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Synthesis and Biochemical Evaluation of 3-Phenoxy-1,4-diarylazetidin-2-ones as Tubulin-Targeting Antitumour Agents

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Abstract

Structure-activity relationships for a series of 3-phenoxy-1,4-diarylazetidin-2-ones were investigated leading to the discovery of a number of potent antiproliferative compounds, including *trans*-4-(3-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**78b**) and *trans*-4-(3-amino-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**90b**). X-ray crystallography studies indicate the potential importance of the torsional angle between the 1-phenyl 'A' ring and 4-phenyl 'B' ring for potent antiproliferative activity, and that a *trans* configuration between the 3-phenoxy and 4-phenyl rings is generally optimal. These compounds displayed IC_{50} values of 38 nM and 19 nM respectively in MCF-7 breast cancer cells, inhibited the polymerization of isolated tubulin *in vitro*, disrupted the microtubular structure in MCF-7 cells as visualised by confocal microscopy, and caused G_2/M arrest and apoptosis. Compound **90b** possessed a mean GI_{50} value of 22 nM in the NCI60 cell line screen, displayed minimal cytotoxicity and was shown to interact at the colchicine-binding site on β-tubulin. Phosphate and amino acid prodrugs of both **78b** and **90b** were synthesised, of which the alanine amide **102b** retained potency and is a promising candidate for further clinical development.

Introduction

Tubulin polymerization plays a critical role in mitosis and cell division, and antimitotic drugs including vincristine, vinblastine, paclitaxel, docetaxel are one of the major classes of cytotoxic drugs in clinical use for treatment of cancers. The combretastatins are a group of diarylstilbenes isolated from the bark of the South African tree *Combretum caffrum*. Combretastatin A-4 (CA-4, **2**, Figure 1) demonstrates potent cytotoxic activity against a range of human cancer cell lines e.g. leukaemia, ovarian and colon cancer including multidrug resistant (MDR) lines overexpressing P-glycoprotein (Pgp). It binds to the colchicine site of tubulin, inhibits tubulin polymerisation, and inhibits angiogenesis.[1] A water-soluble, phosphate prodrug, combretastatin A-4 phosphate [CA-4P, **3**, Figure 1 (also known as fosbretabulin disodium)], has been designated orphan drug status for the treatment of anaplastic thyroid cancer by both the FDA and the European Medicines Agency (EMA), and for the treatment of ovarian cancer by the FDA.[2] The related amino acid amide **4** [(Figure 1 (also known as AC7700 or AVE8026)] was recently investigated in phase III trials for treatment of advanced soft-tissue sarcomas but, in combination with cisplatin, it did not show a sufficient clinical benefit in patients to support its use as a therapeutic option.[3] Combretastatin A-1 diphosphate (also known as CA-1P) is under investigation for relapsed and refractory acute myelogenous leukemia, myelodysplastic syndromes and advanced solid tumors. Crolibulin [**5**, Figure 1 (also known as EPC2407 and crinobulin)] is another tubulin-disrupting agent interacting at the colchicine-binding site and is in early phase I/II trials in combination with cisplatin focusing on anaplastic thyroid cancer.[4]

The *cis* alkene configuration in **2** is essential for anti-cancer activity as it positions the aromatic rings at the optimal distance from each other and gives the optimal dihedral angle for interaction with the colchicine-binding site of tubulin (55° for **2** and 53° for **1**).[5-6] Isomerization to the inactive *trans* configuration is observed on storage and *in vivo*

metabolism.[7] To optimize potency and pharmacokinetic properties, many structural modifications of the combretastatin stilbene structure have been investigated, including the conformationally restricted analogues obtained by incorporating the 1,2-diarylalkene bridge into a carbocyclic or heterocyclic ring system. Heterocombretastatin derivatives have been synthesized based on, amongst others, five membered aromatic heterocyclic rings such as tetrazole, pyrazole, imidazole, triazole, isoxazole, thiazole and thiophene rings.[8-10] Interest in small-molecule tubulin polymerization inhibitors continues unabated, with new analogues reported regularly (e.g. compound **6**, Figure 1).[11-13]

We have previously reported the antiproliferative activity of selective estrogen receptor modulator (SERM)-type compounds containing the azetidin-2-one (β-lactam) scaffold.[14] Previous research has indicated the potential of the azetidin-2-one ring as a template for antiproliferative agents, and we have demonstrated potent antiproliferative activity for series of 1,4-diarylazetidin-2-ones and *trans*-1,3,4-triarylazetidin-2-ones in human breast cancer cell lines.[15-18] Our studies were furthered to design, synthesise and biologically evaluate a novel panel of compounds containing an aryloxy substituent at C-3 of the β-lactam ring. We wished to explore the role of the C-3 phenoxy substituent to determine if this flexible, electron-withdrawing substituent could provide potent antiproliferative compounds. Introduction of the phenoxy substitutent at C-3 also allowed us to examine the role of the *cis*/*trans* configuration of azetidin-2-ones on their antiproliferative effects.

Chemistry and Stability Studies

The preparation of the target azetidinones is illustrated in Schemes 1-8. The majority of compounds identified for synthesis contain a 3,4,5-trimethoxyaryl ring at N-1, present in ring A of **2**, and an aryl ring with various substitution patterns at the C-4 position of the β-lactam ring (Ring B). Imines **14-39** were obtained by condensation of the appropriately substituted benzaldehydes and anilines in refluxing ethanol (Scheme 1). Nitrobenzaldehydes **7** and **8**

were prepared by nitration of isovanillin at either the 2 or 6 position using concd nitric acid (Scheme 1). The reaction was optimised to produce both isomers in reasonable amounts (1:4 of **7**:**8**) by slow addition of the acid at room temperature. Nitrobenzaldehyde **11** was obtained in three steps (Scheme 1). Esterification of isovanillin to **9** was carried out to moderate the directing group effect of the hydroxy group. Ester **9** was nitrated with concd nitric acid, and the acetate group in **10** was cleaved with 5% sodium hydroxide to yield benzaldehyde **11** (Scheme 1). Azidobenzaldehyde **13** was obtained in two steps; aminobenzyl alcohol was treated with sodium azide to afford alcohol **12**, which yielded **13** upon oxidation with PCC (Scheme 1). Phenolic imines **31**-**39** were protected as *tert*-butyldimethylsiloxy (TBDMS) ethers (**40**-**48**, Scheme 1), which can be removed under mild conditions without degradation of the β-lactam ring. It is also feasible to firstly protect the phenolic benzaldehyde with the TBDMS group and then form the imine.

The β-lactam products **49**-**74** were obtained as racemic mixtures upon Staudinger reaction of the appropriate imine with phenoxyacetyl chloride in the presence of triethylamine (Scheme 2). The *cis* isomer was isolated in all cases; the minor *trans* isomer was isolated for compounds **50**, **55**, **58** and **61** with *cis/trans* ratios determined as 1.5:1, 2:1, 1.5:1 and 2:1 respectively, as indicated by *J* values of ~5 Hz (*cis*) and 1.5-2.2 Hz (*trans*) in the proton NMR spectra. Oxidation of the thioether **64** with *m*-CPBA resulted in the isolation of the sulfoxide **76**, while the sulfone **77** was obtained when excess *m*-CPBA was used (Scheme 2). Introduction of the acrylate ester in compound **75** was achieved by olefinic coupling between bromo-substituted β-lactam **54** and ethyl acrylate with 1,3-(diphenylphosphino)propane/palladium(II) acetate as catalyst. The 4-azidophenylazetidinone **65** was prepared as a potential photoaffinity probe (Scheme 2).

Deprotection of silylated β-lactams **66**-**74** with TBAF afforded phenolic products **78**-**86** (Schemes 3 and 4). Reduction of the nitro group in compounds **61**-**63** and **84**-**86** was achieved by treatment with zinc dust in acetic acid for 7 days to yield the corresponding amines **87**-**92** in high yields (Scheme 4). To investigate the effect of introduction of the 3,4,5-trimethoxyphenyl ring at the C-3 position of the β-lactam, 3,4,5-trimethoxyphenol was reacted with chloroacetic acid in the presence of sodium hydride to yield **93**. Acid **93** was subsequently activated successfully with triphosgene in a one-pot Staundinger reaction with imine **40** to form **94**. The silyl ether protecting group was removed using TBAF to give phenolic β-lactam **95** in 36% yield (Scheme 5).

The effect of the reaction conditions on the stereochemical outcome of the Staudinger reaction was investigated. Many factors can affect the stereochemical outcome of the Staudinger reaction, including the nature of the ketene intermediate, the substitutents on the imine and the reaction conditions.[19] The stereochemistry of 3-phenoxy β-lactams has been reported as differing depending on the reaction conditions.[16, 20-22] β-Lactam **78** was obtained by direct reaction of imine **40** and phenoxyacetic acid in dichloromethane using triphosgene as an acid-activating agent; only the *cis* isomer **78a** was isolated (53% yield). β-Lactam **50** was obtained using microwave irradiation (general method III.B), with a *cis/trans* ratio of 5:1 in dichloromethane at 40 °C (yield 32%) and 10:1 in dichloroethane at 80 °C (yield 34%) compared with a *cis/trans* ratio of 1.5:1 with the conventional thermal method (yield 54%). In refluxing toluene, with a change in the order of addition of reagents, *trans* isomers **50a** and **61a** were obtained as the major isomer (*trans:cis* ratios 7:1 and 5:1 respectively)(general method III.C). These results are in agreement with other studies where reaction conditions had a significant impact on the stereochemical outcome of the Staudinger reaction.[16, 20-22]

To examine the contribution of the azetidinone ring to the antiproliferative activity and stability of these compounds, reduction of **78a** to azetidine **96** was achieved by treatment with diisobutylaluminium hydride. The IR spectrum of **96** indicated that the β-lactam carbonyl absorption at 1739 cm^{-1} was no longer present. To our knowledge, this is the first report of the azetidine ring as a scaffold for CA4 analogues. The novel thione analogue **97** was obtained on reaction of **78a** with Lawesson's reagent; the characteristic C=S absorption was observed at 1592 cm^{-1}.

Introduction of a cytotoxic alkylating functionality to CA-4 has been previously reported[23] and is potentially useful for the development of conjugates to overcome multidrug resistance. The phenolic (**78a**) and amino (**90a**) azetidinones were coupled with the alkylating cytotoxic drug chlorambucil using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI)/DMAP to give **98** and **99** respectively (Scheme 6). The *cis* and *trans* isomers of amino β-lactam (**90a** and **90b**) were coupled with the Fmoc-protected amino acids alanine and glycine to afford amides **100a**, **100b** and **101**, which were deprotected using 1-octanethiol and TBAF to afford amines **102a**, **102b** and **103** (Scheme 7).[24] Dibenzylphosphate esters **104a** and **104b** were obtained from phenolic azetidinones (**78a** and **78b**) by treatment with dibenzylphosphite. Removal of the benzyl group is achieved by hydrogenation (Pd/C) to afford the phosphates **105a** and **105b** respectively (Scheme 8).

We have previously demonstrated stability of 3-phenyl and 3-thienyl β-lactams over the pH range 4-9.[18, 25] Preliminary stability studies of the 3-phenoxy β-lactams **78b** and **90b** were carried out at acidic, neutral and basic conditions (pH 4, 7.4 and 9). The half-life ($t_{½}$) was determined to be greater than 24 h at pH 7.4 for compound **90b**. Half-lives were shorter at pH 4 and 9 (18 h for both). Compound **78b** was less stable than **90b** at all pH values tested ($t_{½}$ =

12, 8 and 11 h respectively at pH 4, 7.4 and 9). Based on this stability study, amino-containing β-lactam **90b** would be the most suitable for further development.

Biological Results and Discussion

Antiproliferative effects

The β-lactam products synthesized were screened for their antiproliferative activity in a cell viability assay with MCF-7 human breast cancer cell line using the MTT assay, with the results expressed as IC_{50} values (the drug concentration required to inhibit cell growth by 50%)(Table 1). The β-lactams were also screened for their cytotoxicity using an LDH assay, with the results expressed as percentage cell death at a concentration of 10 μM (Table 1). Although the MTT assay alone cannot differentiate between growth inhibition and cytotoxic cell death, the LDH assay is specific for cell death, as it measures the concentration of the cytosolic protein lactate dehydrogenase (LDH), released upon cell lysis, in the supernatant. If a compound does not cause significant cell death, this suggests that it acts as a growth inhibitor. The β-lactams examined in this study were designed to contain the 3,4,5-trimethoxyphenyl substituent (Ring A) at the N-1 position of the β-lactam ring as this was found to be optimal.[18] The importance of the trimethoxyphenyl ring was reconfirmed by synthesis and evaluation of **83**, which was over 100-fold less potent than compared to **78a** (IC_{50} = 49 μM, 0.38 μM respectively) (Table 1). The majority of compounds prepared contain a phenoxy ring at position C-3 of the β-lactam.

A number of methoxy-containing substituents located on the C-4 aryl ring were evaluated. Amongst the *cis* isomers, antiproliferative potencies decreased in the order of **52** > **49** > **50a** > **51** (IC_{50} = 26, 29, 35, 171 μM respectively). For **50**, with a 4-methoxyphenyl substituent at C-4, chromatographic separation of the *cis* and *trans* isomers facilitated the identification of the *trans* **50b** product as having a superior IC_{50} value (IC_{50} = 6.9 μM) compared with the *cis*

product **50a** (IC_{50} = 35 μM). Amongst the *cis* compounds with different substituents at the 4-position of the C-4 aryl ring, SCH_3-containing compound **64** displayed the most impressive potency (**64** > **76** ≈ **65** > **57** ≈ **58a** > **54** ≈ **79** > **77** ≈ **63** > **75** > **92**; IC_{50} values 7.7, 20, 23, 31, 31.6, 48, 48, 62, 63, 87, 160 μM respectively). A thiomethyl analogue of **2** with a 3-fold reduction in anti-tubulin activity and a 2-fold loss in potency compared to the corresponding 4-methoxy analogue is known.[26] Potent CA-4 analogues with a naphthalene ring have previously been reported,[27] but in this series compounds **59** and **60** displayed poor activity with IC_{50} values in excess of 50 μM. The inclusion of the 3,4-methylenedioxyphenyl substitution pattern, present in steganacin and podophyllotoxin, also led to reduced potency for compound **53** (IC_{50} = 48 μM).

β-Lactam analogues containing 3,4-substitution patterns on the C-4 aryl ring were next examined. Amongst the *cis*-orientated analogues, the potency decreases in the order **78a** > **81** ≈ **55a** > **56** > **90a** > **80** > **61a** (IC_{50} values of 0.38, 4.5, 5.1, 6.3, 12.2, 32, 69 μM respectively, Table 1). Compound **78a** has a B-ring substitution pattern identical to **2** and is the most potent of this mini-series, with a submicromolar IC_{50} value (0.38 μM). Both halogen-containing compounds **55a** and **56** displayed low micromolar activity (IC_{50}=5.1, 6.3 μM). The fluorine group in **56** has the potential advantage of blocking Phase I metabolism mediated by CYP450. The *cis* bromo isomer **55a** was 6-fold more potent than *trans* isomer **55b** (IC_{50} = 5.1, 31 μM respectively). Reduction of the nitro (**61**) to the amino group (**90**) leads to a large increase in potency for both the *cis* and *trans* isomers (2.5-fold and 26-fold respectively). Compound **90b** was also highly potent in MDA-MB-321 breast cancer cells (IC_{50} = 0.015 μM).

Further investigations into the role of the aromatic hydroxyl and amino groups at C-4 were carried out to explore the requirements for activity. Firstly, compounds **82** and **91** were

synthesized, lacking the 4-methoxy group. Compound **82** displayed a considerable loss in potency compared to **78a** (IC_{50} = 54 and 0.38 μM), while **90a** was slightly more potent than **91** (IC_{50} = 12.2 and 17 μM) (Table 1). In combination with the results above, this indicates that having both the hydroxyl and the methoxy groups in their respective positions on ring "B" is neccessay for activity of these β-lactam compounds. Three compounds with methoxy (**50a**), hydroxyl (**79**; 3:1 *cis*:*trans*) and amino (**92**) substitutents at the 4-position of the C-4 aryl ring were evaluated, and their relative antiproliferative potencies confirm the 4-methoxy group as optimal (IC_{50} = 35, 48, 160 μM respectively).

An interesting series of CA-4 analogues, retaining the 3-hydroxy, 4-methoxy substitution pattern but additionally including an amino group substituted at the remaining free positions of the C-4 aryl ring have been reported.[28] A series of similarly substituted *cis* β-lactams were synthesized and evaluated (**87**, **88**, **89** and their nitro precursors **84**, **85** and **86**). As noted above for *cis* and *trans* isomers of compounds **61** and **90**, reduction of the nitro to the amino yields compounds with increased potency. Compounds **87**, **88** and **89** were only obtained as the *cis* isomer, and in comparison to **90a** they all display increased potency (IC_{50} = 18.5, 20, 8.8 and 27 μM respectively). It is possible that the *trans* isomers of these compounds may be even more potent.

A number of clinical trials of CA-4P in combination with an established chemotherapeutics are in progress.[4] Compounds **78a** and **90a** were successfully coupled to chlorambucil via ester and amide linkages respectively to yield **98** and **99** respectively. Ester compound **98** had an equipotent antiproliferative effect when compared to **78a**, and much greater than the reported IC_{50} value for chlorambucil in MCF-7 cells (97 μM).[29] There was a marked increase in cell death from 2.8% to 8% compared to **78a**, most likely due to the toxic effects of chlorambucil. In contrast, amide **99** showed a huge loss in antiproliferative potency (IC_{50} = 145μM). One explanation for these observations was that the ester linkage of **98** was cleaved

in situ by cellular esterases, whilst amide **99** would not be subject to this effect, would remain intact and hence would be unable to inhibit the polymerization of tubulin.

Phosphate esters **105a** and **105b** were prepared to improve water solubility, and demonstrated reduced antiproliferative effects (IC_{50} = 42 and 23 μM respectively) when compared with the corresponding phenols **78a** and **78b** (Table 1). It is likely that rapid *in vivo* dephosphorylation would occur for **105a** and **105b**, analogous to that reported for CA-4P.[30] Interestingly, amino acid prodrugs **102a** and **103** displayed improved potency compared to the parent amine **90a**, while *trans* amide **102b** demonstrates submicromolar antiproliferative activity (IC_{50} = 0.23 μM). These amides are expected to be hydrolysed to the active amine *in vivo* due to the widespread presence of aminopeptidases in blood, in a similar manner to that reported for the CA-4 type compound **4**.[31]

Previous research has demonstrated that an intact β-lactam ring is required for potent antiproliferative activity.[18] A major question arising from these findings concerns the role of the β-lactam ring itself, particularly the role of the carbonyl group at C-2. A major degradation pathway for the β-lactam ring in penicillins and monobactams is hydrolysis. If the C-2 of the ring proved amenable to modification without affecting biochemical activity it may provide access to a series of compounds that would show increased stability *in vivo*. Transformation of the carbonyl to a thione (**97**) and reduction to an azetidine (**96**) were investigated. However large decreases in potency were observed for both compounds **97** and **96** compared to **78a** (Table 1; IC_{50} values 7.5, 3.3 and 0.38 μM respectively). Compound **96** also induced a large increase in cell death to nearly 20% (compared to 2.8% for **78a**). This indicates that the carbonyl group at C-2 is crucial to antiproliferative effects of these β-lactams.

Further Antiproliferative Screening and *In Vivo* Studies

Following a review of the initial antiproliferative data obtained, compounds **78a** and **90b** were selected as representative examples for evalution in the National Cancer Institute's Division of Cancer Treatment and Diagnosis (DCTD) Development Therapeutics Program (DTP).[32] Analysis of their drug-like (Lipinski) properties from a Tier-1 profiling screen, together with predictions of permeability, metabolic stability, blood brain barrier partition, plasma protein binding and human intestinal absorption properties, indicated that they are moderately lipophilic-hydrophilic drugs and are suitable candidates for further investigation (Tables S1 and S2, Supporting Information).

In the initial NCI one-dose (10 μM) cell line screen, compounds **78a** and **90b** displayed promising activity, particularly against leukemia and colon cancer cell lines. Subsequent five-dose screening to evaluate GI_{50} (50% growth inhibition) and LC_{50} (50% lethal concentration) was carried out in NCI panel of 56 cell lines using the sulphorhodamine B (SRB) protein assay (Table S3, Supporting information). The GI_{50} measures the growth inhibitory power of the test agent, while the LC_{50} value signifies a cytotoxic effect. For phenolic compound **78a**, the GI_{50} values were in the submicromolar range for 29 of the panel cell lines investigated, including all of the leukemia and prostate cancer cell lines, four renal cancer lines and five melanoma lines. The mean GI_{50} value across all cell lines tested was 1.45 μM. Particularly of interest was the activity of the compound against MCF-7 (GI_{50} = 0.36 μM) which is in close agreement with the IC_{50} value of 0.38 μM determined in-house. The significant activity of amino compound **90b** displayed in the one-dose screen translated into excellent broad-spectrum antiproliferative activity in the five-dose assay with GI_{50} values below 10 nM in all but four of the panel cell lines tested. It was active against all of the leukemia, prostate and renal cancer cell lines tested indicating the potential of this compound for a wide range of therapeutic applications. The activity of the compound against MCF-7 (GI_{50} <10 nM) is in agreement with the IC_{50} value of 13 nM determined in-house. The mean GI_{50} value for

compound **90b** across all cell lines in the panel is 22.4 nM (Table S3, Supporting information).

