under a nitrogen

atmosphere was added hexamethyldisilazane (0.95mL, 4.50 mmol) followed by $TiCl_4$ (0.25 mL, 2.278 mmol) and the reaction mixture was allowed to stir for 20h after which time $NaCNBH_3$ (0.50 g, 8.1 mmol) dissolved in freshly dried methanol was added dropwise. The reaction mixture was poured slowly over crushed ice. 200 ml of 15% NaOH was added and the mixture was extracted with DCM (5 x 100 mL). The solvent was dried over anhydrous sodium sulphate and removed under vacuum resulting in a residue that was purified by flash chromatography over silica gel (50:50 methanol:diethylether) to afford the product as an oil which solidified (48%). IRυ_{max} (film) cm^{-1}: 3433, 1528, 801. ^{1}H NMR ($CDCl_3$) δ: 0.78-0.83 (t, J=7.5 Hz, 3H, *CH_3*), 0.88-0.91 (t, J=7.0 Hz, 3H, *CH_3*), 1.26-1.44 (m, 4H, *CH_2CH_2*-), 1.61-1.72 (m, 4H, *CH_2*, *CH_2*), 2.81 (s, 2H, *NH_2*) 2.88-2.92 (t, J=7.5 Hz, 2H, S*CH_2*), 3.73-3.76 (t, J=7.0 Hz, 1H, *CH*), 7.21-7.23 (d, J=8.0 Hz, 2H, ArH), 7.26-7.29 (d, J=8.0 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ: 10.73, 13.87, 22.14, 28.75, 30.89, 31.76, 33.59, 57.12, 126.94, 128.87, 135.27, 142.92. HRMS (EI) Found 239.1597; $C_{14}H_{25}NS$ requires 239.1707.

4.2 Biochemistry

4.2.1 Materials

DG-75 and MUTU-I (c179) BL cell lines were gifts from Dr. Dermot Walls (School of Biotechnology, Dublin City University, Ireland) and Prof. Martin Rowe (Division of Cancer Studies, The University of Birmingham, UK) respectively. The SHSY-5Y cell line was purchased from the European Collection of Cell Cultures (ECACC Lot04/c/011p17) and the TREx (+rSERT) cell line was created as in [84] and a gift from by Dr. Chris Tate (MRC Laboratory of Molecular Biology, Cambridge, UK). The HEK293 cell line stably overexpressing the human SERT was a gift from Dr. Patrick

Schloss (Central Institute for Mental Health, Mannheim, Germany) and the HL-60 cell line was a gift from Dr. D. Zisterer (School of Biochemistry and Immunology, Trinity College, Dublin). RPMI-1640, DMEM, FBS, HEPES, sodium pyruvate, gentamycin (G418) and glutamine were from Gibco (Invitrogen. Biosciences Ltd. Ireland). Alamar Blue and LymphoPrep were from Biosciences Ltd. and all other chemicals were purchased through Sigma-Aldrich Inc. Ireland.

4.2.2 Cell Culture

The DG-75 cell line is a B-lymphocyte, Burkitt's lymphoma line derived from a metastatic pleural effusion (lung) of a sporadic case of EBV negative Burkitt's lymphoma and has been that expresses SERT [62] (Supplementary figure 1). The MUTU-I (c179) cell line is an isogenic stable group I BL cell line derived from a BL biopsy that was EBV positive and expresses SERT [85] (Supplementary figure 1). The SHSY-5Y cell line SH-SY5Y is a thrice cloned (SK-N-SH -> SH-SY -> SH-SY5 -> SH-SY5Y) subline of the human neuroblastoma cell line SK-N-SH established from a metastatic bone tumour [63]. The HL-60 cell line is a promyelocytic cell line derived from a 36-year-old Caucasian female with acute promyelocytic leukaemia [86]. The above cell lines were cultured in RPMI-1640 medium containing phenol red and supplemented with 10% (v/v) foetal bovine serum (FBS), L-glutamine (2mM), penicillin and streptomycin (100μg/ml). The MUTU-I c179 cell line required the additional supplements of alpha-thioglycerol (5mM in PBS with 20μM bathocuprione disulphonic acid), sodium pyruvate (100mM) and HEPES (1mM). The TREx (-) and TREx (SERT) cell lines were derived from HEK293 cells stably expressing the TetR protein [84] that turns off rat SERT-FLAG expression.

TREx cells were cultured in DMEM containing 10% (v/v) Tet-System approved FBS, L-glutamine (2mM), gentamycin (100μg/ml), blasticidin (5μg/ml) and zeocin (200μg/ml). SERT-FLAG expression was induced by adding tetracycline (1μg/ml) and incubating for 16-24h. The HEK293 cells lines stably overexpressing human SERT was cultured in DMEM supplemented with 10% (v/v) FBS, L-glutamine (2mM), penicillin/streptomycin (100mg/ml) and Geneticin (500mg/ml). Stable expression was valid up to 30 passages. Cells were maintained in a 72cm^2 tissue culture flasks at 37°C in a humidified atmosphere of 95% Oxygen, and 5% carbon dioxide.

4.2.3 Generation of human peripheral blood mononuclear cells

Blood was obtained from a healthy donor, transferred into a 50 ml falcon tube and diluted 1:2 with phosphate buffered saline (PBS). LymphoPrep was used to separate the blood into red blood cells, white blood cell ring and serum. The blood was slowly added to 20ml of ficoll pague plus. The tubes were centrifuged at 1700×g for 30 min. The white blood cell ring was transferred into a new 50 ml tube. The volume was adjusted to 50ml and the samples were centrifuged again at 1700×g for 10 min. The supernatant was removed. This step was repeated again, the pellet was then resuspended in 10 ml of complete IMDM media (10 % FCS, penicillin/streptomycin (100mg/ml)). Cells were counted and seeded at an appropriate concentration of cells/ml.

4.2.4 5-HT Uptake Assay

SERT inhibition was tested as previously described [84]. Briefly, TREx cells (1 X 10^5) were plated on poly-L-lysine coated (0.1mg/ml) plates. SERT-FLAG expression was

induced by adding tetracycline (1μg/ml) and incubating for 24h. When confluent, medium was removed from the cells and washed twice with 0.5ml (per well) of TB buffer (10mM HEPES, 150mM NaCl, 2mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$) pre-warmed to 37°C. Cells were incubated in TB buffer containing the drug of interest at various concentrations or equal volumes of vehicle control for 5min at 37 °C. Specific activity of [^{3}H] 5-HT was diluted with unlabelled 5-HT. Cells were incubated with various concentrations of 5-HT containing approximately 1 Ci/mmol [^{3}H] 5-HT at room temperature. After 2min uptake was stopped by removing the 5-HT solution and immediately adding cold TB buffer containing 1mM paroxetine (to inhibit 5HT transport). Cells were washed twice with cold TB and then lysed in 2% SDS. Radioactivity in the lysates was determined by liquid scintillation counting. The ^{3}H isotope was measured using a Packard Tricarb 2100 TR liquid scintillation analyser. The cocktail used was the commercial scintillant Ecoscint^{TM}, for aqueous samples. Non-specific uptake was defined as the uptake in the presence of 10μM paroxetine subtracted from the total uptake to obtain specific uptake. Cells in the absence of any test compound (untreated cells) represented the 100% re-uptake and the SSRI, citalopram was used as a positive control for SERT inhibition.

4.2.5 Neutral Red Assay for In vitro Cytotoxicity

The Neutral Red Assay was carried out as previously described [87]. Briefly, 5x 10^4 cells per well (200μl) were seeded in a 96 well plate until sub-confluent (24-36h) and treated with the appropriate compound for 48h. Following exposure of cells to drug the supernatant was removed and the cells incubated for 3 $\pm$ 1 h with 250μl neutral red dye

solution under sterile conditions. NR solution was removed carefully and the cells washed before the addition of 100µl of NR assay Solubilisation solution (50% ethanol-1% acetic acid solution in dH_20) .Plates were left to incubate in the dark for 20-30 min at room temperature with gentle shaking. The absorbance of each plate was read at 540nm and at 690nm (background) within 1h. Relative cell viability was expressed as percent of vehicle treated cells. Sodium azide and Triton-X were used as positive controls for cytotoxicity, where 30mM sodium azide and 2% Triton-X resulted in 80-90% cytotoxicity on all cell lines. Untreated cells represented 0% cytotoxicity (100% viability).