Cytotoxicity (determined as the LC_{50} value) was determined to be greater than 100 μM in all but 5 cell lines (compound **78a**), and in all but 4 cell lines (compound **90b**), indicating minimal toxicity (Table S3, Supporting information). This supports the results of our in-house cytotoxicity results, where **78a** caused only 2.8% cell death (at 10 μM) and **90b** resulted in 10.2% cell death (Table 1). Based on the excellent *in vitro* results for compound **90b**, it was progressed to *in vivo* studies performed by the NCI with 12 cancer types using the hollow fibre assay.[33] The maximum tolerated dose (MTD) for **90b** in athymic nude mice was determined to be 100 mg/kg/dose (IP), and from this the high dose for the hollow fibre assay was calculated to be 37.5 mg/kg/dose [=(MTD×1.5)/4]. A positive result (50% or greater reduction in net percent cell growth) was found for one cancer type when injected subcutanteously (UACC-62 melanoma).

The NCI matrix COMPARE analysis,[34] which measures the correlation between two compounds with respect to their differential antiproliferative activity, demonstrated good correlation between **78a**, **90b** and CA-4 (r = 0.53 and 0.63 respectively; r = correlation coefficient). The COMPARE algorithm was also used to compare the differential antiproliferative activities of **90b** to compounds with known mechanisms of action in the NCI Standard Agent Database and it showed the highest correlations to tubulin-targeting agents including maytansine [an analogue of which, in conjugation with trastuzumab (T-DM1), has been recently approved by the FDA for late-stage, HER-2 positive breast[35]], rhizoxin and the clinically-used vinca alkaloids vincristine sulfate and vinblastine sulfate (Table S4, Supporting information).

Effects of β-Lactams on Tubulin Polymerisation, Microtubule Struture in MCF-7 Cells and Bisthioalkylation of β-Tubulin by EBI

Due to the results of the COMPARE analysis, and the structural similarity of these compounds to previously reported tubulin-targeting agents, the effects of selected β-lactams on both tubulin polymerization and the microtubular structure of MCF-7 cells were investigated. A number of the CA-4 β-lactam analogues (compounds **57**, **58b**, **78a**, **78b** and **90b**) were evaluated for their ability to inhibit the assembly of isolated, purified bovine tubulin. The effect of the test compounds on tubulin polymerization was determined by recording the absorbance at 340 nm over time, as light is scattered by microtubules to an extent that is proportional to the concentration of microtubule polymer. V_{max} values (maximal rate of polymerization) were calculated for each compound as the V_{max} value gives a sensitive indication of tubulin/ligand interactions. The fold changes in the V_{max} values for the inhibition of tubulin polymerization with reference to the vehicle control were calculated for each compound (Table 2). Compounds **78a**, **78b** and **90b** were shown to inhibit the polymerization of tubulin at 10 μM; **78b** was the most potent with a reduction in the V_{max} value of 3.2 fold when compared with **2** which demonstrated a 6-fold reduction in the rate of tubulin polymerization. A 1.6-fold reduction in the V_{max} for tubulin polymerization was observed for compound **90b,** while a 1.1-fold reduction was observed for the less potent *cis* isomer **78a**. Compounds **57** and **58b** did not cause any change in the V_{max} value compared to vehicle control, indicating that they do not inhibit the polymerization of tubulin and explaining their poor antiproliferative activity in MCF-7 cells.

Subsequently, we investigated alterations induced by β-lactam **90b** on the microtubule network of MCF-7 cells by confocal microscopy. Confocal analysis of MCF-7 cells stained with α-tubulin mAb demonstrated a well organised microtubular network in cells treated with

vehicle control (Figure 2) and in untreated cells (data not shown). Exposure to the microtubule-stabilising agent paclitaxel induced the formation of microtubule bundles and pseudo asters (Figure 2).[36] Cells exposed to **2** or β-lactam **90b** for 16 h were characterised by complete loss of microtubule formation consistent with depolymerised microtubules (Figure 2). Additionally, cells treated with **2** or β-lactam **90b** contained multiple micronuclei, a phenomenon described as mitotic catastrophe.[37] The findings are in agreement with previous reports that **2** induced mitotic catastrophe in non-small cell lung cancer cells,[38-39] human endothelial cells (HUVEC),[40] human lung carcinoma cells (H460)[40] and human breast cancer cells (MCF-7).[41] Taken in conjuction with the effects on polymerization of isolated tubulin, the confocal imaging results confirm that β-lactam **90b** is targeting tubulin.

Due to the structural similarities between our compounds, **2**, and many other analogues of CA-4, we carried out a whole-cell based assay to characterize the binding-site of these compounds on tubulin. *N,N'*-ethylene-bis(iodoacetamide)(EBI) is an alkylating agent that crosslinks cysteine residues at positions 239 and 354 in the colchicine-binding site of tubulin, forming a β-tubulin-EBI adduct. This adduct is detectable by Western blotting as an immunoreactive band that migrates faster than β-tubulin. Cells that are pretreated with a colchicine-site inhibitor, such as **1** and **2**, prevent the formation of the β-tubulin-EBI adduct.[42-43] MCF-7 cells were treated with a selected β-lactam **90b** (10 μM) for 2 h followed by EBI for an additional 1.5 h (Figure 3). Control samples show the presence of the β-tubulin-EBI adduct at a lower position, indicating that EBI has crosslinked Cys239 and Cys354 on β-tubulin. Adduct formation is inhibited in cells treated with β-lactam **90b**, indicating that **90b** is binding at the colchicine-site of tubulin.

Cell Cycle Analysis by Flow Cytometry

Further biochemical investigations were carried out to investigate the effects of compounds **78b** and **90b** on the cell cycle distribution of MCF-7 breast cancer cells after 24, 48 and 72 hr of treatment. Cell cycle analysis was carried out by staining cells with propidium iodide and analyzing their DNA content by flow cytometry. A statistically significant increase in the percentage of cells in the G_2/M phase after both 24 and 48 hr was noted for compound **90b** (57% and 73% respectively) compared to the vehicle control (25% and 26%)(Figure 4), indicating mitotic blockade. After 72 hr, there was a significant increase in the percentage of apoptoic cells, as indicated by the sub-G_0G_1 peak, for both compounds **78b** and **90b** compared to the vehicle control (34%, 35% and 4% respectively)(Figure 4). The cell cycle analysis results for **90b** are typical of tubulin-targeting compounds, which characteristically cause G_2/M arrest followed by apoptosis.[44]

X-Ray Structural Study

An X-ray crystallography study of eight of the β-lactam products was undertaken to confirm the *cis*/*trans* stereochemical assignments and explore possible important structural features for potent activity. ORTEP diagrams for *trans* compounds **78b** and **90b** are presented in Figure 5, and diagrams for *cis* compounds **49**, **60**, **77** and **55a** and *trans* compounds **50b** and **58b** are shown in Figure S1 (Supporting Information).

Generally, a *trans* arrangement between C-3 and C-4 of the β-lactam ring was found to confer superior antiproliferative activity compared to the corresponding *cis* compound (Table 1). The X-ray structures determined demonstrated a conformation for the azetidinones where the rings A and B (i.e. aromatic rings located at N-1 and C-4 of the β-lactam ring) are not coplanar. The β-lactam ring forms a rigid scaffold which accommodates the planar hydrophobic aryl rings A and B, required for interaction with the colchicine binding site of tubulin. The torsional angles (Ring A/B) observed for *cis* compounds **49**, **55a**, **60** and **77** were

calculated to be 70.9°, 56.3°, -75.0° (-62.8°) and 72.0° respectively, while the corresponding values for the *trans* compounds **50b**, **58b**, **78b** and **90b** were 56.3°, 56.2° (-54.2°), 60.2°, and -62.1° respectively (Table S5, Supporting Information; numbers in parentheses refer to the second crystallographically independent molecule in the asymmetric unit). The Ring A/B torsional angle in most cases was found to be greater for the *cis* compounds than for the *trans* compounds. The exception is compound **55a**, which interestingly was more potent in antiproliferative assays than the corresponding *trans* isomer **55b**. The torsional angle for the most potent compounds **90a** and **78a** at -62.1°, 60.2° compares with the torsional angle (Ring A/B) determined for **1** and **2** of 55° and 53° respectively. As expected, the Ring B/C torsional angles were low for the *cis* compounds **49**, **55a**, **60** and **77** (10.8°, -1.6°, 0.4° (-7.4°) and -6.1°) while the Ring B/C torsional angles for the *trans* compounds **50b**, **58b**, **78b** and **90b** and were determined to be -122.91°, 120.5° (124.7°), -122.9° and 128.5° (Table S5, Supporting Information; numbers in parentheses refer to the second crystallographically independent molecule in the asymmetric unit). This result indicates that a *trans* arrangement of rings B and C generally facilitates a more favourable interaction of rings A and B with the residues of the β–tubulin binding site. It also highlights the potential importance of the ring A/B torsional angle, where compounds with values greater than 70° possess poor antiproliferative activity. It should be noted that the unbound X-ray crystallographic structure may not be the bioactive conformation *in vivo*.

Molecular modelling in the colchicine-binding site of tubulin

Tubulin is a heterodimer of α and β subunits, with the colchicine site buried mostly in the β-subunit but with some interactions with the α-subunit. Binding of DAMA-colchicine leads to the α-subunit of tubulin being unable to adopt its favoured straight conformation with β-tubulin. It does this by steric interference with residues 101 and 181 in α-tubulin., forcing the

T7 loop and H8 helix to clash with the α-tubulin, and forcing the Lys β352 side chain to interfere with the α-tubulin. Two important residues (Val β318 and Cys β241) in the site have been identified from biochemical data. Modification of the Val β318 residue has been shown to reduce the sensitivity of tubulin to colchicine.[45] Colchicine derivatives with groups substituted at the methoxy positions can crosslink with Cys β241.[46]

The 3-bromo-4-methoxy compounds (**55a** and **55b**) displayed reasonable activity in the low micromolar range, with *cis* isomer **55a** displaying better antiproliferative activity than *trans* isomer **55b** (IC_{50} = 5.1 μM and 31 μM respectively). By examining the ligand interactions for the docked compounds it becomes apparent that **55a** maintains the important interactions with Cys β241 (polar), Val β318 (polar), Lys β352 (basic), and Val α181 (hydrophobic). The *trans* compound **55b** does not maintain the interaction with Val β318 (Figure S2, Supporting Information). This may account for the fall in antiproliferative activity observed for **55b**.

The most potent compounds, phenolic **78b** and amino **90b**, displayed submicromolar activity. The *trans* isomer was favoured in both cases, in contrast to the observation for **55a** and **55b**, although this may be explained by their ring A/B torsional angles as described above. For compound **78**, IC_{50} values of 0.38 μM and 0.038 μM were obtained for the *cis* and *trans* isomers respectively. Both isomers interact as expected with the residues reported by Ravelli,[6] but there are small differences in the poses adopted by both isomers in the colchicine site (Figure 6). In the trimethoxy region of the two isomers, **78b** more closely resembles the trimethoxy area of colchicine (**78b** is offset from DAMA-colchicine by 1.25 Å compared to 2.13 Å for **78a**). In a similar manner, the methoxy group of the C-4 ring of **78b** is placed 1.11 Å from that of DAMA-colchicine compared to 1.49 Å for **78a**. It has been noted that, for many CA-4 analogues, the *p*-methoxy group of the B ring is essential for activity and the docked solution presented here indicates that the *p*-methoxy group plays a role in sterically interacting with the Lys β352. For the amino compounds (**90a** and **90b**), *cis*

90a is considerably less potent than phenolic **78a** whilst the *trans* isomer, **90b**, is more active than **78b**. The docked solution for the **90b** shows that it maintains the same important contacts as **78b**, but adopts a slightly different pose in the colchicine site. The trimethoxy region is offset by an average of 1.4 Å compared to 1.25 Å for the hydroxy compound (less than the offset of 2.13 Å for **78a**). Finally, molecular docking for azetidine **96** (IC_{50}=7.45μM) and thione **97** (IC_{50}=3.3μM), both of which lack the β-lactam carbonyl group, shows that both compounds are no longer interacting with Val β318. From the previous examples the β-lactam carbonyl appears to interact with three residues – Ala β250 (hydrophobic), Leu β255 (hydrophobic), and Leu β248 (hydrophobic) which are members of the T7 loop H8 helix. It may be important for a ligand to interact in this way, since colchicine also interacts with these residues causing the T7 loop and H8 helix to move and interfere with α-tubulin. Removal of the carbonyl group may adversely affect biochemical activity by reducing these interactions.

Conclusion

We have presented a series of novel 3-phenoxy substituted β-lactams and evaluated their antiproliferative activity and cytotoxicity in MCF-7 breast cancer cells. The most potent compounds discovered via the antiproliferative assays were phenolic **78b** and amino-substiuted **90b**, and were also found to be minimally cytotoxic. Depolymerization of isolated tubulin was demonstrated and confocal microscopy indicated disruption of the tubulin network in MCF-7 cells; taken together, these results confirm that the β-lactams are targeting tubulin. Further experiements demonstrated that β-lactam **90b** interacts with the colchicine-binding site on β-tubulin. Cell-cycle analysis indicated that **90b** caused G_2/M arrest and both **78b** and **90b** caused apoptosis, typical of microtubule-destabilizing agents. X-Ray crystallography studies of selected compounds reveal that the torsional angle between the aryl

rings at N-1 and C-4 of the β-lactam is crucial for potent antiproliferative activity. Phosphate and amino acid prodrugs of both **78b** and **90b** were synthesised, of which compound **102b** retained potency and is a promising candidate for further clinical development.

Experimental Section

All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous solvents were obtained by distillation from the indicated systems immediately prior to use: THF from sodium/benzophenone, dichloromethane from calcium hydride and toluene from sodium. IR spectra were recorded as thin films on NaCl plates or as KBr discs on a Perkin-Elmer Paragon 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX 400 instrument at 20^{o}C, 400.13 MHz for ^{1}H spectra, 100.61 MHz for ^{13}C spectra, in either $CDCl_3$, CD_3COCD_3 or CD_3OD (internal standard: TMS). Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD GC–MS system in EI mode, while HRMS for all final compounds were obtained on a Micromass Time of Flight mass spectrometer (TOF) equipped with electrospray ionization (ES) interface operated in positive ion mode at the High Resolution Mass Spectrometry Laboratory by Dr. Martin Feeney in the School of Chemistry, Trinity College Dublin. TLC was performed using Merck Silica gel 60 TLC aluminium sheets with fluorescent indicator visualizing with UV light at 254nm. Flash chromatography was carried out using standard silica gel 60 (230-400 mesh) obtained from Merck. All products isolated were homogenous on TLC. The purity of the tested compounds was determined by HPLC or combustion analysis and, unless otherwise stated, the purity level was $\geq$95%. Elemental analyses were performed on an Exetor Analytical CE4400 CHN analyser in the microanalysis laboratory, Department of Chemistry, University College Dublin. Analytical high-performance liquid chromatography (HPLC) was performed using a Waters 2487 Dual Wavelength Absorbance detector, a Waters 1525 binary HPLC pump and a Waters 717plus Autosampler. The column used was a Varian Pursuit XRs C18 reverse phase 150 x 4.6mm chromatography column. Samples were detected using a wavelength of 254 nm. All samples were analyzed using acetonitrile (70%): water (30%) over 10 min and a flow rate of 1 mL/min.

3-Hydroxy-4-methoxy-2-nitrobenzaldehyde (8). HNO_3 (conc, 12 mmol) was added to a stirred solution of isovanillin (10 mmol) in acetone (40 mL) at rt. The reaction mixture was stirred for 30 min, poured onto ice water (200 mL) and resulting precipitate filtered via a sinter glass funnel. Bright yellow solid; yield 42% yield; mp 186 °C.[47] IR (KBr): $\tilde{\nu}$ = 1671 cm^{-1} (s; ν(C=O)), 3326 cm^{-1} (s; ν(OH)). ^{1}H NMR ($CDCl_3$): δ 4.09 (s, 3H), 7.15 (d, J=8.5 Hz, 1H), 7.51 (d, J=8.5 Hz 1H), 10.10 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 59.0, 119.1, 123.6, 129.8, 139.8, 141.3, 153.3, 187.2. HRMS (ESI): *m/z* calcd for $C_8H_7NO_5 + Na^+$ $[M + Na]^+$: 220.0216; found: 220.0209. The filtrate was reduced *in vacuo* and a further precipitate formed was found to contain 4:1 mixture of the 3-hydroxy-4-methoxy-2-nitrobenzaldehyde (**8**) and 3-hydroxy-4-methoxy-6-nitrobenzaldehyde (**7**); **5-Hydroxy-4-methoxy-2-nitrobenzaldehyde (7)** was isolated as a brown solid; yield 31%, mp 142 °C. IR (KBr): $\tilde{\nu}$ = 1694 cm^{-1} (s; ν(C=O)), 3332 cm^{-1} (s; ν(OH)). ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 3H), 6.25 (s, 1H), 7.49 (s, 1H), 7.68,(s, 1H), 10.44 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 58.3, 109.5, 119.1, 123.5, 144.3, 150.0, 158.6, 188.7. HRMS (ESI): *m/z* calcd for $C_8H_7NO_5 + Na^+$ $[M + Na]^+$: 220.0216; found: 220.0231.

Acetic acid 5-formyl-2-methoxyphenyl ester (9). Acetic anhydride (40 mmol), dissolved in diethyl ether (40 mL), was added dropwise to a solution of isovanillin (40 mmol), and KOH (40 mmol) in water (200 mL) at 0 °C. The reaction was stirred at 0 °C for a further 30 min before the resulting precipitate was filtered. White powder; yield 93%, mp 73 °C.[48] IR (KBr): $\tilde{\nu}$ = 1684 cm^{-1} (s; ν(C=O)), 1769 cm^{-1} (s; ν(C=O ester). ^{1}H NMR ($CDCl_3$): δ 2.33 (3H, s), 3.91 (3H, s), 4.82 (1H, s), 7.06 (1H, d, J=8.5 Hz), 7.58-7.76 (2H, m), 9.82, (s, 1H). ^{13}C NMR ($CDCl_3$): δ 20.1, 55.8, 111.6, 122.9, 129.8, 139.7, 155.9, 168.3, 189.7.

Acetic acid 5-formyl-2-methoxy-3-nitrophenyl ester (10). To a stirring solution of HNO_3 (conc., 360 mmol) at -18 °C, **9** (36 mmol) was added in portions over 10 min, and the mixture was stirred at -18 °C for 2 min, then at -5 °C for a further 20 min, before being poured onto

150 cm^3 of crushed ice. The resulting slurry was retained at 5 °C overnight, then filtered. The filter cake was washed with water (10 mL×5) and dried to afford the product as a pale yellow solid; yield 82%; mp 65 °C. IR (KBr): $\tilde{\nu}$ = 1698 cm^{-1} (s; *v*(C=O)), 1778 cm^{-1} (s; *v*(C=O ester). ^{1}H NMR ($CDCl_3$): δ 2.41 (3H, s), 4.02 (3H, s), 7.85 (1H, d, *J*=2.0 Hz), 8.20 (1H, d, *J*=2.0Hz), 9.93 (1H, s). ^{13}C NMR ($CDCl_3$): δ 20.2, 62.5, 123.6, 127.4, 130.8, 145.1, 150.3, 167.7, 187.8.

3-Hydroxy-4-methoxy-5-nitrobenzaldehyde (11). Compound **10** (23.5 mmol) was dissolved into warm NaOH (5% aq. soln.). The resulting dark red solution was cooled to rt, acidified to pH 1 with conc. HCl, which resulted in loss of colour and the formation of a precipitate. This mixture was stirred at rt, then cooled to 0 °C. The precipitate was isolated by filtration to afford the product as an off-white solid; yield 8%; mp 52-56 °C. IR (KBr) υ max: 1690 (C=O), 3415 (OH) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.08 (3H, s), 6.59 (1H, s (br), 7.74 (1H, d, *J*=2.0 Hz), 7.98 (1H, d, *J*=1.5 Hz), 9.94 (1H, s). ^{13}C NMR ($CDCl_3$): δ 62.3, 118.5, 118.8, 131.3, 142.3, 145.5, 150.9, 188.9. HRMS (ESI): *m/z* calcd for $C_8H_7NO_5$ + Na^+ $[M + Na]^+$: 220.0216; found: 220.0217.

4-(Azidophenyl)methanol (12). $NaNO_2$ (7.5 mmol) in water (4 mL) was added dropwise to a stirring suspension of 4-(aminobenzyl)alcohol (5 mmol) in 2 M H_2SO_4 (6 mL) at 0 °C; after the addition the reaction became clear and colourless. The solution was stirred for 15 min before NaN_3 (7.5 mmol) in water (4 mL) was added carefully dropwise over 5 min with evolution of gas. The reaction was stirred at rt for a further hour, extracted with diethyl ether (10 mL×4); the organic layer was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. Clear oil; yield 90%.[49] IR (NaCl): $\tilde{\nu}$ = 3321 cm^{-1} (s; *v*(OH)), 2117 cm^{-1} (s; *v*(N_3)). ^{1}H NMR ($CDCl_3$): δ 2.32 (1H, s, broad), 4.64 (2H, s), 7.00 (2H, d, *J*=8.4 Hz), 7.32 (2H, d, *J*=8.6 Hz). ^{13}C NMR ($CDCl_3$): δ 64.1, 118.6, 128.1, 137.1, 138.9.

4-Azidobenzaldehyde (13). To a stirred solution of **12** (4.47 mmol) in dry CH_2Cl_2 (20 mL) under nitrogen was added pyridinium chlorochromate (7.6 mmol), and the resulting solution was stirred at rt for 1 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered through a 1 cm silica column and the solvent removed *in vacuo* to yield the product as an orange oil; yield 78%.[49] IR (NaCl): $\tilde{\nu}$ = 1694 cm^{-1} (s; ν(C=O)), 2110 cm^{-1} (s; $\nu(N_3)$). ^{1}H NMR ($CDCl_3$): δ 7.10 (2H, d, *J*=8.3 Hz), 7.83 (2H, d, *J*=8.3 Hz), 9.89 (1H, s). ^{13}C NMR ($CDCl_3$): δ 119.0, 131.1, 132.7, 190.2.

General method I: imine preparation

The appropriately substituted benzaldehyde (10 mmol) and substituted aniline (10 mmol) were refluxed together in ethanol (50 mL) for 5 h with a catalytic amount of concentrated H_2SO_4. The reaction mixture was reduced *in vacuo* and the resulting solid product was recrystallised from ethanol. Imines **14**, **17**, **31** and **40**,[17] **20** and **22**,[41] **15**,[18] **24**, **25** and **26**[25] were prepared and characterised as described previously by us.

(3-Methoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (16) was prepared according to general method I from 3-methoxybenzaldehyde and 3,4,5-trimethoxyaniline. Colourless solid; yield 88%, mp 59 °C. IR (KBr): $\tilde{\nu}$ = 1587 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H), 3.90 (s, 6H), 3.92 (s, 3H), 6.51 (s, 2H), 7.32-7.52 (m, 4H), 8.46 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 54.9, 55.7, 60.6, 97.7, 111.3, 117.9, 121.8, 129.3, 135.9, 137.0, 147.4, 153.1, 159.2, 159.6. HRMS (ESI): *m/z* calcd for $C_{17}H_{19}NO_4 + H^+$ $[M + H]^+$: 302.1387; found: 302.1403.

Benzo[1,3]dioxol-5-methylene-(3,4,5-trimethoxyphenyl)amine (18) was prepared according to general method I from 3,4,5-trimethoxyaniline and 3,4-methylenedioxybenzaldehyde. Pale yellow solid; yield 68%; mp 101 °C.[15] IR (KBr): $\tilde{\nu}$ = 1629 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 3.89 (s, 6H), 5.93 (s, 2H), 6.05-7.42 (m,

5H), 8.35 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.4, 55.6, 60.5, 97.6, 101.2, 106.3, 107.8, 125.3, 130.5, 135.7, 147.5, 148.0, 150.1, 153.4, 158.4. HRMS (ESI): *m/z* calcd for $C_{17}H_{17}NO_5 + H^+$ $[M + H]^+$: 316.1179; found: 316.1186.

(4-Bromobenzylidene)-3,4,5-trimethoxyphenylamine (19) was prepared according to general method I from 4-bromobenzaldehyde and 3,4,5-trimethoxyaniline. Pale yellow solid; yield 70%; mp 110-112 ˚C. IR (KBr): $\tilde{\nu}$ = 1624 cm^{-1} (s; *v*(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 3.92 (s, 6H), 6.51 (s, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H), 8.45 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.6, 97.7, 125.5, 129.7, 130.5, 131.6, 132.0, 147.9, 153.2, 157.8. HRMS (ESI): *m/z* calcd for $C_{16}H_{16}BrNO_3 + H^+$ $[M + H]^+$: 350.0386; found: 350.0382.

(3-Fluoro-4-methoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (21) was prepared according to general method I from 3,4,5-trimethoxyaniline and 3-fluoro-4-methoxybenzaldehyde White solid; yield 84%; mp 100 ºC. IR (KBr): $\tilde{\nu}$ = 1609 cm^{-1} (s; *v*(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.82, (s 3H), 3.91, (s 6H), 3.97 (s 3H), 6.48 (s, 2H), 7.62-7.75, (m, 3H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.4, 55.6, 60.5, 97.7, 112.3, 114.7, 125.8, 129.2, 135.9, 147.2, 149.4, 150.8, 153.3, 157.1. HRMS (ESI): *m/z* calcd for $C_{17}H_{18}FNO_4 + H^+$ $[M + H]^+$: 320.1293; found: 320.1284.

4-[(3,4,5-Trimethoxyphenylimino)methyl]benzonitrile (23) was prepared according to general method I from 3,4,5-trimethoxyaniline and 4-formylbenzonitrile. Pale yellow solid; yield 92%; mp 134 ºC.[50] IR (KBr): $\tilde{\nu}$ = 1580 cm^{-1} (s; *v*(C=N)), 2223 cm^{-1} (s; *v* (C≡N)). ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H), 3.92 (s, 6H), 6.55-8.02 (m, 6H), 8.53 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.2, 61.1, 98.4, 114.3, 118.5, 129.1, 132.6, 137.2, 139.9, 146.8, 153.7, 157.1. HRMS (ESI): *m/z* calcd for $C_{17}H_{16}N_2O_3 + H^+$ $[M + H]^+$: 297.1234; found: 297.1250.

(3-Nitrobenzylidene)-(3,4,5-trimethoxyphenyl)amine (27) was prepared according to general method I from 3-nitrobenzaldehyde and 3,4,5-trimethoxyaniline. Yellow solid; yield

93%; mp 160 ºC. IR (KBr): $\tilde{\nu}$ = 1588 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 3.93 (s, 6H), 6.56 (s, 2H), 6.66-6.72 (m, 4H), 8.76 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.6, 97.9, 122.9, 125.1, 129.4, 133.6, 136.7, 137.3, 146.1, 148.2, 153.2, 155.9. HRMS (ESI): *m/z* calcd for $C_{16}H_{16}N_2O_5 + H^+$ $[M + H]^+$: 317.1132; found: 317.1143.

(4-Nitrobenzylidene)-(3,4,5-trimethoxyphenyl)amine (28) was prepared according to general method I from 4-nitrobenzaldehyde and 3,4,5-trimethoxyaniline. Yellow solid, yield 82%; mp 158 ºC. IR (KBr): $\tilde{\nu}$ = 1587 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H), 3.94 (s, 6H), 6.57 (s, 2H), 8.09 (d, *J*=8.0 Hz, 2H), 8.34 (d, *J*=8.0 Hz, 2H), 8.59 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.2, 61.1, 98.5, 124.1, 129.3, 137.4, 141.5, 146.7, 149.2, 153.7, 156.5. HRMS (ESI): *m/z* calcd for $C_{16}H_{16}N_2O_5 + H^+$ $[M + H]^+$: 317.1132; found: 317.1124.

(4-Methylsulfanylbenzylidene)-(3,4,5-trimethoxyphenyl)amine (29) was prepared according to general method I from 3,4,5-trimethoxyaniline and 4-methylthiobenzaldehyde Yellow solid; yield 88%; mp 96 ºC. IR (KBr): $\tilde{\nu}$ = 1628 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H), 3.87 (s, 3H), 3.89 (s, 6H), 6.48-7.80 (m, 6H), 8.41 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5, 55.7, 60.6, 97.7, 125.2, 132.2, 135.8, 142.9, 147.5, 153.1, 158.5. HRMS (ESI): *m/z* calcd for $C_{17}H_{19}NO_3S + H^+$ $[M + H]^+$: 318.1158; found: 318.1162.

(4-Azidobenzylidene)-(3,4,5-trimethoxyphenyl)amine (30) was prepared from 4-azidobenzaldehyde (**13**) and 3,4,5-trimethoxyaniline according to general method I. Yellow solid; yield 18%; mp 74 ºC. IR (KBr): $\tilde{\nu}$ = 1584 cm^{-1} (s; ν(C=N)), 2120 cm^{-1} (s; $\nu(N_3)$). ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3), 3.92 (s, 6H), 6.51 (s, 2H), 7.14 (d, *J*=8.3 Hz, 2H), 7.9 (d, *J*=8.6, 2H), 8.45 (s, 1H). ^{13}C NMR ($CDCl_3$): 55.9, 57.2, 98.1, 122.6, 127.4, 128.4, 129.9, 133.3, 135.4, 151.5, 161.1. HRMS (ESI): *m/z* calcd for $C_{16}H_{16}N_4O_3 + H^+$ $[M + H]^+$: 313.1295; found: 313.1225.

4-[(3,4,5-Trimethoxyphenylimino)methyl]phenol (32) was prepared according to general method I from 3,4,5-trimethoxyaniline and 4-hydroxybenzaldehyde. Pale yellow solid; yield 69% yield; mp 158 °C. IR (KBr): $\tilde{\nu}$ = 1624 cm^{-1} (s; ν(C=N)), 3398 cm^{-1} (s; ν (OH)). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 3.89 (s 6H), 6.48, (s, 2H), 7.26-7.76 (m, 4H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.6, 55.6, 60.6, 97.7, 108.0, 113.9, 124.8, 128.4, 135.6, 146.8, 147.6, 148.7, 153.5, 159.1. HRMS (ESI): *m/z* calcd for $C_{16}H_{17}NO_4 + H^+$ $[M + H]^+$: 288.1230; found: 288.1235.

4-[(3,4,5-Trimethoxyphenylimino)methyl]benzene-1,2-diol (33) was prepared according to general method I from 3,4,5-trimethoxyaniline and 3,4-dihydroxybenzaldehyde. White solid; yield 82%; mp 58 °C. IR (KBr): $\tilde{\nu}$ = 1645 cm^{-1} (s; ν(C=N)), 3401 cm^{-1} (s; ν (OH)). ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 3H), 3.88 (s, 6H), 4.90 (s, (br), 1H), 6.55 (s, 2H), 7.31-7.42 (m, 3H), 8.38 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 54.4, 54.7, 59.4, 97.6, 112.9, 114.4, 117.6, 122.6, 127.5, 135.3, 142.1, 135.7, 153.0, 160.6. HRMS (ESI): *m/z* calcd for $C_{16}H_{17}NO_5 + H^+$ $[M + H]^+$: 304.1179; found: 304.1181.

2-Methoxy-4-[(3,4,5-trimethoxyphenylimino)methyl]phenol (34) was prepared according to general method I from 3,4,5-trimethoxyaniline and 4-hydroxy-3-methoxybenzaldehyde. Pale yellow solid; yield 61%; mp 140 °C. IR (KBr): $\tilde{\nu}$ = 1613 cm^{-1} (s; ν(C=N)), 3403 cm^{-1} (s; ν (OH)). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 6H), 3.91 (s, 6H), 3.07 (s, 3H), 6.48-7.61 (m, 5H), 6.36 (s (br), 1H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.0, 61.0, 98.1, 108.5, 114.4, 125.3, 128.8, 136.1, 147.3, 148.1, 149.2, 153.6, 159.7. HRMS (ESI): *m/z* calcd for $C_{17}H_{19}NO_5 + H^+$ $[M + H]^+$: 318.1336; found: 318.1341.

3-[(3,4,5-Trimethoxyphenylimino)methyl]phenol (35) was prepared according to general method I from 3-hydroxybenzaldehyde and 3,4,5-trimethoxyaniline. White solid; yield 73%, mp 121 °C. IR (KBr): $\tilde{\nu}$ = 1583 cm^{-1} (s; ν(C=N)), 3327 cm^{-1} (s; ν (OH)). ^{1}H NMR ($CDCl_3$): δ

3.76 (s, 3H), 3.88 (s, 6H), 6.50 (s, 2H) 7.33-7.42 (m, 4H), 7.00 (s(br), 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.6, 97.8, 114.1, 118.8, 121.5, 129.6, 136.1, 136.7, 147.1, 153.1, 156.0, 159.8. HRMS (ESI): *m/z* calcd for $C_{16}H_{17}NO_4$ + H^+ $[M + H]^+$: 288.1230; found: 288.1249.

2-Methoxy-5-[(4-methoxyphenylimino)methyl]phenol (36) was prepared according to general method I from 4-methoxyaniline and 3-hydroxy-4-methoxybenzaldehyde. Pale yellow solid; yield 95%; mp 112-114 °C.[51] IR (KBr): $\tilde{\nu}$ = 1576 cm^{-1} (s; *ν*(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H), 3.91 (s, 3H), 5.86, (br s, 1H), 6.92-7.51 (m, 7H), 8.38 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.1, 55.6, 109.9, 113.3, 113.9, 121.5, 121.7, 129.8, 144.6, 145.5, 148.7, 157.6, 157.6. HRMS (ESI): *m/z* calcd for $C_{15}H_{15}NO_3$ + H^+ $[M + H]^+$: 258.1125; found: 258.1122.

6-Methoxy-2-nitro-3-[(3,4,5-trimethoxyphenylimino)methyl]phenol (37) was prepared from **8** and 3,4,5-trimethoxyaniline according to general method I. Yellow powder; yield 23%; mp 136 °C. IR (KBr): $\tilde{\nu}$ = 1578 cm^{-1} (s; *ν*(C=N)), 3275 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 1.73 (1H, s), 3.87 (3H, s), 3.91 (6H, s), 4.01 (3H, s), 6.51 (2H, s), 7.11 (1H, d, *J*=8.5 Hz), 7.66 (1H, d, *J*=8.5 Hz), 8.61 (1H, s). ^{13}C NMR ($CDCl_3$): δ 55.4, 56.5, 60.6, 97.9, 113.8, 120.4, 122.9, 135.8, 136.4, 142.3, 146.6, 150.4, 153.4, 154.3. HRMS (ESI): *m/z* calcd for $C_{17}H_{18}N_2O_7$ + H^+ $[M + H]^+$: 363.1187; found: 363.1180.

2-Methoxy-4-nitro-5-[(3,4,5-trimethoxyphenylimino)methyl]phenol (38) was prepared from **7** and 3,4,5-trimethoxyaniline according to general method I. Yellow powder; yield 10%, mp 188 °C. IR (KBr): $\tilde{\nu}$ = 1583 cm^{-1} (s; *ν*(C=N)), 3428 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.88 (3H, s), 3.92 (6H, s), 4.03 (3H, s), 6.56 (2H, s), 7.67 (1H, s), 7.76 (1H, s), 9.01 (1H, s). ^{13}C NMR ($CDCl_3$): δ 55.5, 55.7, 60.6, 98.1, 106.9, 113.9, 126.5, 136.5, 141.4,

146.5, 147.5, 150.1, 153.1, 155.2. HRMS (ESI): *m/z* calcd for $C_{17}H_{18}N_2O_7 + H^+$ $[M + H]^+$: 363.1187; found: 363.1178.

2-Methoxy-3-nitro-5-[(3,4,5-trimethoxyphenylimino)methyl]phenol (39) was prepared from **11** and 3,4,5-trimethoxyaniline according to general method I. Yellow solid; yield 47%; mp 105 °C. IR (KBr): $\tilde{\nu}$ = 1592 cm^{-1} (s; *v*(C=N)), 3420 cm^{-1} (s; *v*(OH)). ^1H NMR ($CDCl_3$): δ 3.83 (s, 3H), 3.90 (s, 6H), 4.05 (s, 3H), 6.53 (s, 2H), 7.82 (d, *J*=1.7 Hz, 1H), 7.99 (d, *J*=1.7 Hz, 1H), 8.42 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.4, 55.7, 60.6, 62.4, 97.9, 117.0, 118.6, 131.9, 136.6, 142.3, 142.8, 146.2, 150.5, 153.2, 155.5. HRMS (ESI): *m/z* calcd for $C_{17}H_{18}N_2O_7 + H^+$ $[M + H]^+$: 363.1187; found: 363.1184.

General method II: synthesis of TBDMS-protected imines

DBU was added dropwise to a solution of imine (5 mmol) and *tert*-butyldimethylsilylchloride (6 mmol) stirring in anhydrous CH_2Cl_2 (40 mL) under a nitrogen atmosphere. The reaction was stirred under N_2 at rt until the disappearance of starting material (as indicated by TLC; 2 - 4 h). The reaction was diluted with CH_2Cl_2 (50 mL) and washed with water (100 mL×2), 0.1M HCl (50 mL×2), and saturated aq. $NaHCO_3$ (50 mL×2), before drying over anhydrous Na_2SO_4. The solvent was removed *in vacuo* to yield TBDMS-protected imine, which was used in the subsequent β-lactam syntheses without further purification. Imines **40**[17] and **43**[18] was characterised as described by us previously.

[4-(*tert*-Butyldimethylsilanyloxy)benzylidene]-(3,4,5-trimethoxyphenyl)amine (41) was was prepared from **32** according to general method II. Amber oil; yield 91%. IR (NaCl): $\tilde{\nu}$ = 1581 cm^{-1} (s; *v*(C=N)). ^1H NMR ($CDCl_3$): 0.21 (6H, s), 1.01 (9H, s), 3.88 (3H, s), 3.91 (6H, s), 6.48 (2H, s), 6.94 (2H, d), 7.79 (2H, d), 8.41 (1H, s). ^{13}C NMR ($CDCl_3$): δ -5.9, 18.1, 25.3, 55.1, 56.0, 61.0, 98.1, 108.5, 114.4, 125.3, 128.8, 136.1, 147.3, 148.1, 149.3, 153.6, 159.7. HRMS (ESI): *m/z* calcd for $C_{22}H_{31}NO_4Si^+$ $[M]^+$: 401.2022; found: 401.2031.

[3,4-Bis-(*tert*-butyldimethylsilanyloxy)benzylidene]-(3,4,5-trimethoxyphenyl)amine (42) was prepared from **33** according to general method II. Colourless oil, yield 88%. IR (NaCl): $\tilde{\nu}$ = 1598 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 0.25 (12H, s), 1.05 (18H, s), 3.87 (3H, s), 3.92 (6H, s), 6.47 (2H, s), 7.28 (1H, s), 7.40 (2H, m), 8.32 (1H, s). ^{13}C NMR ($CDCl_3$): δ -5.9, -5.9, 18.0, 18.1, 25.3, 54.9, 55.4, 60.6, 91.9, 111.2, 120.0, 123.7, 129.0, 137.6, 142.4, 147.0, 151.5, 154.6, 158.8. HRMS (ESI): *m/z* calcd for $C_{28}H_{45}NO_5Si_2^+$ $[M]^+$: 531.2836; found: 531.2839.

[3-(*tert*-Butyldimethylsilanyloxy)benzylidene]-(3,4,5-trimethoxyphenyl)amine (44) was prepared from **35** according to general method II and was used without further characterization to prepare **69**. Amber oil; yield 92%. IR (NaCl): $\tilde{\nu}$ = 1597 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): 0.23 (s, 3H), 0.24 (s, 3H), 1.00 (s, 9H), 3.87 (s, 3H), 3.90 (s, 6H), 6.50 (s, 2H), 6.97 (1H, dd, *J*=2.2 Hz, 7.7 Hz), 7.34-7.41 (m, 3H), 8.42 (s, 1H). ^{13}C NMR ($CDCl_3$): δ -4.9, -4.8, 17.7, 25.2, 53.0, 55.6, 60.5, 97.7, 119.3, 121.9, 122.9, 129.3, 135.9, 137.1, 147.5, 153.1, 155.7, 159.2.

1-(3-((tert-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-N-(4-methoxyphenyl)methanimine (45) was prepared from **36** according to general method II and was used without further characterization to prepare **74**. Clear oil; yield 83%. IR (NaCl): $\tilde{\nu}$ = 1574 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 0.22 (s, 6H), 1.05 (s, 9H), 3.85 (s, 3H), 3.91 (s, 3H), 6.93-7.45 (m, 7H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$): δ -4.5, 18.5, 27.7, 55.4, 55.5, 111.4, 114.3, 120.2, 122.1, 123.7, 129.7, 145.2, 153.8, 153.8, 157.9.