4.2.6 Data Analysis of NR assay

Each compound was screened over a 1µM-1mM concentration range in triplicate on two independent days with activity expressed as percentage cell viability compared to vehicle treated controls. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability) (Tables 1-4). All data points (expressed as means ± S.E.M.) were analysed using GRAPHPAD Prism (version 4) software (Graphpad software Inc., San Diego, CA). To determine if the pEC_{50}'s calculated for each drug differed significantly in each cell line, statistical analysis was carried out using a one way ANOVA Test comparing each pEC50 value. A P value of <0.05 was considered to reflect a significant difference. The means for different treatment groups were then compared using a two-way ANOVA test with no matching followed by a Bonferroni Post Test to compare replicate means by row to the control cell line HEK293. P values of <0.05 were considered to reflect a significant difference.

4.2.7 Alamar Blue Assay

1-5x10^4 cells/ well were seeded in a 96-well plate and treated with the respective drug for the desired length of time. Each well was then treated with 10µl of Alamar Blue and left to incubate at 37°C in the dark for 4-6h. Fluorescence was read using an emission of 590nM and excitation 544nm. The background fluorescence of the media without cells + alamar blue was taken away from each group, and the control untreated cells represented 100% cell viability and10µM Taxol was used as a positive control.

4.2.8 Quantification of Apoptosis. Propidium Iodide FACS analysis.

750,000 cells were seeded in 5ml, treated with the appropriate amount of compound and incubated for a specified time. Cells were harvested by centrifugation at 300xg for 5min and washed with 5ml of ice-cold PBS. The pellet was resuspended in 200µl PBS and 2ml of ice-cold 70% ethanol and cells were fixed overnight at 4°C. Cells were pelleted by centrifugation at 300xg for 5min and resuspended in PBS with 25µl of RNAse A (10mg/ml stock) and 75µl of propidium iodide (1mg/ml). The tubes were incubated in the dark at 37°C for 30min.Cell cycle analysis was performed using appropriate gates counting 10,000 cells and analysed using CELLQUEST software package. Untreated cells had <5% cells in the pre-G1 phase of the cell cycle and 10µM Taxol was used as a positive control for cell death.

4.3 Computational Studies

To date no crystal structure of hSERT exists to avail of in the docking process. A recent homology model of hSERT was constructed using LeuT as a template by Jorgensen et al [28]. Subsequent investigation of the flexibility of the binding site in complex with the natural substrate (5-HT) was undertaken to determine key protein ligand interactions through MD simulation in a membrane environment. FlexE [48] was selected as a docking platform to allow these binding site variations to be taken into account. The FlexE approach is based on flexibly docking a ligand(s) into a united protein model generated from the superimposed structures of an ensemble. Initially, from a 17ns trajectory the set was reduced to a more manageable size by selecting every 1000th stable frame. Taking the first frame as a template, the remaining 30 structures were aligned and superposed in MOE v2007.09 [88] with rmsd calculated for all residues of the binding sites (within 6.5Å of ligand). A set of three structures were chosen for the ensemble from a clustering based on the outputted rmsd. These structures were converted to PDB using Babel3 [89], to ensure backbone and side-chain atoms are ordered correctly. An ensemble description file was subsequently generated to allow automated docking within a script with all default values retained except set INST_EXT_ACT_RADIUS=0 and superposing of active sites only for the creation of the united protein model. The top 20 docked complexes were outputted and an svl script run within MOE on these complexes to determine key H-bonding interactions. The highest ranking solution with appropriate H-bonding incorporating Asp98 were then selected for further refinement using LigX (MOE v2007.09).

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Figure Captions

Figure 1. **The structures of methylenedioxyamphetamine, (MDA), methylenedioxymethamphetamine (MDMA) and 4-methylthioamphetamine (4-MTA)**

Figure 2. **Derivatives 4a (4-MTA) and 9a display similar key interactions in the 5-HT SERT binding site to 5-HT, MDMA and escitalopram**

2-D rendering of ligand-protein interactions using LigX module of MOE was used to create docked structures of 5-HT, 4-MTA, MDMA, **9a**, and escitalopram in the 5-HT binding site using a recent homology model of hSERT, constructed by Jorgensen et al [28] containing escitalopram as a bound ligand. Clearly similar contacts are observed in all cases with the exception of an arene-cation bond formed with Tyr95 in the escitalopram docked structure.

Figure 3. The interaction of 4-MTA analogues with Asp98 and Phe335 is important for SERT inhibition activity.

A positive charge at Asp98 is a crucial determinant of SERT reuptake, where a weaker H-bond with Asp98 through the oxygen of the hydroxylamine **4m** when compared with interactions proposed for primary amine **4a** is important for SERT inhibition (A). Interactions of a hydroxyl group with Asn101 and Asp98 and the nitrogen with Asp98

and Tyr176 is important SERT binding activity, illustrated by the docked structure of the S-ethylsubstituted compound **15c** (Type III) in the SERT binding site (B). The contacts of the nitrogen with Phe335 and Asp98 are preserved for the 4-MTA analogues, contributing to their SERT binding abilities illustrated by the docked structure of compound **19b** in the SERT binding site (C). 2-D rendering of ligand-protein interactions using LigX module of MOE was used using a recent homology model of hSERT, constructed by Jorgensen et al [28] containing escitalopram as a bound ligand.

Figure. 4. **The majority of 4-MTA derivatives are cytotoxic to hSERT and wildtype HEK cells with EC_{50} values in the low micromolar range.**

5x 10^4 cells were seeded and treated for 48h. Cells were incubated for 3 $\pm$ 1 h with neutral red dye solution. Absorbance was read at 540nM (690nm background). Relative cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by an EC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves where EC_{50} is the dose at 50% cell viability. Derivatives **4s**, **15f**, **15g** and **25b** were not included as EC_{50} values were greater than 100μM.

Figure.5 Derivative 9e is a potent antiproliferative pro-apoptotic agent

1-5x10^4 cells/ 200μl (A and B) and 7x10^5 cells/5ml (C) were seeded and treated with the 10μM or 50μM (C I) 9e and 25a (50μM) (CII) for the indicated times. 10μl of Alamar Blue reagent was added to each well (A and B), fluorescence was read as emission 590nm/excitation 544nm and values represent the mean value $\pm$ the S.E.M. of six data

points (recording in triplicate on two independent days) (A and B). Cells were harvested by centrifugation and fixed overnight in 70% ethanol (C). FACS analysis was carried out upon incubation with propidium iodide and RNase A. 10,000 cells were counted using appropriate gates (C). Values represent the mean ± SEM of three independent experiments.

Figure.6 The effects of derivatives 9e and 25a on peripheral blood mononuclear cells

1-5x10^4 cells/ 200ml were seeded and treated with 0.1-100μM **35**(A), **34** (B) for 24h. 10μl of Alamar Blue reagent was added to each well, fluorescence was read as emission 590nm/excitation 544nm and values represent the mean value + the S.E.M. of six data points (recording in triplicate on two independent days) .

Tables: 1-5

Table 1. **The effects of the 4-Methylthioamphetamine derivatives, 4a-i, 4l-p, (Type I) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.**

TREx SERT-FLAG cells (1x10^5) were incubated with drug and [^{3}H] 5-HT. After 2min, uptake was stopped by removing the 5-HT solution and immediately adding cold buffer containing 1mM paroxetine (to inhibit 5HT transport). Cells were washed, lysed and radioactivity determined by liquid scintillation counting. Non-specific uptake was defined as the uptake in the presence of 10μM paroxetine subtracted from the total uptake to obtain specific uptake. For the Neutral Red Assay, 5x10^4cells/well were seeded treated with for 48h. Supernatant was removed and the cells incubated for 3 ± 1 h with neutral

red dye solution. NR solution was removed, washed before the addition NR assay solubilisation solution. Absorbance was read at 540nm (690nm, background) within 1h. Cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability).