[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxy-2-nitrobenzylidene]-(3,4,5-trimethoxy-phenyl)amine (46) was prepared from **37** according to general method II and was used without further characterization to prepare **71**. Amber oil; yield 88%. IR (NaCl): $\tilde{\nu}$ = 1579 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 0.26 (6H, s), 1.04 (9H, s), 3.89 (3H, s), 3.93 (6H, s), 3.97 (3H, s), 6.56 (2H, s), 7.64 (d, *J*=2 Hz, 2H), 8.99 (1H, s). ^{13}C NMR ($CDCl_3$): δ -4.9, 17.3,

25.1, 55.5, 55.7, 60.6, 98.1, 107.7, 119.6, 123.4, 136.4, 142.5, 146.9, 149.8, 152.1, 153.1, 154.9.

[5-(*tert*-Butyldimethylsilanyloxy)-4-methoxy-2-nitrobenzylidene]-(3,4,5-trimethoxy-phenyl)amine (47) was prepared from **38** according to general method II and was used without further characterization to prepare **73**. Amber oil; yield 87%. IR (NaCl): $\tilde{\nu}$ = 1583 cm^{-1} (s; *v*(C=N)). ^{1}H NMR ($CDCl_3$): δ 0.25 (6H, s), 1.06 (9H, s), 3.86 (3H, s), 3.94 (6H, s), 4.08 (3H, s), 6.59 (2H, s), 7.64 (1H, s), 7.81 (1H, s), 8.11 (1H, s). ^{13}C NMR ($CDCl_3$): δ -4.8, 17.4, 25.2, 55.5, 55.8, 60.9, 98.0, 119.9, 122.9, 128.4, 133.8, 137.3, 147.6, 149.7, 151.1, 153.9, 158.8.

[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxy-5-nitrobenzylidene]-(3,4,5-trimethoxy-phenyl)amine (48) was prepared from **39** according to general method II and was used without further characterization to prepare **72**. Amber oil; yield 91%. IR (NaCl): $\tilde{\nu}$ = 1596 cm^{-1} (s; *v*(C=N)). ^{1}H NMR ($CDCl_3$): δ 0.23 (s, 6H), 0.96 (s, 9H), 3.86 (s, 3H), 3.93 (s, 6H), 4.03 (s, 3H), 6.52 (s, 2H), 7.04 (d, *J*=1.7 Hz, 1H), 7.68 (d, *J*=1. Hz, 1H), 8.27 (s, 1H). ^{13}C NMR ($CDCl_3$): δ -4.6, -4.0, 18.1, 25.1, 55.3, 55.4, 56.4, 60.6, 97.8, 120.1, 121.2 , 131.9, 137.7, 146.7, 151.1, 152.3, 153.1, 154.2, 161.3.

Synthesis of β-Lactams; General Method III.A: To a refluxing solution of the appropriate imine (5 mmol) and triethylamine (6 mmol) in anhydrous CH_2Cl_2 (40 mL) was added a solution of the appropiate acid chloride (6 mmol) in anhydrous CH_2Cl_2 (10 mL) over 45 min under nitrogen. The reaction was refluxed for 5 h, continuously stirred under nitrogen, and stirred overnight until the starting material had disappeared as monitored by TLC. The reaction mixture was cooled to rt and washed with water (100 mL×2), dried over anhydrous Na_2SO_4 and solvent was removed *in vacuo*. The crude product was purified by flash chromatography over silica gel (1:1; *n*-hexane: ethyl acetate).

General Method III.B: A solution of the imine (1 mmol) and triethylamine (1.2 mmol) in dichloroethane (10 mL) heated to 40 ºC by microwave irradiation at atmospheric pressure and under a nitrogen atmosphere. To this solution, the appropriate acid chloride (1.2 mmol) in dichloroethane (5 mL) was added dropwise over 45 min. Upon completion of the addition, the reaction temperature was maintained at 40 ºC by continous microwave irradiation, while maintaining the nitrogen atmosphere. A characteristic darkening of the reaction was noticed upon completion of the reaction. The reaction was washed with water (100 mL×2), dried over Na_2SO_4 before the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (1:1, *n*-hexane: ethyl acetate).

General method III.C: The imine (5 mmol) and phenoxyacetyl chloride (5 mmol) were dissolved in dry toluene (15 mL), under nitrogen and stirring at 0 ºC. The solution was allowed to reach rt and warmed to 100 ºC. Triethylamine (5.5 mmol) was added dropwise. The mixture was refluxed at 100 ºC for 5 h. The solution was concentrated *in vacuo* and the residue was purified by flash chromatography over silica gel eluted with 4:1 *n*-hexane: ethyl acetate as eluent.

***Cis*-3-Phenoxy-1,4-bis-(3,4,5-trimethoxyphenyl)azetidin-2-one (49)** was obtained from **14** according to general method III.A. White solid; yield 32%; mp 132-134 ºC. IR (KBr): $\tilde{\nu}$ = 1762 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.75 (s 12H), 3.79 (s, 6H), 5.20 (d, 1H, *J*=5.0 Hz), 5.60 (d, 1H, *J*=5.0 Hz), 6.57 (s, 2H), 6.98-7.19 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 55.0, 55.5, 60.5, 62.0, 80.5, 95.0, 102.5, 115.0, 122.0, 127.5, 129.0, 132.5, 134.5, 138.2, 152.5, 153.1, 156.0, 162.7. HRMS (ESI): *m/z* calcd for $C_{27}H_{29}NO_8$ + Na^+ $[M + Na]^+$: 518.1785; found: 518.1786.

4-(4-Methoxyphenyl)-3-phenoxy-(3,4,5-trimethoxyphenyl)azetidin-2-one (50) was obtained from **15** according to general method III.A. *Trans* isomer **50b** was isolated as white solid; yield 21%; mp 112-114 ºC. IR (KBr): $\tilde{\nu}$ = 1740 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ

3.72 (s, 6H), 3.79 (s, 3H), 3.87 (s, 3H), 4.95 (d, 1H, *J*= 2.2 Hz), 5.13 (d, 1H, *J*= 2.2 Hz), 6.58-7.28 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 54.9, 55.6, 63.6, 60.5, 86.7, 94.9, 114.4, 114.9, 121.8, 126.9, 127.4, 129.2, 132.7, 134.4, 153.0, 156.5, 159.8, 162.1. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_6 + Na^+$ $[M + Na]^+$: 458.1574; found: 458.1580.

Cis isomer **50a** was isolated as a white solid; yield 33%; mp 155 ºC. IR (KBr): $\tilde{\nu}$ = 1741 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 6H), 3.78 (s, 3H), 3.79 (s, 3H), 5.34 (d, 1H, *J*=5.0 Hz), 5.54 (d, 1H, *J*=5.0 Hz), 6.58-7.28 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 55.2, 56.1, 60.9, 62.1, 81.1, 95.3, 113.9, 115.7, 122.2, 124.4, 129.3, 129.5, 133.1, 134.9, 153.5, 156.9, 159.9, 163.0. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_6 + Na^+$ $[M + Na]^+$: 458.1574; found: 458.1576.

***Cis*-4-(3-Methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (51)** was obtained from **16** according to general method III.A. Colourless solid; yield 34%; mp 158 ºC. IR (KBr): $\tilde{\nu}$ = 1749 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6H), 3.81 (s, 6H), 5.32 (d, *J*=5.1 Hz, 1H), 5.57 (d, *J*=5.1 Hz, 1H), 6.67 (s, 2H), 6.82-7.29 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 56.1, 60.9, 62.5, 81.0, 95.3, 110.3, 114.2, 115.6, 121.9, 122.3, 124.4, 129.4, 133.2, 134.9, 146.2, 146.7, 153.5, 156.9, 163.0. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_6 + Na^+$ $[M + Na]^+$: 458.1574; found: 458.1574.

***Cis*-4-(3,4-Dimethoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (52)** was obtained from **17** according to general method III.A. White solid; yield 56%, mp 152 ºC. IR (KBr): $\tilde{\nu}$ = 1751 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.79 (s, 6H), 3.85 (s, 3H), 5.33 (d, *J*=5.0 Hz, 1H), 5.58 (d, *J*= 4.5 Hz, 1H), 6.66-6.99 (10H, m). ^{13}C NMR ($CDCl_3$): δ 55.1, 55.9, 60.3, 62.6, 80.2, 95.9, 110.9, 115.4, 120.1, 122.4, 124.7, 129.7, 133.3, 134.2, 148.9, 156.4, 162.1. HRMS (ESI): *m/z* calcd for $C_{26}H_{27}NO_7 + Na^+$ $[M + Na]^+$: 488.1680; found: 488.1697.

***Cis*-4-Benzo[1,3]dioxol-5-yl-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (53)** was obtained from **18** according to general method III.A. Pale yellow soild; yield 71%; mp 156 ºC. IR (KBr): $\tilde{\nu}$ = 1747 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.79 (s, 3H), 5.32 (d, 1H, J_{cis}=5.0 Hz), 5.51 (d, 1H , J_{cis}=5.0 Hz), 5.95 (s, 2H), 6.64-7.28 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 56.0, 60.5, 62.1, 80.6, 95.4, 101.7, 107.5, 115.8, 121.5, 122.3, 126.4, 129.6, 132.7, 134.9, 147.4, 153.4, 156.7, 162.8. HRMS (ESI): *m/z* calcd for $C_{50}H_{46}N_2O_{14}$ + Na^+ [2M + Na]$^+$: 921.2841; found: 921.2844.

***Cis*-4-(4-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-phenoxyazetidin-2-one (54)** was obtained from **19** according to general method III.A. Colourless solid; yield 61%; mp 125-127 ˚C. IR (KBr): $\tilde{\nu}$ = 1759 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.79 (s, 3H), 5.35 (d, *J* = 5.0 Hz), 5.58 (d, *J* = 5.0 Hz), 6.59 (s, 2H), 6.80-7.47 (m, 9H), ^{13}C NMR ($CDCl_3$): δ 55.6, 60.5, 61.2, 80.4, 94.7, 115.1, 122.0, 122.5, 128.9, 129.3, 131.3, 131.4, 132.3, 134.6, 153.1, 156.3, 162.3. HRMS (ESI): *m/z* calcd for $C_{24}H_{22}BrNO_5$ + H^+ [M + H]$^+$: 484.0754; found: 484.0735.

4-(3-Bromo-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (55) was obtained from **20** according to general method III.A. *Cis* isomer **55a** was isolated as a white solid; yield 61%; mp 158ºC. IR (KBr): $\tilde{\nu}$ = 1756 cm^{-1} (s; ν(C=O)).^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6H), 3.80 (s, 3H), 3.87 (s, 3H), 5.29 (d, *J* = 5.2 Hz), 5.55 (d, *J* = 5.2 Hz, 1H), 6.62-7.35, (m, 10H). ^{13}C NMR ($CDCl_3$): δ 55.7, 55.8, 60.5, 60.9, 80.5, 94.8, 111.1, 111.2, 115.2, 121.9, 125.6, 127.9, 128.9, 132.4, 132.6, 134.6, 153.1, 155.7, 156.3, 162.3. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}BrNO_6$ + Na^+ [M + Na]$^+$: 536.0679; found: 536.0701.

Trans isomer **55b** was isolated as a white solid; yield 31%; mp 170ºC. IR (KBr): $\tilde{\nu}$ = 1753 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$) δ 3.73, (s, 6H), 3.79 (s, 3H), 3.94 (s, 3H), 4.92 (d, *J*=2.0 Hz, 1H), 5.12 (d, *J*=2.0 Hz, 1H), 6.57-7.64 (m, 11H)**.** ^{13}C NMR ($CDCl_3$): δ 55.6, 55.9, 60.5, 62.8, 86.7, 94.9, 111.9, 112.3, 114.9, 120.0, 126.3, 128.6, 129.3, 131.0, 132.4, 134.7, 153.1,

156.1, 156.5, 161.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}BrNO_6$ + Na^+ $[M + Na]^+$: 536.0679; found: 536.0676.

***Cis*-4-(3-Fluoro-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (56)** was obtained from **21** according to general method III.A. White solid; yield 56%; mp 120 ºC. IR (KBr): $\tilde{\nu}$ = 1752 cm^{-1} (s; *v*(C=O)). ^1H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.86 (s, 3H), 3.86 (s, 3H), 5.30 (d, 1H, *J*=5.0 Hz), 5.52 (d, 1H, *J*=5.0 Hz), 6.6-7.1 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 55.5, 60.3, 61.5, 80.3, 94.5, 112.7, 115.8, 115.5, 121.2, 113.3, 125.7, 129.6, 132.6, 134.8, 147.2, 153.9, 156.6, 162.0. HRMS (ESI): *m/z* calcd for $C_{50}H_{48}F_2N_2O_{12}$ + Na^+ $[2M + Na]^+$: 929.3068; found: 929.3057.

***Cis*-4-(4-Dimethylaminophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (57)** was obtained from **22** according to general method III.A. Pale brown solid; yield 34%; mp 166 ºC. IR (KBr): $\tilde{\nu}$ = 1744 cm^{-1} (s; *v*(C=O)). ^1H NMR ($CDCl_3$): δ 2.92 (s, 6H), 3.72 (s, 6H), 3.74 (s, 3H), 5.32 (d, *J*=5.1 Hz, 1H), 5.51 (d, *J*=5.1 Hz, 1H), 6.62 (s, 2H), 6.87-7.11 (9H, m). ^{13}C NMR ($CDCl_3$): δ 40.5, 55.1, 60.9, 62.7, 81.7, 97.9, 111.4, 114.9, 119.4, 121.7, 128.1, 129.3, 132.5, 134.7, 150.5, 152.4, 156.6, 162.9. HRMS (ESI): *m/z* calcd for $C_{26}H_{28}N_2O_5$ + H^+ $[M + H]^+$: 449.2071; found: 449.2096.

4-[4-Oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl]benzonitrile (58) was obtained from **23** according to general method III.A. *Cis* isomer **58a** isolated as a white solid; yield 37%; mp 174 ºC. IR (KBr): $\tilde{\nu}$ = 1765 cm^{-1} (s; *v*(C=O)), 2237 cm^{-1} (s; *v*(C≡N)). ^1H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.80 (s, 3H), 5.44 (d, *J*=5.0 Hz, 1H), 5.64 (d, *J* = 5.0 Hz, 1H), 6.56-7.63 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.5, 61.0, 80.5, 94.7, 112.3, 114.9, 117.9, 122.2, 128.3, 129.1 131.8, 132.1, 135.1, 137.9, 153.3, 155.9, 161.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{22}N_2O_5$ + Na^+ $[M + Na]^+$: 453.1421; found: 453.1427.

Trans isomer **58b** isolated as an off-white solid; yield 24%; mp 162 °C. IR (KBr): $\tilde{\nu}$ = 1760 cm^{-1} (s; *v*(C=O)), 2227 cm^{-1} (s; *v*(C≡N)). ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 6H), 3.79 (s, 3H), 5.08 (d, *J*=1.5 Hz, 1H), 5.12 (d, *J*=1.5 Hz, 1H), 6.50 (s, 2H), 6.88-7.76 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.5, 63.0, 86.6, 94.8, 112.9, 115.0, 117.6, 122.4, 126.7, 129.4, 132.0, 132.8, 134.9, 140.6, 153.3, 156.3, 161.5. HRMS (ESI): *m/z* calcd for $C_{25}H_{22}N_2O_5 + Na^+$ $[M + Na]^+$: 453.1421; found: 453.1435.

***Cis*-4-Naphthalen-2-yl-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (59)** was obtained from **24** according to general method III.A. White solid; yield 42%; mp 162 °C. IR (KBr): $\tilde{\nu}$ = 1746 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.71 (s, 6H), 3.78 (s, 3H), 5.57 (d, *J*=5.2 Hz, 1H), 5.67 (d, *J*=5.2 Hz, 1H), 6.68-7.81 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 55.5, 60.5, 62.1, 80.9, 94.8, 115.3, 121.9, 124.8, 125.9, 127.3, 127.4, 127.9, 128.9, 129.9, 132.6, 132.7, 134.5, 153.1, 156.5, 162.7. HRMS (ESI): *m/z* calcd for $C_{28}H_{25}NO_5 + Na^+$ $[M + Na]^+$: 478.1625; found: 478.1647.

***Cis*-4-Naphthalen-1-yl-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (60)** was obtained from **25** according to general method III.A. Off-white solid; yield 98%; mp 182-186 °C. IR (KBr): $\tilde{\nu}$ = 1757 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.70 (s, 6H), 3.84 (s, 3H), 5.77 (d, 1H), 6.17 (d, 1H), 6.68-7.85, (m, 14H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.6, 81.7, 94.8, 116.1, 121.9, 124.9, 125.4, 125.9, 127.7, 127.8, 128.6, 130.9, 132.9, 132.9, 133.2, 134.3, 153.2, 156.9, 163.3. HRMS (ESI): *m/z* calcd for $C_{28}H_{25}NO_5 + Na^+$ $[M + Na]^+$: 478.1625; found: 478.163.

4-(4-Methoxy-3-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (61) was obtained from **26** according to general method III.A. *Cis* isomer **61a** was isolated as a pale brown solid; yield 52%; mp 136-138 °C. IR (KBr): $\tilde{\nu}$ = 1758 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H), 3.79 (s, 6H), 3.93 (s, 3H), 5.41 (d, *J*=5.0 Hz, 1H), 5.60 (d, *J*=5.0 Hz,

1H), 6.59 (s, 2H), 7.23 -7.92 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 55.7, 56.2, 60.3, 60.4, 80.4, 94.7, 113.3, 114.9, 122.2, 124.7, 125.3, 129.1, 132.2, 133.2, 134.8, 138.7, 152.8, 153.2, 156.0, 162.1. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}N_2O_8 + Na^+$ $[M + Na]^+$: 503.1425; found: 503.1433.

Trans isomer **61b** was isolated as a pale brown solid; yield 26% yield; mp 160 ºC. IR (KBr): $\tilde{\nu}$ = 1753 cm^{-1} (s; *ν*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6H), 3.80 (3H, s), 4.02 (s, 3H), 5.03 (d, *J*=1.5 Hz, 1H), 5.14 (d, *J*=1.5 Hz, 1H), 6.61 (s, 2H), 7.28-7.92 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 55.7, 56.3, 60.5, 62.3, 86.6, 94.9, 114.3, 114.9, 122.3, 123.6, 127.5, 129.4, 131.3, 132.0, 134.9, 139.5, 152.9, 153.2, 156.3, 161.6. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}N_2O_8 + Na^+$ $[M + Na]^+$: 503.1425; found: 503.1418.

***Cis*-4-(3-Nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (62)** was obtained from **27** according to general method III.A. Brown solid; yield 47%; mp 170-174 ºC. IR (KBr): $\tilde{\nu}$ = 1762 cm^{-1} (s; *ν*(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H), 3.80 (s, 6H), 5.53 (d, *J*=5 Hz, 1H), 5.68 (d, *J*=5 Hz, 1H), 6.60 (s, 2H), 7.34-8.27 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.5, 60.7, 80.4, 94.7, 114.9, 122.2, 122.7, 123.5, 129.2, 132.1, 133.4, 134.8, 134.9, 147.7, 153.3, 155.8, 161.9. HRMS (ESI): *m/z* calcd for $C_{24}H_{22}N_2O_7 + Na^+$ $[M + Na]^+$: 473.1319; found: 473.1325.

***Cis*-4-(4-Nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (63)** was obtained from **28** according to general method III.A. Colourless solid; yield 39%, mp 154 ºC. IR (KBr) υ max: 1765 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H), 3.80 (s, 6H), 5.51 (d, *J*= 5.0 Hz, 1H), 5.66 (d, *J*=5.6 Hz, 1H), 6.57-8.20 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.5, 60.8, 80.6, 94.7, 114.9, 122.3, 123.2, 128.5, 129.1, 132.1, 139.9, 147.7, 153.3, 155.9, 161.9. HRMS (ESI): *m/z* calcd for $C_{24}H_{22}N_2O_7 + Na^+$ $[M + Na]^+$: 473.1319; found: 473.1335.

***Cis*-(4-Methylsulfanylphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (64)** was obtained from **29** according to general method III.A. Pale yellow solid; yield 77%; mp

130 ºC. IR (KBr): $\tilde{\nu}$ = 1744 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H), 3.74 (s, 1H), 3.79 (s, 1H), 5.34 (d, 1H), 5.55 (d, 1H), 6.57-7.17 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 15.7, 55.7, 60.4, 61.5, 80.3, 94.6, 115.1, 121.6, 125.6, 128.4, 128.2, 132.8, 134.2, 139.6, 153.9, 156.3, 162.2. HRMS (ESI): *m/z* calcd for $C_{50}H_{50}N_2O_{10}S_2$ + Na^+ $[2M + Na]^+$: 925.2799; found: 925.2841.

***Cis*-4-(4-Azidophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (65)** was obtained from **30** according to general method III.A. Grey solid; yield 64%; mp 134-138ºC. IR (KBr) υ max: 1761 cm^{-1} (C=O), 2136 cm^{-1} (-N_3). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.80 (s, 3H), 5.37 (d, *J*=4.8 Hz, 1H), 5.57 (d, *J*=4.8 Hz, 1H), 6.61 (s, 2H), 6.82 (d, *J*=7.9, 2H), 6.97 (m, 3H, ArH), 7.20 (d, *J*=8.3 Hz, 8.8 Hz, 2H), 7.40 (d, *J*=8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 55.6, 55.7, 60.5, 61.3, 80.5, 94.8, 115.1, 118.7, 121.9, 128.9, 128.9, 129.2, 132.5, 134.6, 140.2, 153.1, 156.3, 162.3. HRMS (ESI): *m/z* calcd for $C_{24}H_{22}N_4O_5$ + Na^+ $[M + Na]^+$: 469.1482; found: 469.1500.

4-[3-(t-Butyldimethylsilanyloxy)-4-methoxyphenyl]-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (66) was obtained from **40** according to general method III.A. *Cis* isomer **66a** was isolated as a white resin; yield 61%. IR (NaCl): $\tilde{\nu}$ = 1729 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 0.04 (6H, s), 0.91 (9H, s), 3.72 (3H, s), 3.74 (6H, s), 3.91 (3H, s), 5.28 (1H, d, *J*=5 Hz), 5.52 (1H, d, *J*=5 Hz), 6.64-6.89 (10H, m). ^{13}C NMR ($CDCl_3$): δ -4.2, , 17.9, 23.5, 55.4, 55.7, 60.6, 61.9, 81.1, 94.9, 113.6, 114.5, 117.4, 119.4, 120.3, 125.1, 128.0, 132.4, 137.8, 142.0, 148.3, 151.2), 156.7, 162.7. HRMS (ESI): *m/z* calcd for $C_{31}H_{39}NO_7Si$ + Na^+ $[M + Na]^+$: 588.2388; found: 588.2402.

Trans isomer **66b** isolated as a white resin; yield 21%. IR (NaCl): $\tilde{\nu}$ = 1729 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 0.02 (6H, s), 1.02 (9H, s), 3.75 (3H, s), 3.77 (6H, s), 3.92 (3H, s), 4.89 (1H, d, *J*=1.5 Hz), 5.10 (1H, d, *J*=1.5 Hz), 6.58-6.87 (10H, m). ^{13}C NMR ($CDCl_3$): δ -4.2, -

4.3, 17.6, 24.1, 54.8, 54.9, 59.2, 62.3, 82.6, 95.2, 111.4, 114.2, 117.6, 119.8, 120.4, 128.2, 131.4, 132.3, 138.3, 143.1, 149.7, 149.9, 157.3, 161.1. HRMS (ESI): *m/z* calcd for $C_{31}H_{39}NO_7Si + Na^+$ $[M + Na]^+$: 588.2388; found: 588.2402.

***Cis*-(4-(3-((tert-Butyldimethylsilyl)oxy)-4-methoxy-2-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (71)** was obtained from **46** according to general method III.A. Amber oil; yield 61%. IR (KBr): $\tilde{\nu}$ = 1758 cm^{-1} (s; *v*(C=O)). ^1H NMR ($CDCl_3$): δ 0.01 (s, 3H), 0.03 (s, 3H), 0.87 (s, 9H), 3.81 (s, 6H), 3.82 (s, 3H), 3.93 (s, 3H), 5.40 (d, *J*=5.7 Hz, 1H), 5.71 (d, *J*=5.4 Hz, 1H), 6.68 (s, 2H), 6.90-6.96 (m, 4H), 7.22-7.28 (m, 2H), 7.82 (s, 1H). ^{13}C NMR ($CDCl_3$): δ -5.3, -5.3, 17.9, 24.9, 55.6, 55.8, 60.5, 58.7, 81.7, 94.7, 108.9, 115.9, 119.9, 122.2, 123.7, 129.0, 132.4, 134.8, 140.8, 150.3, 150.3, 153.5, 156.8, 163.2. HRMS (ESI): *m/z* calcd for $C_{31}H_{38}N_2O_9Si + Na^+$ $[M + Na]^+$: 633.2239; found: 633.2232.

***Cis*-4-(3-(*tert*-Butyldimethylsilanyloxy)-4-methoxy-5-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (72)** was obtained from **48** according to general method III.A. Amber oil; 73% yield. IR (NaCl): $\tilde{\nu}$ = 1753 cm^{-1} (s; *v*(C=O)). ^1H NMR ($CDCl_3$): δ 0.23 (s, 6H), 0.96 (s, 9H), 3.75 (s, 6H), 3.77 (s, 3H), 3.82 (s, 3H), 5.28 (d, *J*=5.0 Hz, 1H), 5.51 (d, *J*=5.0 Hz, 1H), 6.20 (s(br), 1H), 6.64 (s, 2H), 6.71-6.78 (m, 3H), 7.11-7.19 (m, 3H), 7.47 (d, *J*=2.0 Hz) . ^{13}C NMR ($CDCl_3$): -4.8, -4.9, 17.8, 24.1, 55.8, 60.5, 62.1, 60.5, 80.4, 94.8, 115.1, 116.1, 119.3, 122.2, 129.1, 129.3, 132.0, 141.1, 141.8, 150.4, 151.1, 153.3, 155.9, 161.9. HRMS (ESI): *m/z* calcd for $C_{31}H_{38}N_2O_9Si + Na^+$ $[M + Na]^+$: 633.2239; found: 633.2245.

***Cis*-4-(5-((tert-Butyldimethylsilyl)oxy)-4-methoxy-2-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (73)** was obtained from **47** according to general method III.A. Amber oil; yield 58%. IR (NaCl): $\tilde{\nu}$ = 1759 cm^{-1} (s; *v*(C=O)). ^1H NMR ($CDCl_3$): δ 0.12 (s, 3H), 0.15 (s, 3H), 0.96 (s, 9H), 3.79 (s, 3H), 3.82 (s, 3H), 3.84 (s, 6H), 5.27 (d, *J*=4.7 Hz, 1H), 5.54 (d, *J*=4.7 Hz, 1H), 6.70 (s, 2H), 6.84-6.96 (m, 4H), 7.12-7.25 (m, 3H). ^{13}C NMR

($CDCl_3$): δ -4.8, -4.9, 18.0, 25.1, 55.0, 55.7, 60.5, 55.9, 80.8, 94.3, 112.1, 115.1, 115.8, 120.7, 122.0, 129.0, 132.0, 134.5, 136.5, 144.3, 150.7, 153.2, 156.2, 162.0. HRMS (ESI): *m/z* calcd for $C_{31}H_{38}N_2O_9Si + Na^+$ $[M + Na]^+$: 633.2239; found: 633.2231.

4-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-1-(4-methoxyphenyl)-3-phenoxyazetidin-2-one (74) was obtained from **45** according to general method III.A. Colourless resin; yield 87%. IR (NaCl): $\tilde{\nu}$ = 1743 cm^{-1} (s; *ν*(C=O)). ^1H NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.95 (s, 9H), 3.75 (s, 3H), 3.77 (s, 3H), 5.26 (d, *J*=5.0 Hz, 1H), 5.52 (d, *J*=5.0 Hz, 1H), 6.74-6.92 (m, 12H). ^{13}C NMR ($CDCl_3$): δ -5.2, -5.3, 17.9, 25.2, 54.9, 54.9, 61.4, 80.6, 111.2, 113.9, 115.1, 118.6, 120.5, 121.4, 121.6, 124.4, 128.8, 130.0, 144.4, 150.9, 155.9, 156.5, 161.9. HRMS (ESI): *m/z* calcd for $C_{29}H_{35}NO_5Si + Na^+$ $[M + Na]^+$: 528.2177; found: 528.2193.

***Cis*-Ethyl-3-(4-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl) acrylate (75).** β-Lactam **268** (1.5 mmol), triethylamine (7.5 mmol), palladium(II) acetate (0.24 mmol), 1,3-(diphenylphosphino)propane (0.22 mmol) and ethyl acrylate (2.0 mmol) were combined under nitrogen. Anhydrous DMF (15 mL) was added and the mixture was stirred for 18 hours at 100 ºC. The mixture was cooled, diluted with CH_2Cl_2 (200 mL), washed with water (100 mL×6) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and resulting dark brown oil was purified by flash chromatography over silica gel (*n*-hexane-ethyl acetate, 3:1). Colourless solid; yield 16%; mp 139 ˚C. IR (KBr): $\tilde{\nu}$ = 1760 cm^{-1} (s; *ν*(C=O)), 1713 cm^{-1} (s; *ν*(C=O)). ^1H NMR ($CDCl_3$): δ 1.34 (t, *J* = 7.0 Hz, 3H), 3.74 (s, 6H), 3.79 (s, 3H), 4.27 (q, *J* = 7.0 Hz, 2H), 5.40 (d, *J* = 4.5 Hz, 1H, H_4), 5.61 (d, *J* = 5.0 Hz, 1H), 6.42 (d, *J* = 16.0 Hz, 1H), 6.61 (s, 2H), 6.80-6.82 (m, 2H), 6.93-6.97 (m, 1H), 7.18 (d, *J* = 7.5 Hz, 2H), 7.42-7.44 (2H, m), 7.48 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 13.9, 55.6, 60.5, 60.2, 61.5, 80.7, 94.8, 115.2, 118.5, 121.9, 127.7,

128.2, 128.9, 132.4, 134.4, 134.5, 134.6, 143.2, 153.2, 156.3, 162.3, 166.3. HRMS (ESI): *m/z* calcd for $C_{29}H_{29}NO_7 + Na^+$ $[M + Na]^+$: 526.1836; found: 526.1832.

***Cis*-4-(4-Methanesulfinylphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (76).** *m*-CPBA (0.25 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to a stirring solution of β-lactam **64** (0.25 mmol) in anhydrous CH_2Cl_2 (10 mL) under nitrogen and the mixture was stirred for 2 h. The reaction mixture was washed with 5% $NaHCO_3$ aq. solution (50 mL) and water (50 mL), dried over Na_2SO_4, and solvent removed *in vacuo*. Chromatography over silica gel (eluent: 9:1, ethyl acetate: *n*-hexane) yielded **76** as a pale brown solid; yield 63%; mp 156 ºC. IR (KBr): $\tilde{\nu}$ = 1749 cm^{-1} (s; *v*(C=O)), 1051 cm^{-1} (s; *v*(S-O)). ^1H NMR (DMSO-d_6) δ 2.65 (s, 3H), 3.60 (s, 6H), 3.66 (s, 3H), 5.84 (d, *J*=4.7 Hz, 1H), 5.97 (d, *J*=4.7 Hz, H_3), 6.62 (s, 2H), 6.82 (dd, *J*=2.2 Hz, 9.1 Hz, 2H), 6.91-6.94 (m, 1H), 7.19-7.21 (m, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.89 (d, *J*=8.3 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 43.3, 56.2, 60.5, 60.8, 80.7, 95.6, 115.5, 122.3, 123.8, 129.4, 129.7, 132.9, 134.8, 136.5, 146.8, 153.6, 156.5, 162.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_6S + Na^+$ $[M + Na]^+$: 490.1295; found: 490.1280.

***Cis*-4-(4-Methanesulfonylphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (77).** *m*-CPBA (0.66 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to a stirring solution of β-lactam **64** (0.25 mmol) in anhydrous CH_2Cl_2 (10 mL) under nitrogen and the mixture was stirred for 30 min. The reaction mixture was washed with 5% $NaHCO_3$ aq. solution (50 mL) and water (50 mL), dried over Na_2SO_4, and solvent removed *in vacuo*. Chromatography over silica gel (7:3; ethyl acetate:*n*-hexane) yielded **77** as pale brown solid; yield 66%; mp 160-162 ºC. IR (KBr): $\tilde{\nu}$ = 1750 cm^{-1} (s; *v*(C=O)), 1307, 1221 cm^{-1} (s; *v*(S-O)). ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H), 3.77 (s, 6H), 3.81 (s, 3H), 5.49 (d, 1H), 5.67 (d, 1H), 6.59 (s, 2H), 6.81 (d, *J*=8.3 Hz), 6.96 (dd, *J*=8.2 Hz, 2.1 Hz, 1H), 7.20 (dd, *J*=8.1 Hz, 2 Hz, 2H),

7.62 (d, *J*=8.0 Hz, 2H), 7.92 (d, *J*=8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 43.9, 57.8, 60.5, 60.9, 80.6, 94.8, 115.0, 122.2, 127.1, 128.6, 129.0, 132.0, 134.9, 138.9, 140.5, 153.3, 155.9, 161.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_7S + Na^+$ $[M + Na]^+$: 506.1244; found: 506.1262.

General method IV: removal of TBDMS-protecting group.

TBAF (1M solution in hexanes) (2 mmol) was added dropwise to a solution of the β-lactam silyl ether (2 mmol) in anhydrous THF under nitrogen at 0 °C. The reaction was stirred at 0 °C until complete as indicated by TLC. Ethyl acetate (75 mL) was added and the mixture was washed with 0.1 M HCl (100 mL). The aqueous layer was extracted with ethyl acetate (25 mL×2). The combined organic layers were washed with water (100 mL), brine (100 mL), and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the crude reaction mixture was purified by flash chromatography over silica gel (hexane:ethyl acetate gradient).

***Cis*-4-(3-Hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (78a)** was obtained from **66a** according to general method IV. White solid; yield 73%; mp 176-180 °C. IR (KBr): $\tilde{\nu}$ = 1739 cm^{-1} (s; *ν*(C=O)), 3401 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 6H, 2xOCH_3), 3.79 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.29 (d, *J*=5.0 Hz, 1H, H_4), 5.53 (d, *J*=5.0 Hz, 1H, H_3), 6.65 (s, 2H, H_2', H_6'), 6.78-7.21 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$): δ 55.4 (OCH_3), 55.6 (OCH_3), 60.5 (OCH_3), 61.6 (C_4), 80.7 (C_3), 94.8 (C_2', C_6'), 109.9 (C_2'', C_6''), 113.9 (C_2'''), 115.3 (C_5'''), 119.7 (C_4''), 121.8 (C_6'''), 125.1 (C_4'), 128.8 (C_3'', C_5''), 132.7 (C_1'), 145.1 (C_1'), 145.1 (C_1'''), 146.4 (C_4'''), 153.1 (C_3', C_5'), 156.6 (C_1''), 162.5 (C=O). HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_7 + Na^+$ $[M + Na]^+$: 474.1523; found: 474.1529.

***Trans*-4-(3-Hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (78b)** was obtained from **66b** according to general method IV. White solid; yield 68%. IR (KBr): $\tilde{\nu}$ = 1737 cm^{-1} (s; *ν*(C=O)), 3498 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 3H),

3.70 (s, 6H), 3.88 (s, 3H), 5.10 (d, *J*=1.5 Hz, 1H), 5.35 (d, *J*=1.5 Hz, 1H), 6.70 (s, 2H), 6.92-7.31 (m, 8H), 7.87 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 54.9, 54.9, 59.2, 63.0, 86.5, 95.2, 111.3, 112.9, 114.9, 118.3, 121.6, 128.2, 129.1, 132.7, 134.2, 146.8, 147.7, 153.3, 156.8, 161.6. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_7 + Na^+$ $[M + Na]^+$: 474.1523; found: 474.1533.

***Cis*-4-(4-Hydroxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (79).** *Cis*-(4-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**68**) was obtained from **41** according to general method III.A and was deprotected according to general method IV to yield **79**. White solid; yield 40%; mp 142 ºC. IR (KBr): $\tilde{\nu}$ = 1741 cm^{-1} (s; *ν*(C=O)), 3287 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 6H), 3.80 (s, 3H), 5.27 (d, *J*=4.8 Hz, 1H), 5.54 (d, *J*=5.0 Hz, 1H), 6.66 (s, 2H), 6.73-7.18 (9H, m). ^{13}C NMR ($CDCl_3$): δ 56.1, 60.9, 62.4, 81.2, 95.3, 114.3, 115.7, 118.7, 122.3, 129.3, 133.2, 133.8, 134.9, 146.6, 153.5, 157.1, 163.1. HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_6 + Na^+$ $[M + Na]^+$: 444.1418; found: 444.1432.

4-(4-Hydroxy-3-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (80). 4-(4-((*tert*-Butyldimethylsilyl)oxy)-3-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**67**) was obtained from **43** according to general method III.A and was deprotected according to general method IV to yield **80**. White solid; yield 57%; mp 158 ºC. IR (KBr): $\tilde{\nu}$ = 1757 cm^{-1} (s; *ν*(C=O)), 3415 cm^{-1} (s; *ν*(OH)). Mixture of isomers in 2:1 *trans: cis* ratio. ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 3.80 (s, 3H), 3.85 (s, 3H), 4.64 (d, *J*=1.8 Hz, 0.66H), 4.87 (d, *J*=1.8 Hz, 0.66H), 5.40 (d, *J*=5.0 Hz, 0.33H), 5.60 (d, *J*=5.0 Hz, 0.33H), 6.61-7.42 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 56.1, 56.2, 61.7, 60.9, 81.1, 95.3, 97.9, 114.7, 114.9, 115.7, 121.5, 122.1, 122.4, 122.6, 129.3, 130.7, 132.8, 132.9, 134.8, 153.4, 153.6, 156.8, 156.9), 157.8, 162.8, 166.3, 167.2. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_7 + Na^+$ $[M + Na]^+$: 474.1523; found: 474.1528.

4-(3,4-Dihydroxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (81). 4-(3,4-bis((*tert*-Butyldimethylsilyl)oxy)phenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**70**) was obtained from **42** according to general method III.A and was deprotected according to general method IV to yield **81**. Off-white solid; yield 26%; mp 180-182 ºC. IR (KBr): $\tilde{\nu}$ = 1749 cm^{-1} (s; *v*(C=O)), 3426 cm^{-1} (s; *v*(OH)). Mixture of isomers in 2:1 *trans: cis* ratio. ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 9H), 3.71 (s, 6H), 4.88 (d, *J*= 2.0 Hz, 0.65H), 5.09 (d, *J*=2.0 Hz, 0.65H), 5.28 (d, *J*=4.9 Hz, 0.35H), 5.52 (d, *J*=4.9 Hz, 0.35H), 5.69 (s(br), 0.4H), 5.88 (s(br), 0.6H), 6.53-7.24 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 55.5, 55.6, 60.5, 63.9, 62.2, 80.8, 94.9, 94.9, 112.1, 114.1, 114.6, 115.8, 115.4, 119.3, 120.9, 121.9, 127.3, 128.9, 129.2, 132.4, 132.6, 134.4, 143.5, 144.3, 144.4, 152.9, 153.1, 156.4, 156.5, 162.5. HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_7 + Na^+$ $[M + Na]^+$: 460.1367; found: 460.1367.

***Cis*-4-(3-Hydroxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (82).** *Cis*-4-(3-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**69**) was obtained from **44** according to general method III.A and was deprotected according to general method IV to yield **82**. Colourless solid; yield 43%; mp 218-220 ºC. IR (KBr): $\tilde{\nu}$ = 1749 cm^{-1} (s; *v*(C=O)), 3380 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.59 (s, 3H), 3.66 (s, 6H), 5.64 (d, *J*=4.5 Hz, 1H), 5.86 (d, *J*=5.0 Hz, 1H), 6.60 (s, 2H), 6.97.23 (m, 9H), 9.41 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.8, 60.1, 61.1, 80.4, 95.1, 114.6, 115.2, 115.5, 119.2, 121.9, 129.2, 132.8, 134.3, 134.6, 153.2, 156.6, 157.1, 162.7. HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_6 + Na^+$ $[M + Na]^+$: 444.1418; found: 444.1414.

***Cis*-4-(3-Hydroxy-4-methoxyphenyl)-1-(4-methoxyphenyl)-3-phenoxyazetidin-2-one (83).** 4-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(4-methoxyphenyl)-3-phenoxyazetidin-2-one (**74**) was obtained from **45** according to general method III.A and deprotected according to general method IV to yield **83**. Colourless solid; yield 46%; mp 136-138 ºC. IR (KBr): $\tilde{\nu}$ = 1743 cm^{-1} (s; *v*(C=O)), 3379 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ

3.77 (s, 3H), 3.86 (s, 3H), 5.28 (d, *J*=5.0 Hz, 1H), 5.52 (d, *J*=5.0 Hz, 1H), 6.76-6.98 (m, 8H), 7.18-7.32 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 54.8, 54.9, 61.3, 80.6, 111.2, 113.6, 114.8, 116.3, 120.6, 121.0, 121.5, 124.3, 129.0, 129.4, 144.1, 148.6, 155.9, 156.6, 162.0. HRMS (ESI): *m/z* calcd for $C_{23}H_{21}NO_5 + Na^+$ $[M + Na]^+$: 414.1312; found: 414.1313.

***Cis*-4-(3-Hydroxy-4-methoxy-2-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (84)** was obtained from **71** according to general method IV. Off-white solid; yield 22%; mp 136 ºC. IR (KBr): $\tilde{\nu}$ = 1748cm^{-1} (s; *v*(C=O)), 3274 cm^{-1} (s; *v*(OH)). ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 3H), 3.71 (s, 6H), 3.81 (s, 3H), 5.52 (d, *J*=5.1 Hz, 1H), 5.83 (d, *J*=5.1 Hz, 1H), 6.64 (s, 2H), 6.82-6.91 (m, 4H), 7.14-7.26 (m, 3H). ^{13}C NMR (DMSO-d_6): δ 55.8, 55.9, 60.1, 56.2, 80.8, 94.7, 113.1, 115.5, 118.6, 122.2, 129.5, 132.4, 134.4, 136.4, 138.7, 140.9, 147.1, 148.8, 155.3, 162.5. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}N_2O_9 + Na^+$ $[M + Na]^+$: 519.1374; found: 519.1369.