Table 2. The effects of the 4-Methylthioamphetamine derivatives, 9a-e, (Type II) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.

TREx SERT-FLAG cells ($1x10^5$) were incubated with drug and [^{3}H] 5-HT. After 2min, uptake was stopped by removing the 5-HT solution and immediately adding cold buffer containing 1mM paroxetine (to inhibit 5HT transport). Cells were washed, lysed and radioactivity determined by liquid scintillation counting. Non-specific uptake was defined as the uptake in the presence of 10μM paroxetine subtracted from the total uptake to obtain specific uptake. For the Neutral Red Assay, $5x10^4$cells/well were seeded treated with for 48h. Supernatant was removed and the cells incubated for 3 $\pm$ 1 h with neutral red dye solution. NR solution was removed, washed before the addition NR assay solubilisation solution. Absorbance was read at 540nm (690nm, background) within 1h. Cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability).

Table 3. The effects of the 4-Methylthioamphetamine derivatives, 15a-d (Type III), and 15e-i (Type IV) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.

TREx SERT-FLAG cells ($1x10^5$) were incubated with drug and [^{3}H] 5-HT. After 2min, uptake was stopped by removing the 5-HT solution and immediately adding cold buffer containing 1mM paroxetine (to inhibit 5HT transport). Cells were washed, lysed and radioactivity determined by liquid scintillation counting. Non-specific uptake was defined as the uptake in the presence of 10μM paroxetine subtracted from the total uptake to obtain specific uptake. For the Neutral Red Assay, $5x10^4$cells/well were seeded treated with for 48h. Supernatant was removed and the cells incubated for 3 ± 1 h with neutral red dye solution. NR solution was removed, washed before the addition NR assay solubilisation solution. Absorbance was read at 540nm (690nm, background) within 1h. Cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability).

Table 4. The effects of the 4-Methylthioamphetamine derivatives, 19a-h, 19m-n (Type V) and related analogues 25a-b (Type VI) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.

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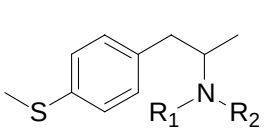
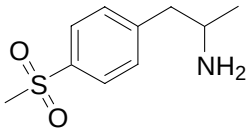
Table. 5 Compounds 9e and 25a induce dose dependent apoptosis in a range of haematopoietic malignancies.

Cells were seeded at a density of $7x10^5$cells/5ml treated with **9e** and **25a** for the indicated time, harvested by centrifugation and fixed overnight in 70% ethanol. FACS analysis was carried out upon incubation with propidium iodide and RNase A. 10,000 cells were counted using appropriate gates. EC_{50} values were determined by non-linear regression analysis of sigmoidal log concentration dependence curves where EC_{50} is the dose at 50% apoptotic effect.

Schemes: 1-5

Table 1. The effects of the 4-Methylthioamphetamine derivatives, **4a-i, 4l-p**, (Type 1) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.





Compnd	R_1	R_2	Yield (%)	cLogP	SERT Reuptake Inhibition IC_{50} (μM)	HEK293 pEC50[a] ± SE	HEK293 hSERT pEC50[a] ± SE	DG-75 pEC50[a] ± SE	SH-SY5Y pEC50[a] ± SE
4a(4-MTA)	H	H	85	2.30	0.207±0.029	4.73 ± 0.14	4.65 ± 0.15	3.466 ± 0.73	3.216± 0.52
4b	CH_3	H	49	2.45	0.417±0.031	4.71 ± 0.13	4.77 ± 0.18	3.89± 0.28	2.81± 0.23
4c	CH_2CH_3	H	35	2.98	0.963±0.076	4.79 ± 0.10	4.90 ± 0.10	3.00± 0.17	2.73 ± 0.21
4d	$CH(CH_3)_2$	H	36	3.29	1.854±0.618	4.51 ± 0.16	4.60 ± 0.11	3.47± 0.24	2.71± 0.29
4e		H	21	2.80	0.702±0.234	4.73 ± 0.10	4.71 ± 0.14	3.50± 0.17	3.43± 0.26
4f	$CH_2C{=}CH_2$	H	23	3.22	0.524±0.121	4.81 ± 0.12	4.76 ± 0.15	3.72± 0.29	3.91± 0.32
4g	—C≡CH	H	31	3.05	0.688±0.110	4.61 ± 0.15	4.62 ± 0.17	4.42± 0.20	2.63± 0.18
4h	CH_2CH_2OH	H	68	1.97	0.868±0.117	5.00 ± 0.09	4.89 ± 0.089	2.75± 0.21	2.64± 0.18
4i	$CH_2CH_2OCH_3$	H	28	2.60	3.583±0.816	4.64 ± 0.13	4.68 ± 0.18	4.48± 0.21	2.87± 0.24
4l	CH_3	CH_3	48	2.99	0.520±0.088	4.70 ± 0.06	4.54 ± 0.14	3.31 ± 0.18	2.73 ± 0.29
4m	OH	CH_3	20	1.82	1.875[c]	4.62 ± 0.12	4.53 ± 0.11	3.13± 0.17	3.64 ± 0.14
4n	OCH_3	CH_3	73	2.96	25.06±3.41	3.28 ± 0.24	2.61 ± 0.24	<2	<2
4o	OH	H	68	1.86	0.436±0.040	4.42 ± 0.13	4.40 ± 0.13	3.4± 0.16	3.24 ±0.29
4p	OCH_3	H	81	2.67	0.664±0.057	4.96 ± 0.10	4.55 ± 0.11	2.74 ± 0.20	2.61± 0.21
4s	-	-	67	0.59	100% reuptake at 100μM	<2	2.67±0.58	<2	<2
MDMA	-	-	-	2.15	1.06	3.61 ± 0.19	3.31 ± 0.21	4.49 ± 0.21	<2
MDA	-	-	-	1.64	0.996	3.49 ± 0.18	3.16 ± 0.34	2.72 ± 0.25	<2
PMA				1.77	0.173	3.34 ± 0.21	2.97 ± 0.24	4.49 ± 0.17	<2

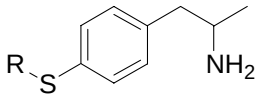
[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA).

[b] At 1μM, 4-MTA is selectively more toxic to the hSERT cell line compared to the control HEK293 cell line ($P<0.05$ based on two-way ANOVA test (GRAPH pad Prism 4) with no matching followed by a Bonferroni Post Test to compare replicate means by row to the control cell line HEK293 for each concentration

[c] Single determination

The selective serotonin reuptake inhibitor, citalopram was used as a positive control for SERT uptake inhibition (IC_{50} of 3.17nM), whereas sodium azide (30mM) and Triton-X (2%) acted as positive controls for cytotoxicity resulting in 80-90% cytotoxicity to all cells.

Table 2. The effects of the 4-Methylthioamphetamine derivatives, **9a-e**, (Type II) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.



Compnd	R	Yield (%)	cLogP	SERT Reuptake Inhibition IC_{50}(μM)	HEK293 pEC50[a]+ SE	hSERT pEC50[a]+ SE	DG-75 pEC50[a]+ SE	SH-SY5Y pEC50[a]+ SE
9a	CH_2CH_3	75	2.83	1.403±0.250	4.82 ± 0.10	4.85 ± 0.12	4.64± 0.19	4.19± 0.16
9b	$C(CH_3)_3$	58	3.69	100%100μM	5.14 ± 0.13	5.4 ± 0.1563	5.11 ± 0.19	4.69± 0.16
9c	$CH_2C_6H_5$	71	3.87	0.679±0.025	5.53 ± 0.10	5.43 ± 0.15	5.31± 0.14	4.71 ± 0.14
9d	$CH_2CH_2N(CH_3)_2$	52	2.23	43.29±9.154	5.06 ± 0.11	5.09 ± 0.13	4.76± 0.18	3.62± 0.16
9e	C_6H_5	47	4.09	14.33±0.355	5.56±0.13	5.60±0.09	5.53± 0.12	4.88± 0.14
MDMA	-	-	2.15	1.06	3.61 ± 0.19	3.31 ± 0.21	4.49 ± 0.21	<2
MDA	-	-	1.64	0.996	3.49 ± 0.18	3.16 ± 0.34	2.72 ± 0.25	<2
PMA			1.77	0.173	3.34 ± 0.21	2.97 ± 0.24	4.49 ± 0.17	<2

[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA).
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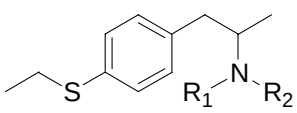
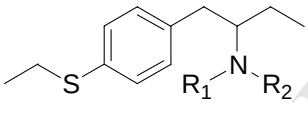
Table 3. The effects of the 4-Methylthioamphetamine derivatives, **15a-d**, (Type III), and **15e-i** (Type IV) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.



15a-d



15e-i

Compd	R_1	R_2	Yld (%)	cLogP	SERT Reuptake Inhibition $IC_{50}(\mu M)$	HEK293 pEC50[a] ± SE	HEK293 SERT pEC50[a] ± SE	DG-75 pEC50[a] ± SE	SH-SY5Y pEC50[a] ± SE
15a	CH_3	H	34	2.99	0.724±0.104	5.12 ± 0.11	4.71 ± 0.13	3.88± 0.12	3.55± 0.19
15b	CH_2CH_3	H	17	3.50	0.478±0.036	5.20 ± 0.07	4.78 ± 0.13	3.85± 0.19	3.52± 0.20
15c	OH	H	86	2.39	0.625±0.165	4.49 ± 0.13	4.59± 0.18	3.91± 0.32	3.15± 0.12
15d	CH_3	OH	35	2.35	1.575±0.030	4.91 ± 0.09	4.62 ± 0.15	4.73 ± 0.21	4.36± 0.15
15e	H	H	73	3.36	1.290±0.247	5.29 ± 0.06	5.23 ± 0.10	4.16± 0.20	4.27± 0.18
15f	CH_2CH_2	H	27	4.03	1.804±0.035	3.60 ± 0.17	4.213 ± 0.14	3.45± 0.16	3.61± 0.17
15g	CH_3	H	28	3.50	>100μM[b]	-[c]	-[c]	-[c]	-[c]
15h	OH	H	73	2.92	1.446±0.482	4.94 ± 0.08	4.82 ± 0.13	4.11± 0.27	2.74± 0.13
15i	CH_3	OH	62	2.89	2.003±0.170	4.29 ± 0.15	4.35 ± 0.14	3.40± 0.15	3.68± 0.13
MDMA	-	-	-	2.15	1.06	3.61 ± 0.19	3.31 ± 0.21	4.49 ± 0.21	<2
MDA	-	-	-	1.64	0.996	3.49 ± 0.18	3.16 ± 0.34	2.72 ± 0.25	<2
PMA				1.77	0.173	3.34 ± 0.21	2.97 ± 0.24	4.49 ± 0.17	<2

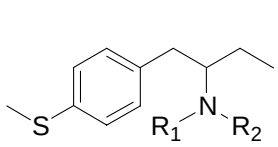
[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA).

[b] 26% reuptake inhibition at 1μM

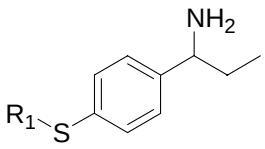
[c] Compound not included in Neutral Red cytotoxicity screen

The selective serotonin reuptake inhibitor, citalopram was used as a positive control for SERT uptake inhibition (IC_{50} of 3.17nM), whereas sodium azide (30mM) and Triton-X (2%) acted as positive controls for cytotoxicity resulting in 80-90% cytotoxicity to all cells.

Table 4. The effects of the 4-Methylthioamphetamine derivatives, **19a-h, 19m-n** (Type V) and related analogues **25a-b** (Type VI) on SERT activity (IC_{50}), on SERT expressing HEK cells (EC_{50}) and on the malignant cell lines, DG-75 and SHSY-5Y.



19a-h, 19m-n



25a-b

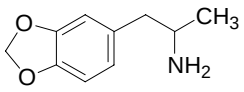
Compd	R_1	R_2	Yld (%)	cLogP	SERT Reuptake Inhibition IC_{50}(μM)	HEK293 pEC50[a] ± SE	HEK293 hSERT pEC50[a] ± SE	DG-75 pEC50[a] ± SE	SH-SY5Y pEC50[a] ± SE
19a	H	H	53	2.83	0.611 ±0.016	4.83 ± 0.11	4.755 ± 0.13	3.87± 0.27	3.36± 0.20
19b	CH_3	H	77	2.99	0.437 ±0.056	4.30 ± 0.15	4.26 ± 0.10	4.33± 0.23	3.08± 0.23
19c	CH_2CH_3	H	28	3.50	0.823 ±0.274	5.00 ± 0.10	4.679± 0.11	3.55± 0.19	3.82± 0.13
19d	$CH_2CH_2CH_3$	H	26	4.03	0.621 ±0.124	3.519 ± 0.17	3.57 ± 0.27	3.59± 0.36	2.52± 0.36
19e	$CH(CH_3)_2$	H	15	3.81	1.939 ±0.628	4.76 ± 0.08	4.67 ± 0.13	3.57± 0.19	3.47± 0.19
19f		H	30	3.33	0.872 ±0.214	4.97± 0.11	4.68 ± 0.16	4.22± 0.25	<2
19g	—C≡CH	H	26	3.58	0.399 ±0.030	4.69 ± 0.12	4.724 ± 0.16	3.80± 0.18	3.405± 0.16
19h	$CH_2CH_2OCH_3$	H	39	3.13	2.821 ±0.081	4.79 ± 0.09	4.718 ± 0.14	2.72± 0.15	2.41± 0.21
19m	OH	H	30	2.39	0.598 ±0.199	5.07 ± 0.07	4.98 ± 0.09	4.11± 0.20	4.50± 0.25
19n	OCH_3	CH_3	51	2.35	0.695 ±0.232	4.92 ± 0.12	4.84 ± 0.13	3.51± 0.23	4.02± 0.28
25a	$-(CH_2)_4CH_3$	-	48	3.98	>25μM	5.50± 0.12	5.59± 0.1	5.32± 0.13	4.71 ±0.14
25b	CH_3	-	46	2.32	65% reuptake at 10μM	2.57± 0.20	2.71± 0.15	<2	<2
MDMA	-	-	-	2.15	1.06	3.61 ± 0.19	3.31 ± 0.21	4.49 ± 0.21	<2
MDA	-	-	-	1.64	0.996	3.49 ± 0.18	3.16 ± 0.34	2.72 ± 0.25	<2
PMA				1.77	0.173	3.34 ± 0.21	2.97 ± 0.24	4.49 ± 0.17	<2

[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA).
The selective serotonin reuptake inhibitor, citalopram was used as a positive control for SERT uptake inhibition (IC_{50} of 3.17nM), whereas sodium azide (30mM) and Triton-X (2%) acted as positive controls for cytotoxicity resulting in 80-90% cytotoxicity to all cells.

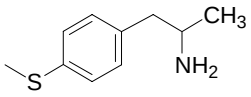
Table. 5 Analogues **9e** and **25a** induce dose dependent apoptosis in a range of haematopoietic malignancies.