***Cis*-4-(3-Hydroxy-4-methoxy-5-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (85)** was obtained from **48** according to general method IV. Pale brown solid; yield 39%; mp 105 ºC. IR (KBr): $\tilde{\nu}$ = 1756 cm^{-1} (s; *v*(C=O)), 3355 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 9H), 3.79 (s, 3H), 4.02 (s, 3H), 5.36 (d, *J*=5.0 Hz, 1H), 5.61 (d, *J*=5.0 Hz, 1H), 6.26 (s(br), 1H) 6.61 (s, 2H), 6.82-6.99 (m, 3H), 7.20-7.30 (m, 3H), 7.50 (d, *J*=2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): 55.8, 60.5, 62.1, 60.5, 80.4, 94.8, 115.1, 116.1, 119.3, 122.2, 129.1, 129.3, 132.0, 141.1, 141.8, 150.4, 151.1, 153.3, 155.9, 161.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}N_2O_9 + Na^+$ $[M + Na]^+$: 519.1374; found: 519.1387.

***Cis*-4-(5-Hydroxy-4-methoxy-2-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (86)** was obtained from **73** according to general method IV. White solid; yield 23%; mp 188 ºC. IR (KBr): $\tilde{\nu}$ = 1757 cm^{-1} (s; *v*(C=O)), 3355 cm^{-1} (s; *v*(OH)). ^{1}H NMR (DMSO-d_6): δ 3.65 (s, 3H), 3.72 (s, 6H), 3.89 (s, 3H), 6.02 (d, *J*=5.3 Hz,

1H), 6.27 (d, *J*=5.3 Hz, 1H), 6.79 (s, 2H), 6.84-7.01 (m, 4H), 7.26 (d, *J*=7.7, 2H), 7.80 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 56.0, 56.1, 60.2, 59.4, 81.8, 95.3, 109.3, 114.1, 116.0, 122.3, 125.7, 129.5, 133.1, 134.5, 139.1, 146.9, 152.4, 153.4, 157.2, 164.1. HRMS (ESI): *m/z* calcd for $C_{25}H_{24}N_2O_9 + Na^+$ $[M + Na]^+$: 519.1374; found: 519.1388.

General method V. Reduction of nitro aryl azetidin-2-ones

Acetic acid (15 mL) was added to β-lactam (0.25 mmol) and zinc powder 10 μm (2.5 mmol) at rt under nitrogen and stirred for 7 days. The reaction mixture was filtered through celite, the solvent was removed from the filtrate *in vacuo* and the resulting residue was purified through by flash column chromatography over silica gel (eluent: dichloromethane).

***Cis*-4-(2-Amino-3-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (87)** was obtained from **84** according to general method V. Brown solid; yield 88%; mp 188 ºC. IR (KBr): $\tilde{\nu}$ = 1756 cm^{-1} (s; *v*(C=O)), 3359 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 2.56 (br s, 2H), 3.74 (s, 9H), 3.78 (s, 3H), 4.91 (s, 3H), 5.24 (d, *J*=5.0 Hz, 1H), 5.54 (d, *J*=5.0 Hz, 1H), 6.59 (s, 2H), 6.79-6.84 (m, 3H), 7.19-7.28 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 55.5, 55.8, 59.1, 56.2, 80.1, 94.8, 113.2, 115.3, 118.2, 121.4, 129.3, 131.2, 120.9, 128.8, 138.0, 138.2, 147.2, 149.0, 156.3, 163.0. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}N_2O_7 + Na^+$ $[M + Na]^+$: 489.1632; found: 489.1646.

***Cis*-4-(3-Amino-5-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (88)** was obtained from **85** according to general method V. Brown solid; yield 91%; mp: 182 ºC. IR (KBr): $\tilde{\nu}$ = 1752 cm^{-1} (s; *v*(C=O)), 3468 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 2.77 (s(br), 2H), 3.74 (s, 6H), 3.76 (s, 3H), 3.80 (s, 3H), 5.17 (d, *J*=5.3 Hz, 1H), 5.52 (d, *J*=5.3 Hz, 1H), 6.39 (d, *J*=2 Hz, 1H), 6.43 (d, *J*=2 Hz, 1H), 6.66 (s, 2H), 6.86-7.21 (m, 5H)**.** ^{13}C NMR ($CDCl_3$): δ 55.3, 58.5, 59.9, 60.2, 80.0, 94.9, 106.9, 107.6, 114.9, 118.9,

127.3, 128.2, 128.5, 129.5, 131.3, 137.3, 151.0, 154.1, 154.3, 161.5. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}N_2O_7 + Na^+$ $[M + Na]^+$: 489.1632; found: 489.1622.

***Cis*-4-(2-Amino-5-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (89)** was obtained from **86** according to general method V. Brown solid; yield 73%; mp 210 ºC. IR (KBr): $\tilde{\nu}$ = 1756 cm^{-1} (s; *v*(C=O)), 3447 cm^{-1} (s; *v*(OH)). ^1H NMR ($CDCl_3$): δ 3.71 (s, 3H), 3.74 (s, 6H), 3.81 (s, 3H), 5.21 (d, *J*=4.9 Hz, 1H), 5.57 (d, *J*=4.9 Hz, 1H), 5.63 (s, 1H), 6.61 (s, 2H), 6.72-6.81 (m, 4H), 7.21-7.27 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 56.0, 56.1, 60.0, 58.8, 80.2, 95.4, 99.5, 113.8, 117.2, 122.2, 125.2, 125.9, 128.9, 136.3, 137.2, 141.4, 146.1, 151.3, 157.6, 164.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}N_2O_7 + Na^+$ $[M + Na]^+$: 489.1632; found: 489.1646.

***Cis*-4-(3-Amino-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90a)** was obtained from **61a** according to general method V. Pale brown solid; yield 83%; mp 158 ºC. IR (KBr): $\tilde{\nu}$ = 1754 cm^{-1} (s; *v*(C=O)), 3368 cm^{-1} (s; *v*(NH_2)). ^1H NMR ($CDCl_3$): δ 3.74 (s, 6H), 3.78 (s, 3H), 3.81 (s, 3H), 5.23 (d, *J*=5.1 Hz, 1H), 5.49 (d, *J*=4.4 Hz, 1H), 6.65-δ6.98 (m, 8H), 7.22-7.27 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 54.9, 55.6, 60.5, 61.9, 80.8, 94.9, 109.4, 113.7, 115.5, 118.3, 121.7, 124.5, 128.8, 132.8, 134.5, 135.8, 147.2, 153.0, 156.8, 162.7. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}N_2O_6 + Na^+$ $[M + Na]^+$: 473.1683; found: 473.1685.

***Trans*-4-(3-Amino-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90b)** was obtained from **61b** according to general method V. Pale brown solid; yield 94%; mp 128-130 ºC. IR (KBr): $\tilde{\nu}$ = 1756 cm^{-1} (s; *v*(C=O)), 3465 cm^{-1} (s; *v*(NH_2)). ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H), 3.78, (s, 3H), 3.90 (s, 3H), 3.93 (s(br), 2H), 4.85 (d, *J*=1.5 Hz, 1H), 5.12 (d, *J*=1.5 Hz, 1H), 6.62 (s, 2H), 6.71- 6.81 (m, 5H), 7.08 (dd, *J*=2.1 Hz, 8.2 Hz, 1H), 7.24-7.29 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 55.1, 55.6, 60.5, 63.9, 86.6, 94.8, 110.1, 111.3,

114.9, 116.5, 121.7, 127.5, 129.2, 132.9, 134.3, 136.7, 147.4, 152.9, 156.6, 162.2. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}N_2O_6 + Na^+$ $[M + Na]^+$: 473.1683; found: 473.1681.

***Cis*-4-(3-Aminophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (91)** was obtained from **62** according to general method V. Brown solid; yield 87%; mp 184 °C. IR (KBr): $\tilde{\nu}$ = 1752 cm^{-1} (s; ν(C=O)), 3453 cm^{-1} (s; $\nu(NH_2)$). ^1H NMR ($CDCl_3$): δ 3.74 (s, 9H), 3.80 (s, 3H), 5.32 (d, J=5.0 Hz, 1H), 5.58 (d, J=5.0 Hz, 1H), 6.64 (s, 2H), 6.82-7.23 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 55.3, 56.1, 60.9, 62.3, 81.1, 95.3, 113.7, 114.4, 115.7, 120.6, 122.3, 129.3, 129.5, 133.1, 134.3, 134.9, 153.5, 156.9, 159.6, 162.9. HRMS (ESI): *m/z* calcd for $C_{24}H_{24}N_2O_5 + Na^+$ $[M + Na]^+$: 443.1577; found: 443.1597.

***Cis*-4-(4-Aminophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (92)** was obtained from **63** according to general method V. Brown solid, yield 79%; mp 172 °C. IR (KBr): $\tilde{\nu}$ = 1753 cm^{-1} (s; ν(C=O)), 3362 cm^{-1} (s; $\nu(NH_2)$). ^1H NMR ($CDCl_3$): 1.7 (s(br), 2H), 3.75 (s, 3H), 3.79 (s, 3H), 5.28 (d, *J*=5.0 Hz, 1H), 5.52 (d, *J*=5.0 Hz, 1H), 6.61 (s, 2H) 7.07-7.21 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 55.3, 56.0, 60.8, 61.1, 81.8, 94.3, 112.7, 114.9, 123.5, 124.3, 119.4, 129.5, 133.8, 134.9, 153.5, 157.3, 160.0, 164.0. HRMS (ESI): *m/z* calcd for $C_{24}H_{24}N_2O_5 + Na^+$ $[M + Na]^+$: 443.1577; found: 443.1576.

***Cis*-4-(3-Hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (95). (3,4,5-Trimethoxyphenoxy)acetic acid (93).** To a stirring solution of the 3,4,5-trimethoxyphenol (25 mmol) and chloroacetic acid (25 mmol) in dry DMF at 0 °C under nitrogen was added 60% NaH (55 mmol) in 6 equal portions. The reaction was stirred at rt for 2 days, after which the solvent was removed and water was added (50 mL). The aqueous layer was washed with diethyl ether (60 mL), acidified to pH 1 with conc. HCl and extracted with diethyl ether (50 mL×4). The organic extracts were dried over Na_2SO_4 and solvent removed *in vacuo* to yield a brown solid; yield 48%.[52] IR (KBr): $\tilde{\nu}$ =

1758 cm^{-1} (s; *v*(C=O)), 3522 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.79 (s, 3H), 3.87 (s, 6H), 4.66 (s, 2H), 6.19 (s, 2H), 7.28 (s(br), 1H). ^{13}C NMR ($CDCl_3$): δ 55.7, 60.6, 64.8, 77.0, 92.1, 132.5, 153.3, 153.7, 173.0. HRMS (ESI): *m/z* calcd for $C_{11}H_{14}O_6 + H^+$ $[M + H]^+$: 243.0863; found: 243.0873.

A solution of **93** (1 mmol) and triphosgene (0.5 mmol) in dry CH_2Cl_2 (10 mL) was heated at reflux under N_2. After 30 min, triethylamine (1.5 mmol) was added, followed by the addition of **40** (0.5 mmol) dissolved in dry CH_2Cl_2 (15 mL) dropwise over 30 min. The reaction was refluxed for a further 6 h, cooled to rt, washed with H_2O (20 mL), saturated $NaHCO_3$ (20 mL×2), and brine (10 mL), before being dried over $MgSO_4$, filtered, and solvent removed *in vacuo*. 4-(3-((tert-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one **94** was isolated by flash chromatography over silica gel (eluent: 1:1, *n*-hexane-ethyl acetate) and was deprotected according to general method IV to yield **95**. Colourless solid; yield 36%; mp 134-136 °C. IR (KBr): $\tilde{\nu}$ = 1745 cm^{-1} (s; *v*(C=O)), 3431 cm^{-1} (s; *v*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.71-3.91 (m, 21H), 5.28 (d, *J*=5.5 Hz, 1H), 5.56 (d, *J*=5.5 Hz, 1H), 5.77 (s, 2H) 6.01 (s, 2H), 6.32-6.39 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 55.7, 56.0, 60.5, 60.7, 61.9, 64.8, 61.4, 81.5, 92.3, 97.5, 113.3, 114.6, 119.9, 122.3, 127.4, 129.9, 133.9, 139.8, 141.6, 146.4, 152.6, 158.5, 168.9. HRMS (ESI): *m/z* calcd for $C_{28}H_{31}NO_{10} + Na^+$ $[M + Na]^+$: 564.1840; found: 564.1859.

2-Methoxy-5-[3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl]phenol (96). DIBAL-H (1M soln., 1.0 mmol) was added to a solution of β-lactam **78a** (0.25 mmol) in anhydrous THF (3 mL) under nitrogen. The reaction mixture was refluxed for 2 h, cooled to rt, and partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The aqueous layer was further extracted with CH_2Cl_2 (10 mL), the combined organic layers were dried over Na_2SO_4 and solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel

(eluent: 1:1, ethyl acetate:*n*-hexane) to afford **96** as a brown oil. Yield 27%. IR (NaCl): $\tilde{\nu}$ = 1598 (C=O), 3412 cm^{-1} (s; *ν*(OH)). ^{1}H NMR ($CDCl_3$): δ 3.52 (m, 2H), 3.72 (s, 6H), 3.78 (s, 3H), 3.83 (s, 3H), 4.79 (d, *J*=5.4 Hz, 1H), 5.29 (m, 1H), 6.02 (s, 2H), 6.88-6.98 (m, 6H), 7.22-7.27 (m, 1H)**.** ^{13}C NMR ($CDCl_3$): δ 53.1, 55.3, 56.1, 61.2, 69.5, 76.4, 98.7, 113.8, 115.2, 116.3, 119.9, 120.9, 125.1, 129.1, 131.3, 141.3, 142.4, 143.6, 150.6, 156.8. HRMS (ESI): *m/z* calcd for $C_{25}H_{27}NO_6 + Na^+$ $[M + Na]^+$: 460.1731; found: 460.1721.

4-(3-Hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidine-2-thione (97). A solution of β-lactam **78a** (0.25 mmol) and Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione) (0.275 mmol) in dry toluene (3 mL) under nitrogen was refluxed for 12 h and stirred at rt overnight. The solvent was removed from the flask using a stream of nitrogen and gentle heating. The resulting solid was purified by flash chromatography over silica gel (elutent: 1:1, *n*-hexane:ethyl acetate). The product was washed with *n*-hexane to remove traces of odour from Lawesson's reagent and **97** was obtained as a pale yellow solid. Yield 63%; mp 110 °C. IR (KBr): $\tilde{\nu}$ = 3336 cm^{-1} (s; *ν*(OH)); 1592 cm^{-1} (s; *ν*(C=S)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6H), 3.81 (s, 3H), 3.86 (s, 3H), 5.32 (d, *J*=4.0 Hz, 1H), 5.61 (s(br), 1H), 5.78 (d, *J*=4.0 Hz, 1H), 6.77 (s, 2), 6.91-7.28 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 53.2, 54.6, 59.9, 69.8, 97.5, 99.7, 111.7, 114.9, 115.7, 119.7, 121.0, 128.4, 131.2, 133.4, 137.7, 141.3, 146.8, 149.7, 157.8, 198.9. HRMS (ESI): *m/z* calcd for $C_{25}H_{25}NO_6S + Na^+$ $[M + Na]^+$: 490.1300; found: 490.1293.

***Cis*-4-{4-[Bis-(2-chloroethyl)amino]phenyl}butyric acid 2-methoxy-4-[4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl]phenyl ester (98).** Chlorambucil (0.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.7 mmol) and DMAP (0.07 mmol) were dissolved in dry CH_2Cl_2 (35 mL) under nitrogen and stirred at rt for 10 min. β-Lactam **78a** (0.5 mmol) in dry CH_2Cl_2 (6 mL) was added dropwise under nitrogen and the reaction

stirred under nitrogen overnight. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (100 mL), saturated $NaHCO_3$ aq. solution (100 mL) and water (100 mL), dried over Na_2SO_4, and the solvent removed *in vacuo*. Chromatography of the residue over silica gel (eluent: 1:1, ethyl acetate: hexane) yielded **98** as an amber oil; yield 53%. IR (NaCl): $\tilde{\nu}$ = 1270 cm^{-1} (s; *v*(CO)), 1760 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.00-2.07 (qt, *J*=7.5 Hz, 2H), 2.58 (t, *J*=7.7 Hz, 2H), 2.66 (t, *J*=7.7 Hz, 2H), 3.63-3.67 (m, 4H), 3.71-3.76 (m, 10H), 3.80 (s, 3H), 3.82 (s, 3H), 5.32 (d, *J*=5.7 Hz, 1H), 5.57 (d, *J*=5.4 Hz, 1H), 6.64-7.28 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 26.4, 32.8, 33.3, 40.1, 53.0, 53.1, 55.4, 55.7, 60.5, 61.3, 80.7, 94.8, 111.7, 115.4, 121.9, 122.6, 124.6, 126.1, 128.9, 129.3, 129.3, 130.0, 132.6, 134.5, 139.3, 143.9, 151.1, 153.1, 156.5, 162.5, 170.8. HRMS (ESI): *m/z* calcd for $C_{39}H_{42}N_2O_8Cl_2$ + Na^+ $[M + Na]^+$: 759.2210; found: 759.2203.

***Cis*-4-{4-[Bis-(2-chloroethyl)amino]phenyl}-N-{2-methoxy-5-[4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl]phenyl}butyramide (99).** Following procedure for synthesis of **98** (above), a solution of **90a** (0.5 mmol) in dry CH_2Cl_2 (6 mL) was added to a solution of chlorambucil (0.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.7 mmol) and DMAP (0.07 mmol) dissolved in dry CH_2Cl_2 (35 mL). The product was obtained as an amber oil; yield 38%. IR (NaCl): $\tilde{\nu}$ = 1686 cm^{-1} (s; *v*(C=O)), 1755 (s; *v*(C=O)) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.04 (q, *J*=6.3 Hz, 13.7 Hz, 2H), 2.41 (dd, *J*=7.5 Hz, 15.2 Hz, 2H), 2.64 (t, *J*=7.6, 14.8 Hz, 2H), 3.63-3.77 (m, 10H), 3.79 (s, 3H), 3.88 (s, 3H), 5.38 (d, *J*=4.9 Hz, 1H), 5.54 (d, *J*=4.5 Hz, 1H), 6.66-6.85 (m, 8H), 7.11-7.20 (m, 5H), 7.72 (s, 1H), 8.59 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 26.6, 33.5, 36.6, 40.0, 53.1, 53.1, 55.3, 55.7, 60.5, 61.73 (C_4), 80.8, 94.8, 109.5, 111.7, 115.4, 119.8, 121.7, 127.0, 128.9, 129.3, 130.0, 132.5, 132.66, 134.4, 143.9, 144.1, 147.2, 151.2, 153.8, 156.8, 162.6, 170.5. HRMS (ESI): *m/z* calcd for $C_{39}H_{43}N_3O_7Cl_2$ + Na^+ $[M + Na]^+$: 758.2370; found: 758.2350.

General method VI: Preparation of Dibenzyl Phosphate Esters

Carbon tetrachloride (85 mmol) was added to a solution of the appropriate phenolic β-lactam (17 mmol) in acetonitrile (100 mL) at 0 ºC. The resulting solution was stirred for 10 min prior to addition of diisopropylethylamine (35 mmol) and DMAP (1.7 mmol), followed by dropwise addition of dibenzyl phosphite (24.5 mmol). Upon completion as indicated by TLC, KH_2PO_4 (0.5 M, aq.) was added and the mixture was allowed to warm to rt. Combined ethyl acetate extracts (3×50 mL) were washed with brine (100 mL, aq.), water (100 mL) and dried using anhydrous Na_2SO_4. The solvent was reduced *in vacuo* and the product was isolated by flash column chromatography over silica gel (eluent: *n*-hexane: ethyl acetate gradient).

***Cis*-(2-Methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) dibenzyl phosphate (104a)** was prepared from **78a** according to general method VI. Brown oil; yield 86%. IR (NaCl): $\tilde{\nu}$ = 1755 cm^{-1} (s; *v*(C=O)), 1298 cm^{-1} (s; *v*(P=O)). ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H), 3.74 (s, 3H), 3.77 (s, 3H), 5.04-5.12 (m, 4H), 5.27 (d, *J* = 5.0 Hz, 1H), 5.52 (d, *J* = 4.5 Hz, 1H), 6.61 (s, 2H), 6.83-7.33 (m, 18H). ^{13}C NMR ($CDCl_3$): δ 55.3, 55.7, 60.3, 61.3, 69.5, 69.6, 80.3, 112.9, 115.6, 120.9, 121.4, 124.4, 123.7, 127.4, 128.8, 128.9, 132.4, 134.3, 136.8, 137.7, 137.3, 147.5, 153.9, 155.9, 162.8. HRMS (ESI): *m/z* calcd for $C_{39}H_{38}NO_{10}P + Na^+$ $[M + Na]^+$: 734.2126; found: 734.2152.