EC_{50} Values (μM)

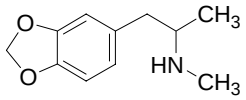
Compound	**Burkitt's lymphoma**		**Human-promyelocytic Leukaemia**
	DG-75 72h	*MUTU- c179 24h*	*HL-60 24h*
9e	23.68	14.21	19.61
25a	33.76	21.02	29.50



Methylenedioxyamphetamine (MDA)



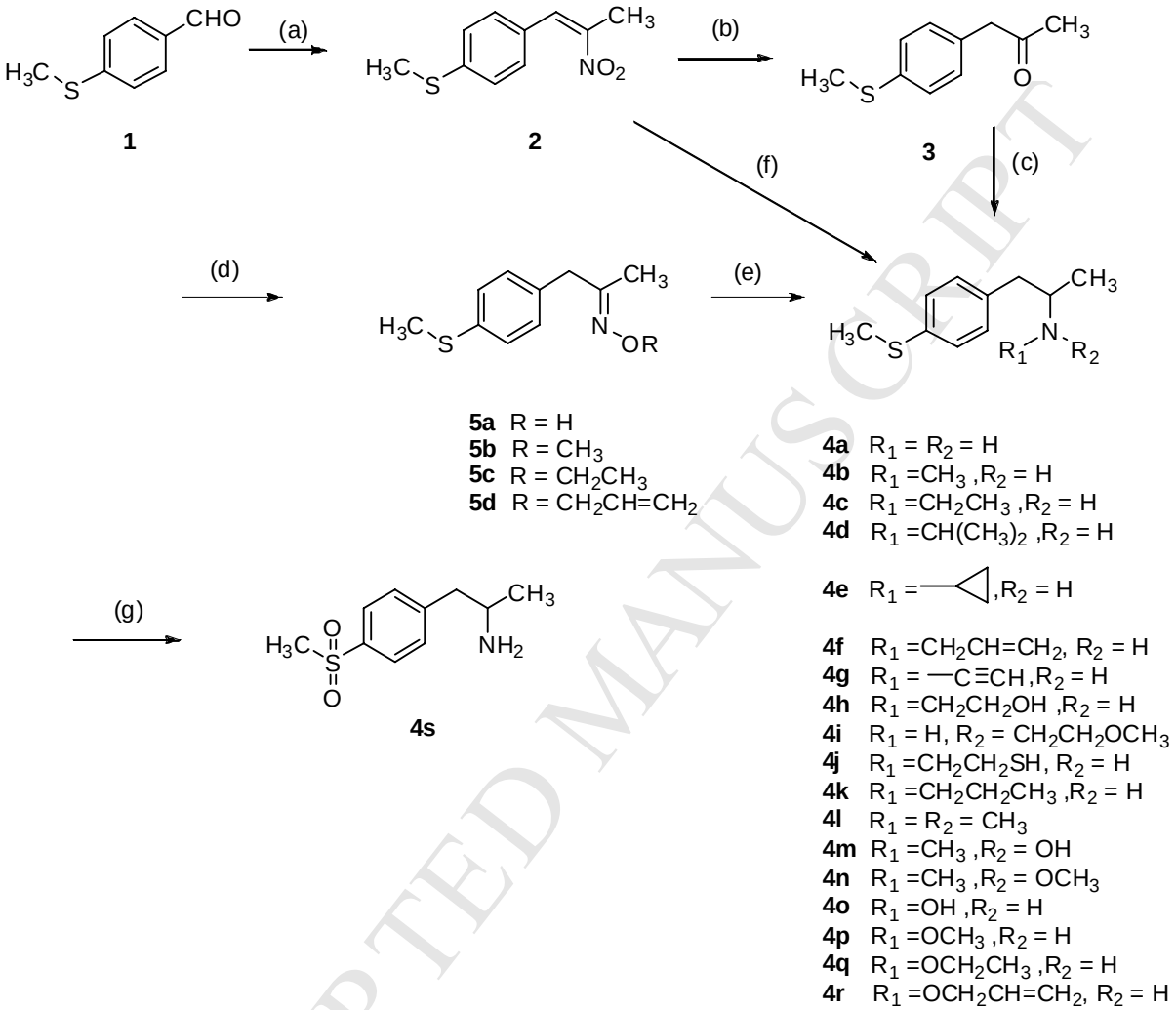
4-Methylthioamphetamine (4-MTA)



Methylenedioxymethamphetamine (MDMA)

Figure 1: The structures of methylenedioxyamphetamine, (MDA), methylenedioxymethamphetamine(MDMA) and 4-methylthioamphetamine (4-MTA)

Scheme 1: Synthesis of 4-Methylthioamphetamine analogues Type 1[a]



1

2

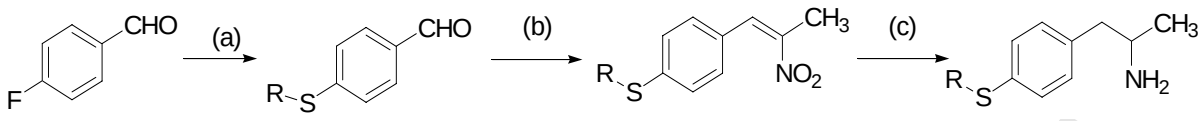
3

5a R = H
5b R = CH_3
5c R = CH_2CH_3
5d R = $CH_2CH{=}CH_2$

4a $R_1 = R_2 = H$
4b $R_1 = CH_3$, $R_2 = H$
4c $R_1 = CH_2CH_3$, $R_2 = H$
4d $R_1 = CH(CH_3)_2$, $R_2 = H$
4e R_1 = cyclopropyl, $R_2 = H$
4f $R_1 = CH_2CH{=}CH_2$, $R_2 = H$
4g $R_1 = C{\equiv}CH$, $R_2 = H$
4h $R_1 = CH_2CH_2OH$, $R_2 = H$
4i $R_1 = H$, $R_2 = CH_2CH_2OCH_3$
4j $R_1 = CH_2CH_2SH$, $R_2 = H$
4k $R_1 = CH_2CH_2CH_3$, $R_2 = H$
4l $R_1 = R_2 = CH_3$
4m $R_1 = CH_3$, $R_2 = OH$
4n $R_1 = CH_3$, $R_2 = OCH_3$
4o $R_1 = OH$, $R_2 = H$
4p $R_1 = OCH_3$, $R_2 = H$
4q $R_1 = OCH_2CH_3$, $R_2 = H$
4r $R_1 = OCH_2CH{=}CH_2$, $R_2 = H$

[a]Reagents and conditions: (a) $CH_3CH_2NO_2$, $(CH_3)_2NH.HCl$, KF, reflux ; (b) Fe, CH_3COOH, 100°, 2h, (c) $NaCNBH_3$, NH_4OCOCH_3 or $R_1R_2NH.HCl$, CH_3OH,rt, 72h; (d) $RONH_2HCl$, Pyridine, EtOH, reflux, 2h; (e) $NaCNBH_3$, MeOH, rt, 72h; (f) $LiAlH_4$, THF, reflux, 12h; (g) mCPBA, CH_2Cl_2.

Scheme 2: Synthesis of 4-Methylthioamphetamine analogues Type II[a]



6

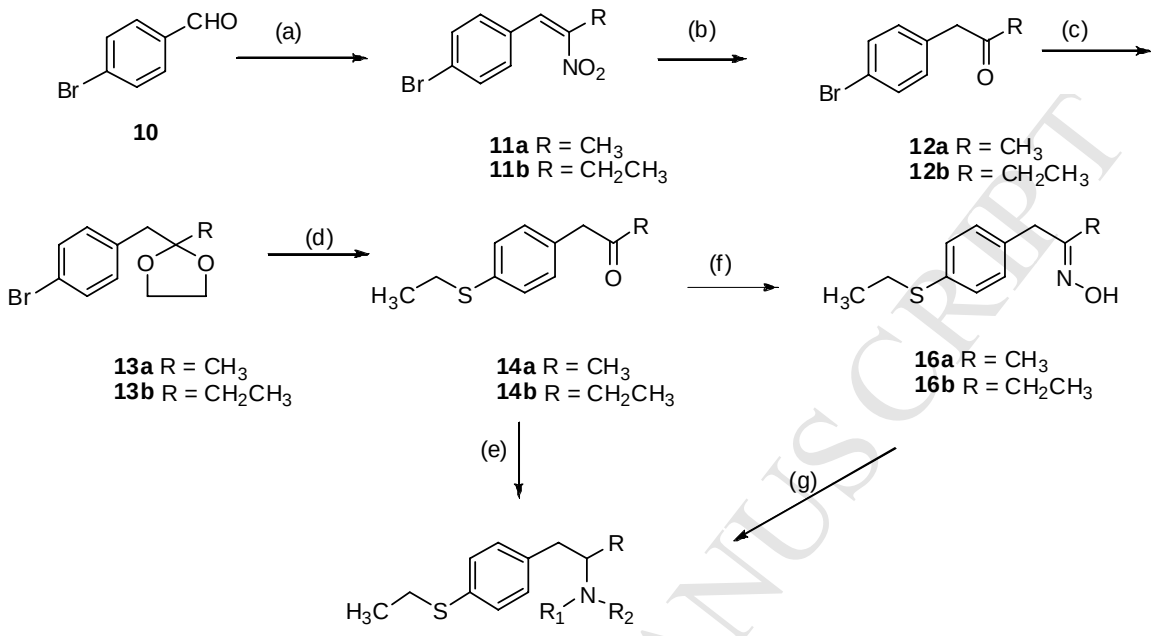
7a R = CH_2CH_3
7b R = $C(CH_3)_3$
7c R = $CH_2C_6H_5$
7d R = $CH_2CH_2N(CH_3)_2$
7e R = C_6H_5

8a R = CH_2CH_3
8b R = $C(CH_3)_3$
8c R = $CH_2C_6H_5$
8d R = $CH_2CH_2N(CH_3)_2$
8e R = C_6H_5

9a R = CH_2CH_3
9b R = $C(CH_3)_3$
9c R = $CH_2C_6H_5$
9d R = $CH_2CH_2N(CH_3)_2$
9e R = C_6H_5

[a]Reagents and conditions: (a) R-SH, K_2CO_3, DMF, 120^o, 4h; (b) $CH_3CH_2NO_2$, $(CH_3)_2NH.HCl$, KF, reflux ; (b) $LiAlH_4$, THF, reflux, 12h.

Scheme 3: Synthesis of 4-Methylthioamphetamine analogues Type III and Type IV[a]



10

11a R = CH_3
11b R = CH_2CH_3

12a R = CH_3
12b R = CH_2CH_3

13a R = CH_3
13b R = CH_2CH_3

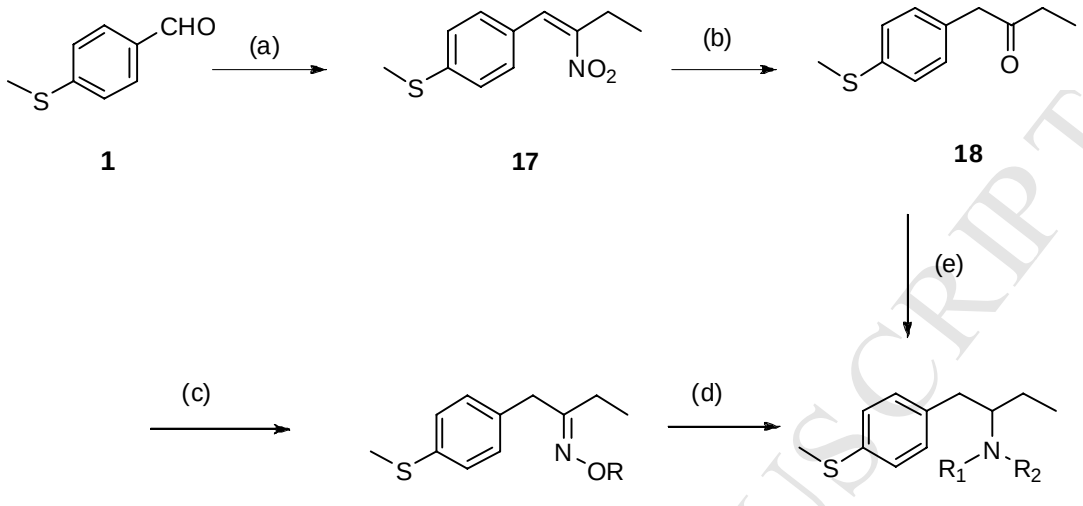
14a R = CH_3
14b R = CH_2CH_3

16a R = CH_3
16b R = CH_2CH_3

15a R = R_1 = CH_3, R_2 = H
15b R = CH_3, R_1 = CH_2CH_3, R_2 = H
15c R = CH_3, R_1 = OH, R_2 = H
15d R = R_2 = CH_3, R_1 = OH
15e R = C_2H_5, R_1 = R_2 = H
15f R = R_1 = C_2H_5, R_2 = H
15g R = C_2H_5, R_1 = CH_3, R_2 = H
15h R = C_2H_5, R_1 = OH, R_2 = H
15i R = C_2H_5, R_1 = OH, R_2 = CH_3

[a]Reagents and conditions: (a) $CH_3CH_2NO_2$or $CH_3CH_2CH_2NO_2$, $(CH_3)_2NH.HCl$, KF, reflux ; (b) Fe, CH_3COOH, 100°, 2h; (c) $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux, 18h; (d) (i) *n*-BuLi, $(CH_3CH_2S)_2$, THF, -78°C (ii) 20%aq HCl, EtOH, reflux, 2h; (e) $NaCNBH_3$, NH_4OCOCH_3 or $R_1R_2NH.HCl$, CH_3OH, rt, 72h; (f) $NH_2OH.HCl$, Pyridine, EtOH, reflux, 2h; (g) $NaCNBH_3$, MeOH, pH 3, rt, 72h.

Scheme 4: Synthesis of 4-Methylthioamphetamine analogues Type V[a]

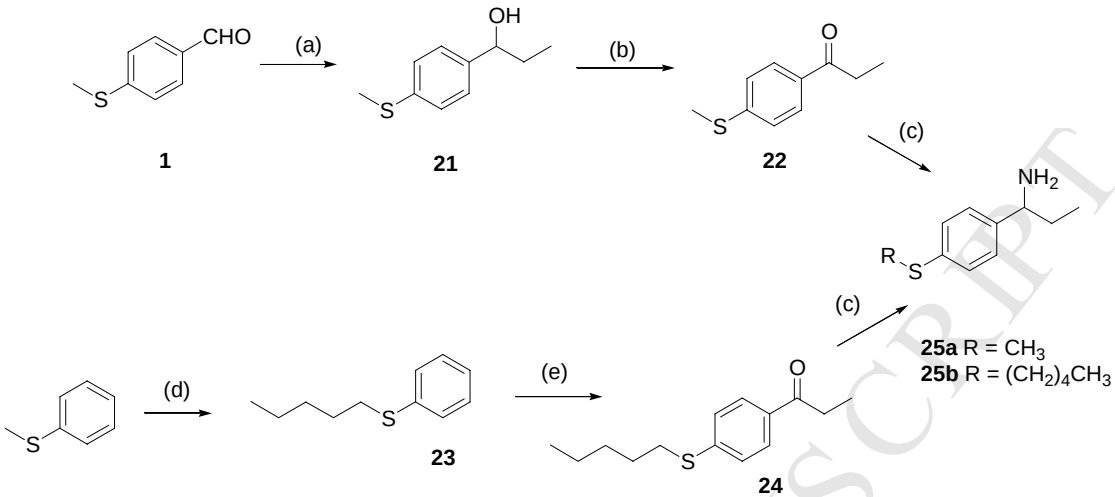


20a R = H
20b R = CH_3
20c R = CH_2CH_3
20d R = $CH_2CH{=}CH_2$

19a $R_1 = R_2 = H$
19b $R_1 = CH_3$, $R_2 = H$
19c $R_1 = CH_2CH_3$, $R_2 = H$
19d $R_1 = CH_2CH_2CH_3$, $R_2 = H$
19e $R_1 = C(CH_3)_3$, $R_2 = H$
19f R_1 =  $R_2 = H$
19g $R_1 = H_2C{-}C{\equiv}CH$, $R_2 = H$
19h $R_1 = CH_2CH_2OCH_3$, $R_2 = H$
19i $R_1 = CH_2CH{=}CH_2$, $R_2 = H$
19j $R_1 = CH_2CH_2OH$, $R_2 = H$
19k $R_1 = R_2 = CH_3$
19l $R_1 = OCH_3$, $R_2 = CH_3$
19m $R_1 = OH$, $R_2 = H$
19n $R_1 = OH$, $R_2 = CH_3$
19o $R_1 = OCH_3$, $R_2 = H$
19p $R_1 = OCH_2CH_3$, $R_2 = H$
19q $R_1 = OCH_2CH{=}CH_2$, $R_2 = H$