***Trans*-(2-Methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) dibenzyl phosphate (104b)** was prepared from **78b** according to general method VI. Yellow oil; yield 9%. IR (NaCl): $\tilde{\nu}$ = 1758 cm^{-1} (s; *v*(C=O)), 1298 cm^{-1} (s; *v*(P=O)). ^1H NMR ($CDCl_3$): δ 3.74 (s, 6H), 3.79 (s, 3H), 3.95 (s, 3H), 4.90 (s(br), 1H), 5.02-5.04 (m, 4H), 5.13 (s(br), 1H), 6.60 (s, 2H), 6.88-7.34 (m, 18H). HRMS (ESI): *m/z* calcd for $C_{39}H_{38}NO_{10}P + Na^+$ $[M + Na]^+$: 734.2126; found: 734.2122.

General Method VII: Deprotection of Dibenzyl Phosphate Esters

The dibenzyphosphate ester protected compound (2 mmol) was dissolved in ethanol: ethyl acetate (50 mL; 1:1 mixture) and hydrogenated over 10% palladium on carbon (1.2g) until reaction was complete on TLC (typically less than 3 h). The catalyst was removed by filtrataion through Celite, the solvent was reduced *in vacuo* and the product was isolated by flash column chromatography over silica gel (eluent: *n*-hexane: ethyl acetate gradient).

***Cis*-2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl dihydrogen phosphate (105a)** was prepared from **104a** according to general method VII. Colourless solid, yield 21%, mp 189-190 ˚C. IR (KBr): $\tilde{\nu}$ = 3478 cm^{-1} (s; *v*(OH)), 1755 cm^{-1} (s; *v*(C=O)), 1312 cm^{-1} (s; *v*(P=O)). ^{1}H NMR ($CDCl_3$): δ 3.48 (s, 3H), 3.64 (s, 6H), 3.69 (s, 3H), 5.26 (d, *J* = 4.0 Hz, 1H), 5.44 (d, *J* = 4.0 Hz, 1H), 6.56 (s, 2H), 6.65-7.12 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 55.2, 55.6, 60.4, 61.1, 80.3, 94.9, 112.0, 115.1, 121.1, 121.8, 124.7, 128.9, 132.4, 133.6, 133.9, 134.3, 149.9, 152.9, 156.3, 162.8. HRMS (ESI): *m/z* calcd for $C_{25}H_{26}NO_{10}P + Na^+$ $[M + Na]^+$: 554.1187; found: 554.1171.

***Trans*-2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl dihydrogen phosphate (105b)** was prepared from **104b** according to general method VII. Yellow oil; yield 47%. IR (NaCl): $\tilde{\nu}$ = 3425 cm^{-1} (s; *v*(OH)), 1756 cm^{-1} (s; *v*(C=O)), 1300 cm^{-1} (s; *v*(P=O)). ^{1}H NMR ($CDCl_3$): δ 3.73 (s, 3H), 3.79 (s, 6H), 3.81 (s, 3H), 4.90 (br s, 1H), 5.12 (br s, 1), 6.59 (s, 2H), 6.73-7.02 (m, 8H). HRMS (ESI): *m/z* calcd for $C_{25}H_{26}NO_{10}P + Na^+$ $[M + Na]^+$: 554.1187; found: 554.1160.

General Method VIII: Preparation of Fmoc-protected azetidin-2-ones

DCC (5.7 mmol), Fmoc-protected amino acid (5.6 mmol) and $HOBt.H_2O$ (7.3 mmol) were added to a stirred solution of amino β-lactam (4.76 mmol) in anhydrous DMF (30 mL) at rt. After 24 h, ethyl acetate (50 mL) was added and the mixture was filtered. DMF was removed by washing with water (5×50 mL). The organic layer containing the product was reduced *in*

vacuo, and the product was isolated by flash column chromatography over silica gel (eluent: dichloromethane: methanol gradient).

***Cis*-(1-((2-Methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl) phenyl)amino)-1-oxopropan-2-yl) carbamaic acid 9*H*-fluoren-9-ylmethyl ester (100a)** was prepared from **90a** and and Fmoc-protected alanine according to general method VIII. Yellow solid; yield 22%; mp 186-187 ˚C. IR (KBr): $\tilde{\nu}$ = 3331 cm^{-1} (s; *v*(NH)), 1757 cm^{-1} (s; *v*(C=O)), 1696 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 1.50 (d, *J* = 6.5 Hz, 3H), 3.76 (s, 6H), 3.78 (s, 3H), 3.80 (s, 3H), 4.25 (t, *J* = 7.0 Hz, 1H), 4.42-4.48 (m, 3H), 5.37 (d, *J* = 5.0 Hz, 1H), 5.54 (d, *J* = 3.5 Hz, 1H), 6.67 (s, 2H), 6.82-7.79 (m, 16H), 8.26 (br s, 1H), 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 13.9, 46.6, 49.1, 55.3, 55.7, 60.5, 61.6, 66.7, 80.7, 94.9, 109.6, 115.3, 119.6, 121.7, 123.1, 123.3, 124.5, 126.6, 127.3, 128.9, 132.6, 134.4, 140.9, 143.2, 147.6, 153.1, 156.6, 157.5, 162.6, 169.8. HRMS (ESI): *m/z* calcd for $C_{43}H_{41}N_3O_9 + Na^+$ $[M + Na]^+$: 766.2735; found: 766.2746.

***Trans*-(1-((2-Methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl) phenyl)amino)-1-oxopropan-2-yl)carbamaic acid 9*H*-fluoren-9-ylmethyl ester (100b)** was prepared from **90b** and and Fmoc-protected alanine according to general method VIII. Yellow oil; yield 79%. IR (NaCl): $\tilde{\nu}$ = 3324 cm^{-1} (s; *v*(NH)), 1759 cm^{-1} (s; *v*(C=O)), 1597 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$): δ 1.52 (d, *J* = 6.5 Hz, 3H), 3.78 (s, 6H), 3.83 (s, 3H), 3.87 (s, 3H), 4.25 (t, *J* = 6.5 Hz, 1H), 4.46 (m, 3H), 4.97 (s(br), 1H), 5.37 (s(br), 1H), 6.67 (s, 2H), 6.91-7.79 (m, 16H), 8.36 (s(br), 1H), 8.53 (s, 1H). HRMS (ESI): *m/z* calcd for $C_{43}H_{41}N_3O_9 + Na^+$ $[M + Na]^+$: 766.2735; found: 766.2762.

***Cis*-(2-((2-Methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl) phenyl)amino)-2-oxoethyl) carbamic acid 9*H*-fluoren-9-ylmethyl ester (101)** was prepared from **90a** and and Fmoc-protected glycine according to general method VIII. Colourless solid; yield 61%; mp 200-201˚C. IR (KBr): $\tilde{\nu}$ = 3331 cm^{-1} (s; *v*(NH)), 1755 cm^{-1}

(s; ν(C=O)), 1623 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6H), 3.79 (s, 3H), 3.79 (s, 3H), 4.05-4.07 (m, 2H), 4.27 (t, J = 7.0 Hz, 1H), 4.47-4.49 (m, 2H), 5.37 (d, J = 4.5 Hz, 1H), 5.54 (d, J = 4.5 Hz, 1H), 6.66 (s, 2H), 6.82-7.80 (m, 16H), 8.20 (s(br), 1H), 8.51 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 45.0, 46.6, 55.3, 55.7, 60.5, 61.6, 66.9, 80.7, 94.8, 109.7, 115.3, 119.6, 119.7, 121.8, 124.5, 124.6, 126.6, 127.4, 128.9, 130.4, 132.6, 134.2, 140.9, 143.2, 152.6, 153.1, 156.8, 157.3, 162.6, 165.2. HRMS (ESI): *m/z* calcd for $C_{42}H_{39}N_3O_9 + Na^+$ $[M + Na]^+$: 752.2579; found: 752.2583.

General Method IX: Removal of Fmoc-protecting group

To the Fmoc-protected β-lactam dissolved in CH_2Cl_2 (minimum volume) was added 1-octanethiol (10 equiv.) and TBAF (2 equiv). The mixture was stirred at rt until reaction was complete on TLC, typically within 10 min. The solvent was reduced *in vacuo* and the product was isolated by flash column chromatography over silica gel (eluent: dichlormethane: methanol gradient) to afford the product.

***Cis*-2-Amino-*N*-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl)propanamide (102a)** was obtained from **100a** according to general method IX. Yellow oil; yield 69%. IR (NaCl): $\tilde{\nu}$ = 3334 cm^{-1} (s; ν(NH)), 1756 cm^{-1} (s; ν(C=O)), 1698 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 1.27-1.28 (m, 3H), 3.75 (s, 6H), 3.78 (s, 3H), 3.78 (m, 1H), 3.86 (s(br), 3H), 5.38 (d, J = 4.7 Hz, 1H), 5.53 (d, J = 4.7 Hz, 1H), 6.67 (s, 2H), 6.83-7.21 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 13.75, 50.1, 55.3, 55.7, 60.5, 61.7, 80.7, 94.8, 109.6, 115.4, 119.7, 121.7, 122.7, 122.8, 128.9, 132.6, 133.6, 148.0, 153.1, 156.6, 162.7, 167.1. HRMS (ESI): *m/z* calcd for $C_{28}H_{31}N_3O_7 - H^+$ $[M - H]^+$: 520.2084; found: 520.2090.

***Trans*-2-Amino-*N*-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl)propanamide (102b)** was obtained from **100b** according to general method IX. Yellow oil; yield 34%. IR (NaCl): $\tilde{\nu}$ = 3305 cm^{-1} (s; ν(NH)), 1724 cm^{-1} (s; ν(C=O)), 1687 cm^{-1}

^{1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 1.26-1.28 (m, 3H), 3.66 (s, 6), 3.70 (s, 3H), 3.79 (s, 3H), 3.81-3.84 (m, 1H), 5.02 (s(br), 1H), 5.12 (s(br), 1H), 6.68 (s, 2H), 6.79-6.99 (m, 8H). HRMS (ESI): *m/z* calcd for $C_{28}H_{31}N_3O_7 + Na^+$ $[M + Na]^+$: 544.2054; found: 544.2043.

***Cis*-2-Amino-*N*-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)-4-oxo-3-phenoxyazetidin-2-yl)phenyl)acetamide (103)** was obtained from **101** according to general method IX. Yellow oil; yield 61%. IR (NaCl): $\tilde{\nu}$ = 3330 cm^{-1} (s; ν(NH)), 1754 cm^{-1} (s; ν(C=O)), 1689 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$): δ 3.66 (s, 6H), 3.70 (s, 3H), 3.79 (s, 3H), 3.82-3.83 (m, 2H), 5.37 (d, J = 5.0 Hz, 1H), 5.51 (d, J = 5.0 Hz, 1H), 6.66 (s, 2H), 6.78-7.17 (m, 8H). ^{13}C NMR (100MHz, $CDCl_3$): δ 46.7, 55.4, 55.7, 60.5, 61.3, 80.5, 94.9, 109.8, 115.3, 119.1, 121.7, 124.7, 128.9, 132.6, 133.5, 133.9, 145.6, 153.0, 154.6, 160.4, 170.1. HRMS (ESI): *m/z* calcd for $C_{27}H_{29}N_3O_7 + H^+$ $[M + H]^+$: 508.2078; found: 508.2075.

Biochemical Evaluation of Activity

Antiproliferative MTT assay. All assays were performed in triplicate for the determination of mean values reported. The human breast tumour cell line MCF-7 was cultured in Eagles minimum essential medium in a 95%O_2/5% CO_2 atmosphere with 10% fetal bovine serum, 2 mM L-glutamine and 100 µg/mL penicillin/streptomycin. The medium was supplemented with 1% non-essential amino acids. MDA-MB-231 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 2mM L-glutamine and 100 µg/mL penicillin/streptomycin (complete medium). Cells were seeded at a density of 2.5 x 10^4 cells/mL in a 96-well plate and incubated at 37 ^{o}C, 95% O_2/5% CO_2 atmosphere for 24 h. After this time they were treated with 2 µL volumes of test compound which had been pre-prepared as stock solutions in ethanol to furnish the concentration range of study, 1 nM–100 µM, and re-incubated for a further 72 h. Control wells contained the equivalent volume of the vehicle ethanol (1% v/v). The culture medium was then removed

and the cells washed with 100 µL phosphate buffered saline (PBS) and 50 µL MTT added, to reach a final concentration of 1 mg/mL MTT added. Cells were incubated for 2 h in darkness at 37 ^{o}C. At this point solubilization was begun through the addition of 200 µL DMSO and the cells maintained at rt in darkness for 20 min to ensure thorough colour diffusion before reading the absorbance. The absorbance value of control cells (no added compound) was set to 100% cell viability and from this graphs of absorbance versus cell density per well were prepared to assess cell viability using GraphPad Prism software.

Cytotoxicity. Cytotoxicity was determined using the CytoTox 96 non-radioactive cytotoxicity assay by Promega following the manufacturer's protocol. The assay quantitatively measures lactate dehydrogenase (LDH) a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatant is measured in a 30 minute coupled enzymatic assay, which results in the conversion of a tetrazolium salt (INT) into a red formazan product. MCF-7 cells were seeded in 96-well plates, incubated for 24 hours and then treated with compounds as described for the MTT antiproliferation assay above. After 72 hours, 20 µL of lysis solution (10X) was added to the 'blank' wells and left for 1 hour to ensure 100% death. 50 µL was removed from each well and transferred to a new 96-well plate. 50 µL of substrate mix from the LDH assay kit was added and the plate was incubated in the dark at rt for 30 minutes. After this period, 50 µL of stop solution was added to each well before reading the absorbance at a wavelength of 490 nm using a Dynatech MR5000 plate reader. Percentage cell death was calculated at 10 µM.

Immunofluorescence Microscopy. Confocal microscopy was used to study the effects of drug treatment on MCF-7 cytoskeleton. For immunofluorescence, MCF-7 cells were seeded at 1×10^5 cells/ml on eight chamber glass slides (BD Biosciences). Cells were either untreated or treated with vehicle [1 % ethanol (v/v)], paclitaxel (1 µM), **2** (100 nM) or **90b** (100 nM) for 16 h. Following treatment cells were gently washed in PBS, fixed for 20 min

with 4 % paraformaldehyde in PBS and permeabilised in 0.5 % Triton X-100. Following washes in PBS containing 0.1 % Tween (PBST), cells were blocked in 5 % bovine serum albumin diluted in PBST. Cells were then incubated with mouse monoclonal anti-α-tubulin−FITC antibody (clone DM1A) (Sigma) (1:100) for 2 hr at rt. Following washes in PBST, cells were incubated with Alexa Fluor 488 dye (1:450) for 1 hr at rt. Following washes in PBST, the cells were mounted in Ultra Cruz Mounting Media (Santa Cruz Biotechnology, Santa Cruz, CA) containing 4,6-diamino-2-phenolindol dihydrochloride (DAPI). Images were captured by Leica SP8 confocal microscopy with Leica application suite X software. All images in each experiment were collected on the same day using identical parameters. Experiments were performed on three independent occasions.

Tubulin Polymerization. Tubulin polymerisation was carried out using an assay kit supplied by Cytoskeleton [Tubulin polymerization HTS assay using >97% pure porcine tubulin, OD-based (BK004P)], based on the principal that light is scattered by microtubules to an extent that is proportional to the concentration of the microtubule polymer. Compounds that interact with tubulin alter its polymerisation, and this can be detected using a spectrophotometer. The absorbance at 340 nm at 37°C is monitored. The assay was performed as described in version 2.2 of the tubulin polymerisation assay kit manual.[53]

Colchicine-Binding Site Assay. MCF-7 cells were seeded at a density of 5×10^4 cells/well in 6-well plates and incubated overnight. Cells were treated with vehicle control [ethanol (0.1 % v/v)], **1** or compound **90b** (both 10 μM) for 2 h. After this time, selected wells were treated with *N,N'*-ethylene-bis(iodoacetamide)(EBI)(Santa Cruz Biotechnology) for 1.5 h. Following treatment, cells were twice-washed with ice-cold PBS and lysed by addition of Laemmli buffer. Samples were separated by SDS-PAGE, trasnsferred to polyvinylidene difluoride membranes and probed with β-tubulin antibodies (Sigma-Aldrich).[42]

Stability Studies. Analytical HPLC stability studies were performed using a Waters In-Line Degasser AF and a Waters 717plus Autosampler. The column used was a hypersil Gold PFP C8 1.9µm (150×4.6 mm) reverse phase chromatography column. Samples were detected using a wavelength of 254 nm. All samples were analysed using acetonitrile: water (50:50) over 10 min and a flow rate of 1 ml/min. Stock solutions are prepared by dissolving 10 mg of compound in 10 ml of mobile phase. Phosphate buffers at the desired pH values (4, 7.4, and 9) were prepared in accordance with the British Pharmacopeia monograph 2010. 200µL of stock solution was diluted with 9.8 mL of appropriate phosphate buffer, shaken and injected immediately. Samples were withdrawn and analysed at t=0 min, and every 3 hours until 24 hours.

Computational Procedure. For ligand preparation, all compounds were built using ACD/Chemsketch v10 to generate SMILES. A single conformer from each string was generated using Corina v3.4 and ensuring Omega v2.2.1 was subsequently employed to generate a maximum of 1000 conformations of each compound. For the receptor preparation, PDB entry 1SA0 was downloaded from the Protein Data Bank (PDB).[6] All waters were retained in both isoforms. Addition and optimisation of hydrogen positions for these waters was carried out using MOE 2011.10 ensuring all other atom positions remained fixed.[54] Using the reported X-ray structure of tubulin co-crystallised with a colchicine derivative, DAMA-colchicine (PDB entry – 1SA0), possible ligand binding orientations were probed with the docking program FREDv2.2.3 (Openeye Scientific Software). Docking was carried out using FREDv2.2.3 in conjunction with Chemgauss3, PLP Scoring function. 3-D ligand conformations were enumerated using CORINAv3.4 (Molecular Networks GMBH) for ligands followed by generation of multiple conformations using OMEGAv2.2.1 (Openeye Scientific Software). Each conformation was subsequently docked and scored with Chemgauss3 PLP as outlined previously. The top binding poses were refined using the LigX

procedure (MOE 2011.10) together with Postdock analysis (SVL script; MOE) of the docked ligand poses.

X-Ray Crystallography. The data sets for all the crystal samples were collected on a Bruker Smart Apex Diffractometer. Suitable crystals from each sample were selected and mounted on a glass fiber tip and placed on the goniometer head in a 123 K N2 gas stream. Each data set was collected using Bruker Smart Version 5.625 software run in multirun mode and 2400 image frames, of 0.3° per frame, were recorded. Data integration and reduction were carried out using Bruker* Saint+ Version 6.45 software and corrected for absorption and polarization effects using Sadabs Version 2.10 software. Space group determinations, structure solutions and refinements were obtained using Bruker Shelxtl Ver. 6.14 software. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms (excluding water) were assigned to calculated positions using a riding model with appropriately fixed isotropic thermal parameters.

***Crystal Data for* 49:** $C_{27}H_{29}NO_8$, M = 495.51, Triclinic, a = 8.9118(5), b = 11.7367(7), c = 12.2126(7) Å, α = 90.8580(10)°, β = 90.4980(10)°, γ = 100.6200(10)°, U = 1255.27(13)Å^3, T = 150 K, space group P-1, Z = 2, μ (Mo Kα) = 0.097 mm^{-1}, ρ = 1.311Mg/cm^3, 10029 reflections collected, 4406 unique, (R_{int} = 0.020), R indices (all data) $^aR_1$ = 0.0491, $wR2$ [$I > 2\sigma(I)$] = 0.1216, *Gof* on F^2= 1.111. CCDC deposition number: 1408845.

***Crystal Data:* 55a:** $C_{25}H_{24}BrNO_6$, M=514.36, Monoclinic, a = 11.4077(8) Å, b = 12.4910(8) Å, c = 16.2876(11) Å; α = 90°, β = 94.226(2)°, γ = 90°; U = 2314.6(3) Å^3, T = 100(2) K, space group P 1 21/c 1, Z = 4, μ (Mo Kα) = 1.54178 Å, , ρ =1.476 g/cm^3, 35486 reflections collected, independent reflections 4048 [R(int) = 0.0366], R indices (all data) R_1 = 0.0289, wR_2 = 0.0863; w=1/[$\sigma^2(F_o^2)$+(0.0555P)^2+2.8462P] where P=(F_o^2+2F_c^2)/3; *Gof* = 0.938. $^aR_1$ = $\Sigma||F_o| - |F_c||/\Sigma|F_o|$, w$R_2$ = $[\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408847.