[a]Reagents and conditions: (a) $CH_3CH_2CH_2NO_2$, $(CH_3)_2NH.HCl$, KF, reflux ; (b) Fe, CH_3COOH, 100°, 2h, (c) $H_2NOR.HCl$, Pyridine, EtOH, reflux, 2h; (d) $NaCNBH_3$, MeOH, rt, 72h, (e) $NaCNBH_3$, NH_4OCOCH_3 or $R_1R_2NH.HCl$, CH_3OH, rt, 72h;

Scheme 5: Synthesis of 4-Methylthioamphetamine analogues Type VI[a]



[a]Scheme reagents and conditions: (a) CH_3CH_2MgBr, Et_2O, 34°, 3h (b) PCC, CH_2Cl_2, 40°, 3h (c) (i) HMDS, $TiCl_4$, CH_2Cl_2, 20h, rt (ii) MeOH, $NaCNBH_3$, rt (d) *sec*BuLi, $CH_3CH_2CH_2CH_2I$, THF, -78°, 3.5h, (e) CH_3CH_2COCl, $AlCl_3$, CH_2Cl_2, 0°, 3h

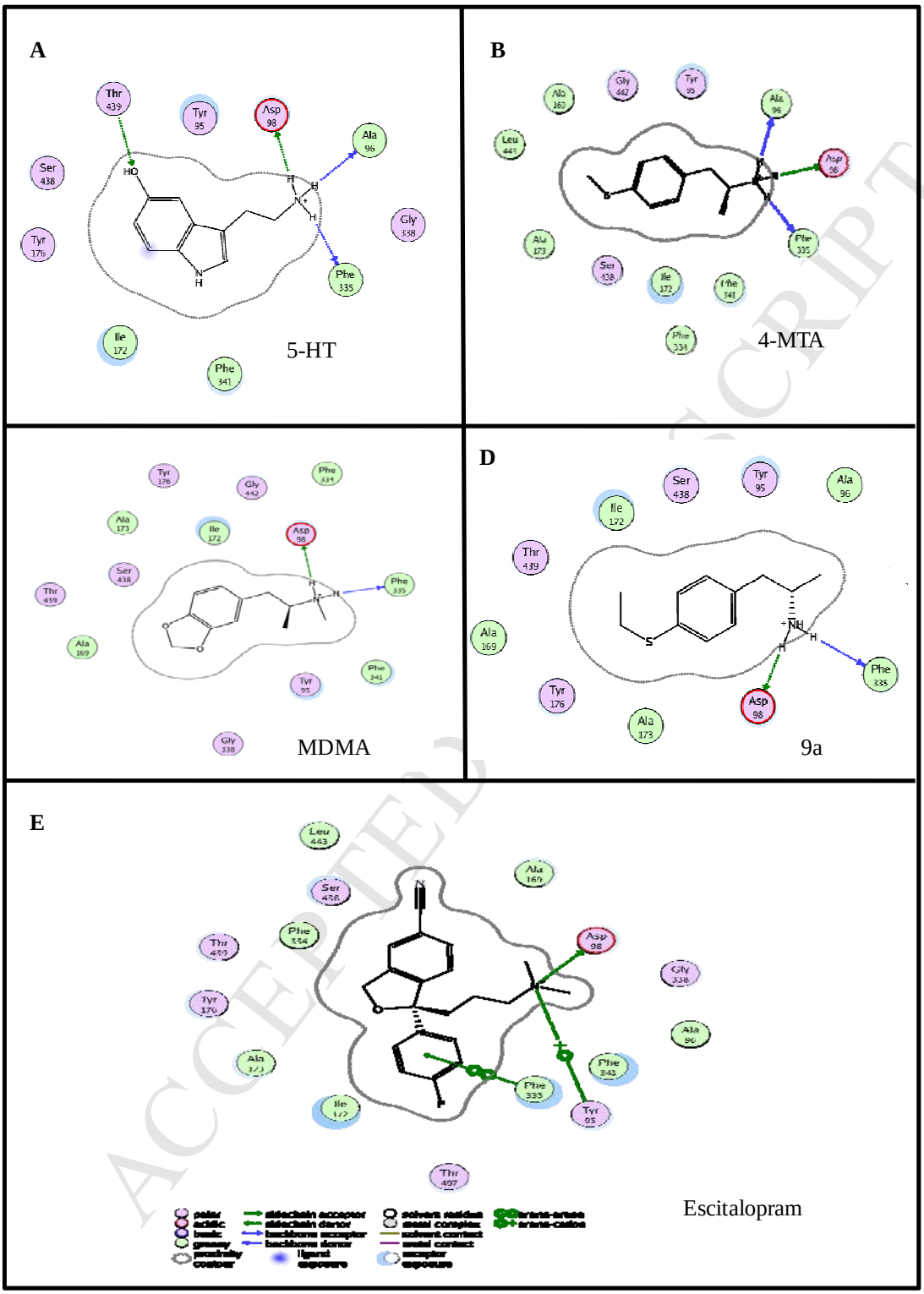


Figure 2. Derivatives 4a (4-MTA) and 9a display similar key interactions in the 5-HT SERT binding site to 5-HT, MDMA and escitalopram

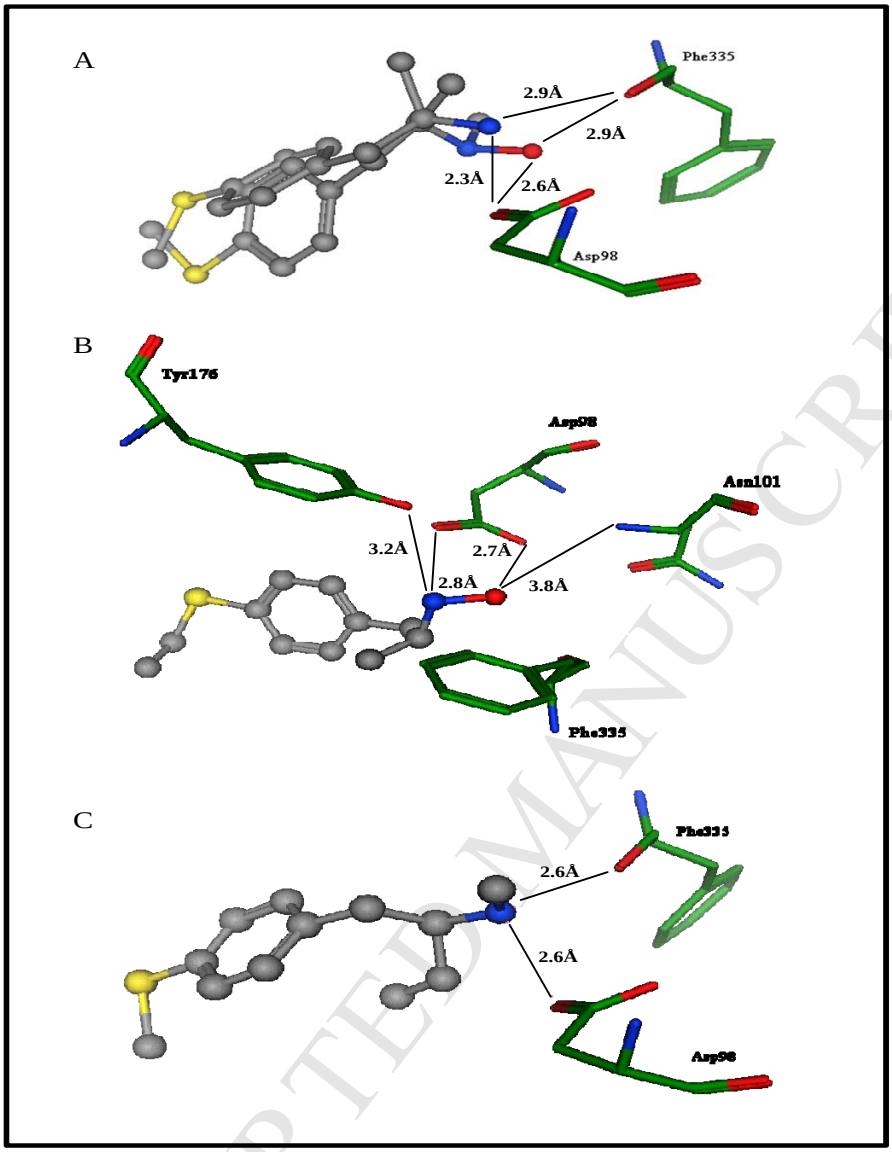


Figure 3: The interaction of 4-MTA analogues with Asp98 and Phe335 is important for SERT inhibition activity.

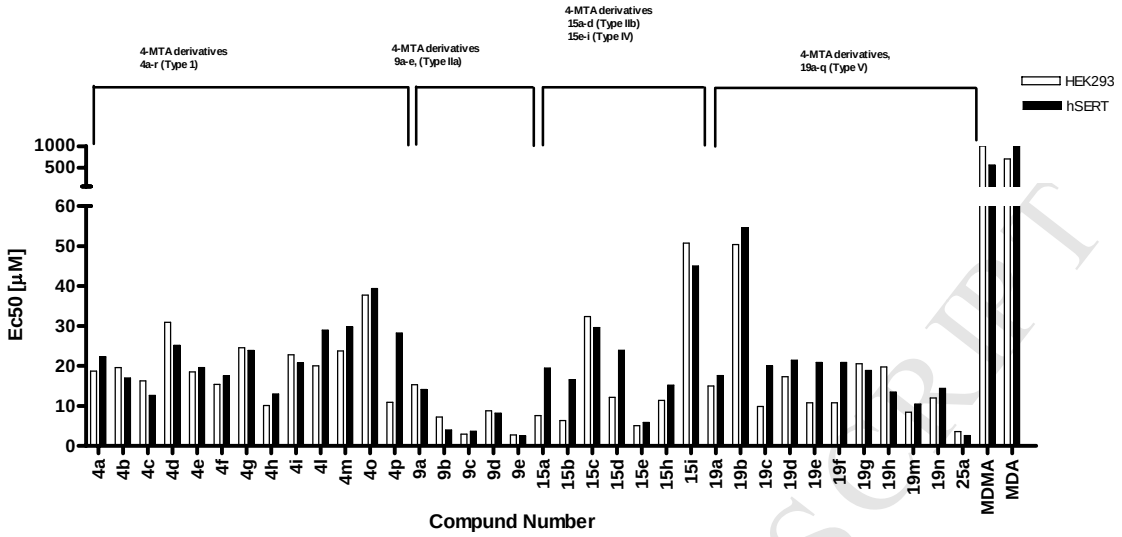


Figure 4. The majority of 4-MTA analogues are cytotoxic to hSERT and wildtype HEK cells with EC_{50} values in the low micromolar range.

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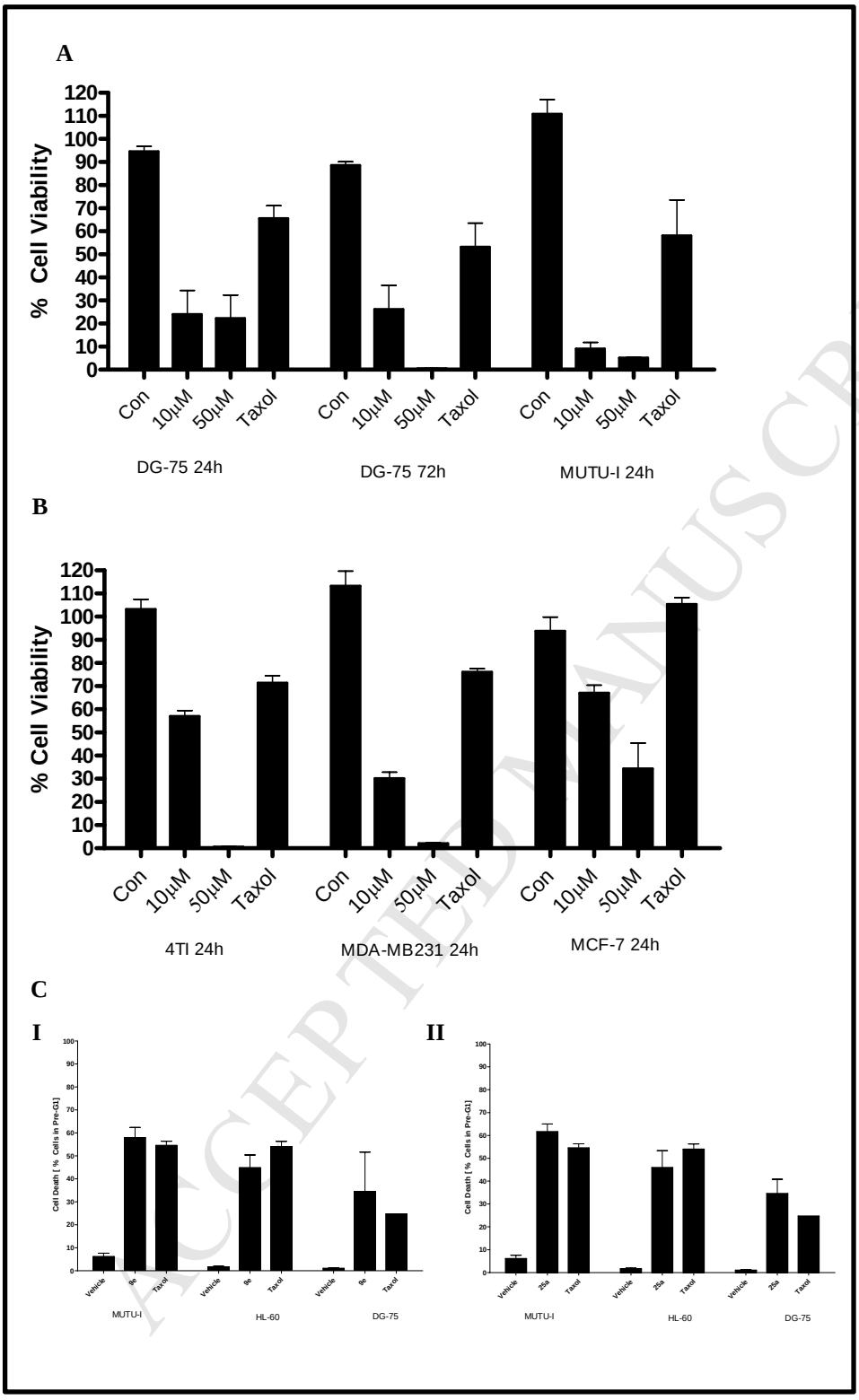

Figure 5. Compound 9e is a potent antiproliferative apoptotic agent

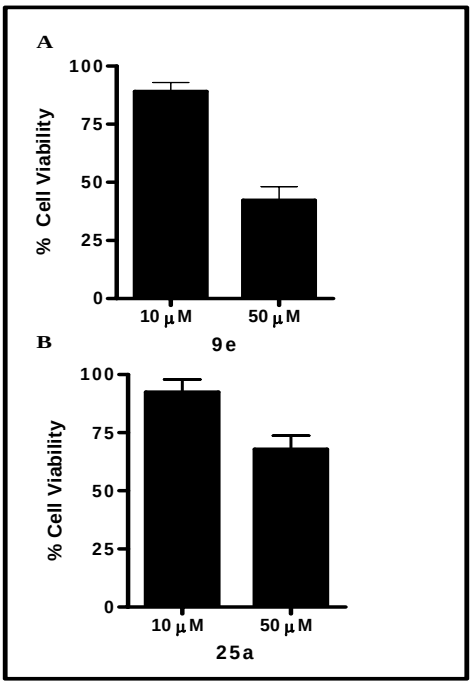

Figure 6. The effects of derivatives 9e and 25a on peripheral blood mononuclear cells