***Crystal Data for* 60:** $C_{28}H_{25}NO_5$, M = 455.49, Triclinic, a = 10.1226(5), b = 14.7272(7), c = 17.3808(8)Å, α= 74.9040(10)°, β= 75.6960(10)°, γ = 74.3650(10)°, U = 2365.1(2)Å^3, T = 150 K, space group P-1, Z = 4, μ (Mo Kα) = 0.088 mm^{-1}, ρ = 1.279Mg/cm^3, 18923 reflections collected, 8330 unique, (R_{int} = 0.0242), R indices (all data) $^aR_1$ = 0.042, $wR2$ [$I > 2\sigma(I)$] = 0.1170, *Gof* = 1.039, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408844.

***Crystal Data for* 77:** $C_{50}H_{50}C_{10}N_2O_{14}S_2$, M = 967.04, Triclinic, a = 9.5683(19), b = 11.422(2), c = 11.892(2)Å, α = 114.97(3)°, β = 101.24(3)°, γ = 93.60(3)°, U = 1140.1(4)Å^3, T = 150 K, space group P-1, Z = 1, μ (Mo Kα) = 0.190 mm^{-1}, ρ = 1.408Mg/cm^3, 11169 reflections collected, 5463 unique, (R_{int} = 0.0203), R indices (all data) $^aR_1$ = 0.1275, $wR2$ [$I > 2\sigma(I)$] = 0.3306, *Gof* = 1.110, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408846.

***Crystal Data for* 50b:** $C_{50}H_{52}N_2O_{13}$, M = 888.94, Monoclinic, a = 19.4658(13), b = 13.4807(9), c = 17.5627(12), Å, α = 90°, β = 106.5660(10)°, γ = 90°, U = 4417.4(5)Å^3, T = 150 K, space group C2/c, Z = 4, μ (Mo Kα) = 0.097 mm^{-1}, ρ = 1.337Mg/cm^3, 17189 reflections collected, 3889 unique, (R_{int} = 0.0267), R indices (all data) $^aR_1$ = 0.0360, $wR2$ [$I > 2\sigma(I)$] = 0.0987, *Gof* = 0.902, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408848.

***Crystal Data for* 58b:** $C_{25}H_{22}N_2O_5$, M = 430.45, Monoclinic, a = 14.9002(9), b = 14.0903(8), c = 21.0318(12)Å, α = 90°, β = 94.6700(10)°, γ = 90°, U = 4400.9 (4)Å^3, T = 150 K, space group P2(1)/c, Z = 8, μ (Mo Kα) = 0.091 mm^{-1}, ρ = 1.299Mg/cm^3, 34424 reflections collected, 7741 unique, (R_{int} = 0.0227), R indices (all data) $^aR_1$ = 0.0328, $wR2$ [$I > 2\sigma(I)$] = 0.1216, *Gof* = 1.111, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408851.

***Crystal Data for* 78b:** $C_{25}H_{25}NO_7$, M = 451.46, Triclinic, a = 10.1929(11), b = 10.4180(11), c = 12.6792(14)Å, α = 90.803(2), β = 110.232(2)°, γ = 117.556(2)°, U = 2052.6(13)Å^3, T = 150 K, space group P-1, Z = 2, μ (Mo Kα) = 0.100 mm^{-1}, ρ = 1.369Mg/cm^3, 8726 reflections collected, 3854 unique, (R_{int} = 0.0151), R indices (all data) $^aR_1$ = 0.0522, $wR2$ [$I > 2\sigma(I)$] = 0.1385, Gof = 1.027, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408850.

***Crystal Data for* 90b:** $C_{25}H_{26}N_2O_6$, M = 450.48, Orthorombic, a = 21.8994(19), b = 7.6060(7), c = 28.035(2)Å, α = β = γ = 90°, U = 4669.7(7)Å^3, T = 150 K, space group Pbca, Z = 8, μ (Mo Kα) = 0.092 mm^{-1}, ρ = 1.282Mg/cm^3, 46377 reflections collected, 4105 unique, (R_{int} = 0.0746), R indices (all data) $^aR_1$ = 0.0432, $wR2$ [$I > 2\sigma(I)$] = 0.1204, Gof = 0.999, $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. CCDC deposition number: 1408849.

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Supporting Information: Tables S1-S5 and Figures S1 and S2 (Tier-1 profiling and Lipinski properties of selected β-lactams, full NCI60 cell line results, COMPARE analysis, X-ray crystal structres and torsional angles, and molecular docking images). The Supporting Information is available free of charge on the ACS Publications website.

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Non-Standard Abbreviations:

CA-4	Combretastatin A-4
CA-4P	Combretastatin A-4 phosphate
DAMA-colchicine	N-Deacetyl-N-(2-mercaptoacetyl)colchicine
EBI	*N,N*'-ethylene-bis(iodoacetamide)
HOBt	Hydroxybenzotriazole
LDH	Lactate dehydrogenase
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TBDMS	*tert*-Butyldimethylsilyloxy

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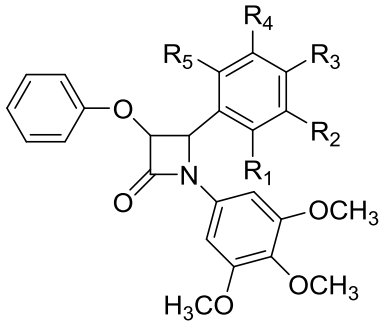
Table 1: Antiproliferative Effects and Cytotoxicity of β-Lactams **49**-**65**, **75**-**92**, **95**-**99**, **102**, **103** and **105** in MCF-7 Cells

						**IC_{50} value (μM)**[a]	**% Cell Death (10 μM)**[b]
	R_1	**R_2**	**R_3**	**R_4**	**R_5**		
49	H	OCH_3	OCH_3	OCH_3	H	29	0
50a	H	H	OCH_3	H	H	35	6.5
50b	H	H	OCH_3	H	H	6.9	7.3
51	H	OCH_3	H	H	H	171	22
52	H	OCH_3	OCH_3	H	H	26	11.2
53	H	$-OCH_2O-$		H	H	48	8.3
54	H	H	Br	H	H	48	nd
55a	H	Br	OCH_3	H	H	5.1	6.4
55b	H	Br	OCH_3	H	H	31	3.9
56	H	F	OCH_3	H	H	6.3	70
57	H	H	$N(CH_3)_2$	H	H	31	5.9
58a	H	H	CN	H	H	31.6	4.8

58b	H	H	CN	H	H	168	2.1
59	H	-C_4H_4-		H	H	282	10.7
60	-C_4H_4-		H	H	H	53	4.6
61a	H	NO_2	OCH_3	H	H	69	5.3
61b	H	NO_2	OCH_3	H	H	4.1	5.2
62	H	NO_2	H	H	H	30	16.7
63	H	H	NO_2	H	H	63	27
64	H	H	SCH_3	H	H	7.7	8.1
65	H	H	N_3	H	H	23	5.8
75	H	H	$C_2H_4CO_2C_2H_5$	H	H	87	nd
76	H	H	$SOCH_3$	H	H	20	1.7
77	H	H	SO_2CH_3	H	H	62	10.2
78a	H	OH	OCH_3	H	H	0.383	2.8
78b	H	OH	OCH_3	H	H	0.038	29
79	H	H	OH	H	H	48	0.1
80	H	OCH_3	OH	H	H	32	5.1
81	H	OH	OH	H	H	4.5	6.0
82	H	OH	H	H	H	54	22
83	*See Scheme 3*					49	10.4
84	NO_2	OH	OCH_3	H	H	89	12.7
85	H	OH	OCH_3	NO_2	H	69	12.1

86	H	OH	OCH_3	H	NO_2	59	9.9
87	NH_2	OH	OCH_3	H	H	19	52
88	H	OH	OCH_3	NH_2	H	8.8	14.0
89	H	OH	OCH_3	H	NH_2	20	70
90a	H	NH_2	OCH_3	H	H	12.2	7.7
90b	H	NH_2	OCH_3	H	H	0.013	10.2
91	H	NH_2	H	H	H	17	48
92	H	H	NH_2	H	H	160	14.7
95	*See Scheme 5*					33	10.9
96	*See Scheme 6*					3.3	19.8
97	*See Scheme 6*					7.5	4.8
98	H	OChlorambucil	OCH_3	H	H	0.41	8.0
99	H	NHChlorambucil	OCH_3	H	H	145	3.2
102a	H	$NHC(O)C(CH_3)NH_2$	OCH_3	H	H	9.2	nd
102b	H	$NHC(O)C(CH_3)NH_2$	OCH_3	H	H	0.23	nd
103	H	$NHC(O)CHNH_2$	OCH_3	H	H	6.5	nd
105a	H	$OP(O)(OH)_2$	OCH_3	H	H	42	nd
105b	H	$OP(O)(OH)_2$	OCH_3	H	H	23	nd
2[c]						0.0039	5.5

[a] IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 cells. Values represent the mean for three experiments performed in triplicate.

[b]Lactate Dehydrogenase assay: Following treatment of the cells, the amount of LDH was determined using LDH assay kit from Promega (G1780). Data is presented as % cell death at a concentration of 10 μM.

[c]The IC_{50} value obtained for **2** (0.0039 μM for MCF-7) is in good agreement with reported values.[26, 55]

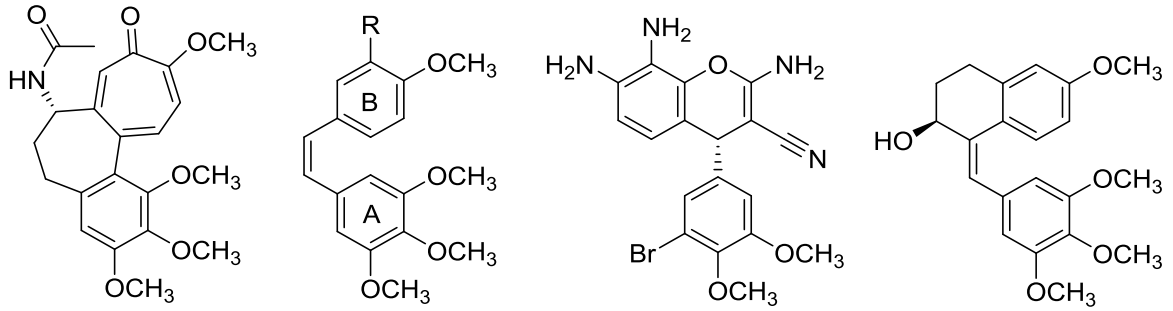
[*]nd = not determined.

Table 2. *In vitro* inhibition of tubulin polymerization for compounds **57**, **58b**, **78a, 78b**, **90b** and **2**[a]

Compound	**Fold-reduction in V_{max}**
57	0
58b	0
78a	1.1
78b	3.2
90b	1.8
2	6.0

[a]Effect of **57**, **58b**, **78a**, **78b**, **90b** and **2** (all at final concentration of 10 μM) on *in vitro* tubulin polymerisation. Purified bovine tubulin and GTP were mixed in a 96-well plate at 37ºC. Ethanol (1% v/v) was used as a vehicle control. The effect on tubulin assembly was monitored in a Spectramax 340PC spectrophotometer at 340 nm at 30 second intervals for 60 minutes at 37 ºC. Fold inhibition of tubulin polymerization was calculated using the V_{max} value for each reaction. The results represent the mean for three separate experiments.

Figures.



1 **2** R=OH **5** **6**

3 R=OPO_3Na_2

4 R=$NHCOCH(NH_2)(CH_2OH).HCl$

Figure 1. Small molecules that interact with the colchicine-binding site of tubulin

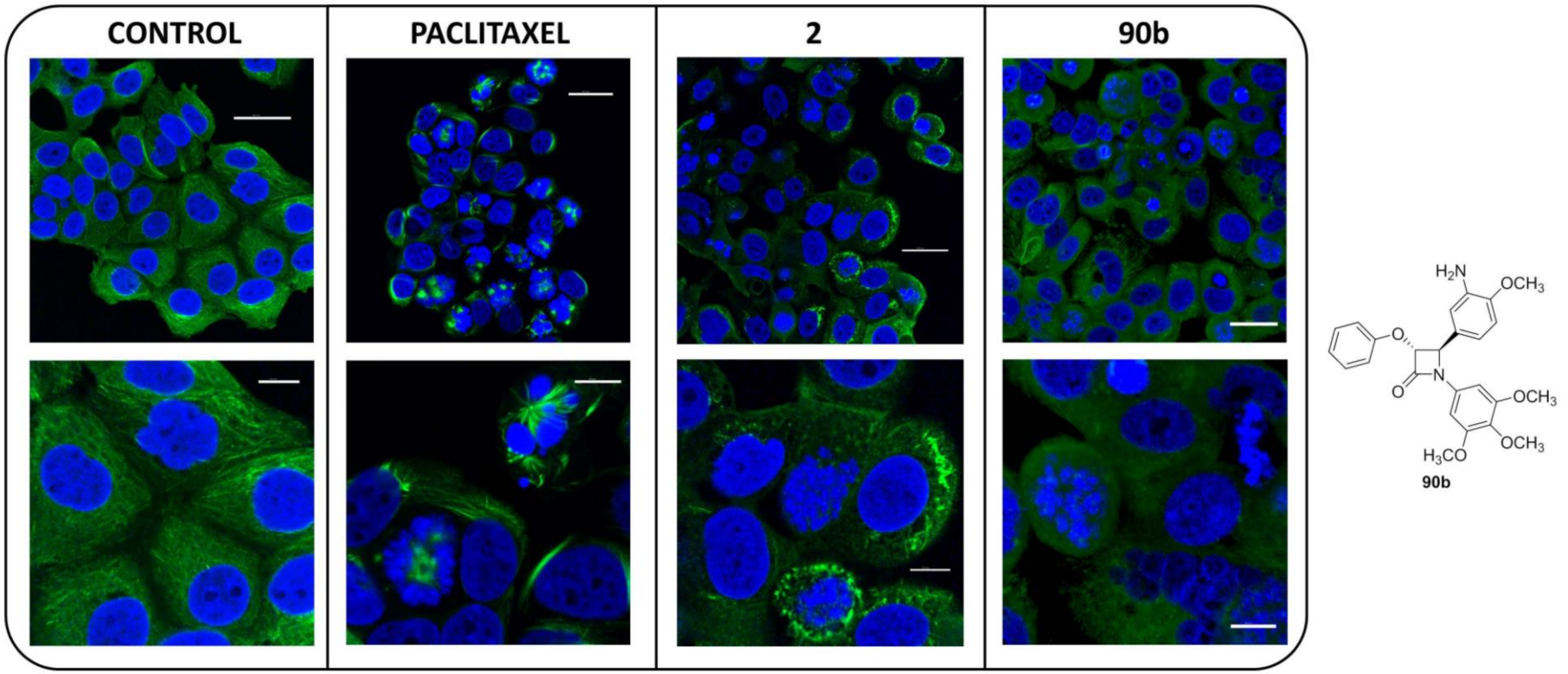


Figure 2. CA-4 **2** and β-lactam **90b** depolymerise the microtubule network of MCF-7 cells. MCF-7 cells were treated with vehicle control [1% ethanol (v/v)], paclitaxel (1 μM), **2** (100 nM) or **90b** (100 nM) for 16 h. Cells were fixed in 4% paraformaldehyde and stained with mouse monoclonal anti-α-tubulin−FITC antibody (clone DM1A) (green), Alexa Fluor 488 dye and counterstained with DAPI (blue). Images were captured by Leica SP8 confocal microscopy with Leica application suite X software. Representative confocal micrographs of three separate experiments are shown. Scale bar: 30 μM (top images); 10 μM (bottom images).

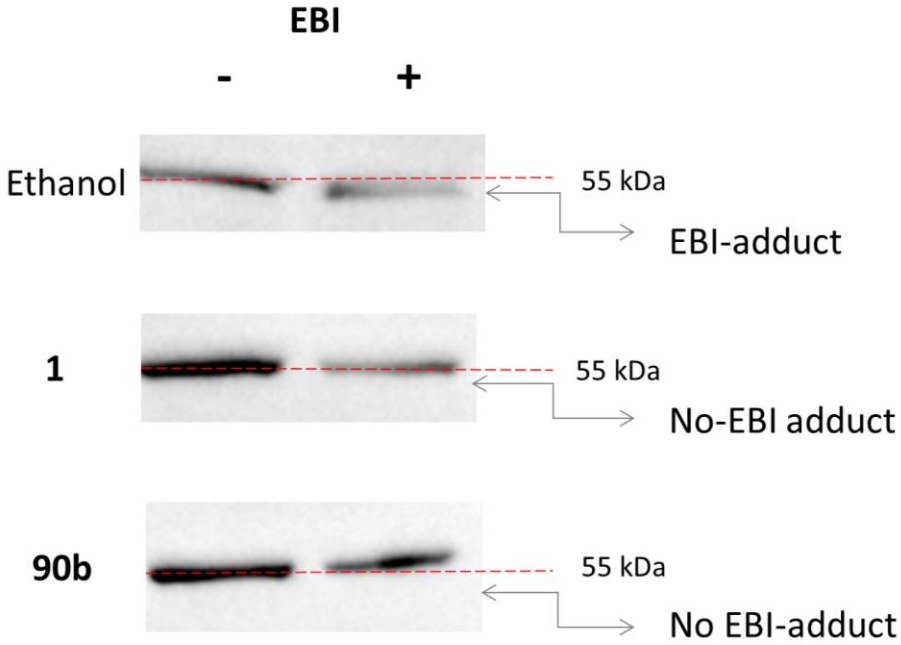


Figure 3. Effects of **1** and β-lactam **90b** on the inhibition of the bisthioalkylation of Cys239 and Cys354 of β-tubulin by *N,N'*-ethylene-bis(iodoacetamide)(EBI) in MCF-7 cells. MCF-7 cells were treated with vehicle control [ethanol 0.1% (v/v)], **1** or **90b** (both 10 μM) for 2 h; selected samples were then treated with EBI for an additional 1.5 h. Cells were harvested, lysed and analysed using sedimentation and Western blotting for β-tubulin and β-tubulin-EBI adduct. Results are indicative of three separate experiments, performed independently.

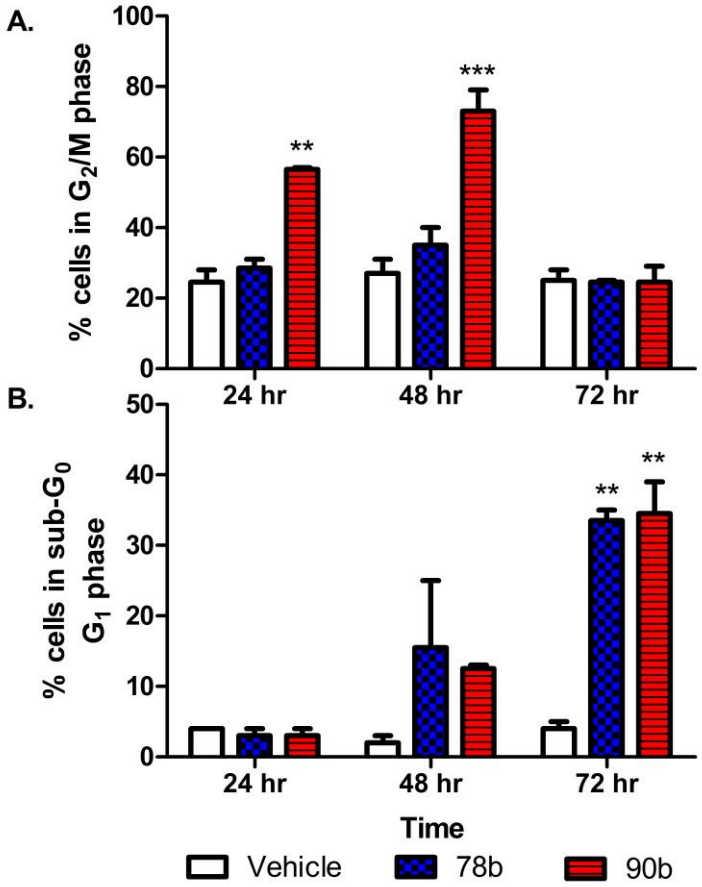


Figure 4. Differential effects of **78b** and **90b** on the cell cycle and apoptosis in MCF-7 cells. Cells were treated with either vehicle [0.1% ethanol (v/v)], **78b** (100 nM) or **90b** (50 nM) for 24, 48 and 72 hr. Cells were then fixed, stained with PI, and analyzed by flow cytometry. Cell cycle analysis was performed on histograms of gated counts per DNA area (FL2-A). The number of cells with <2N (sub-G_1), 2N (G_0G_1), and 4N (G_2/M) DNA content was determined with CellQuest software. Statistical analysis was performed using Prism software. A two-way ANOVA was employed to determine significant differences between and vehicle controls and treated samples. Values represent the mean ± S.E.M. for at least two separate experiments.

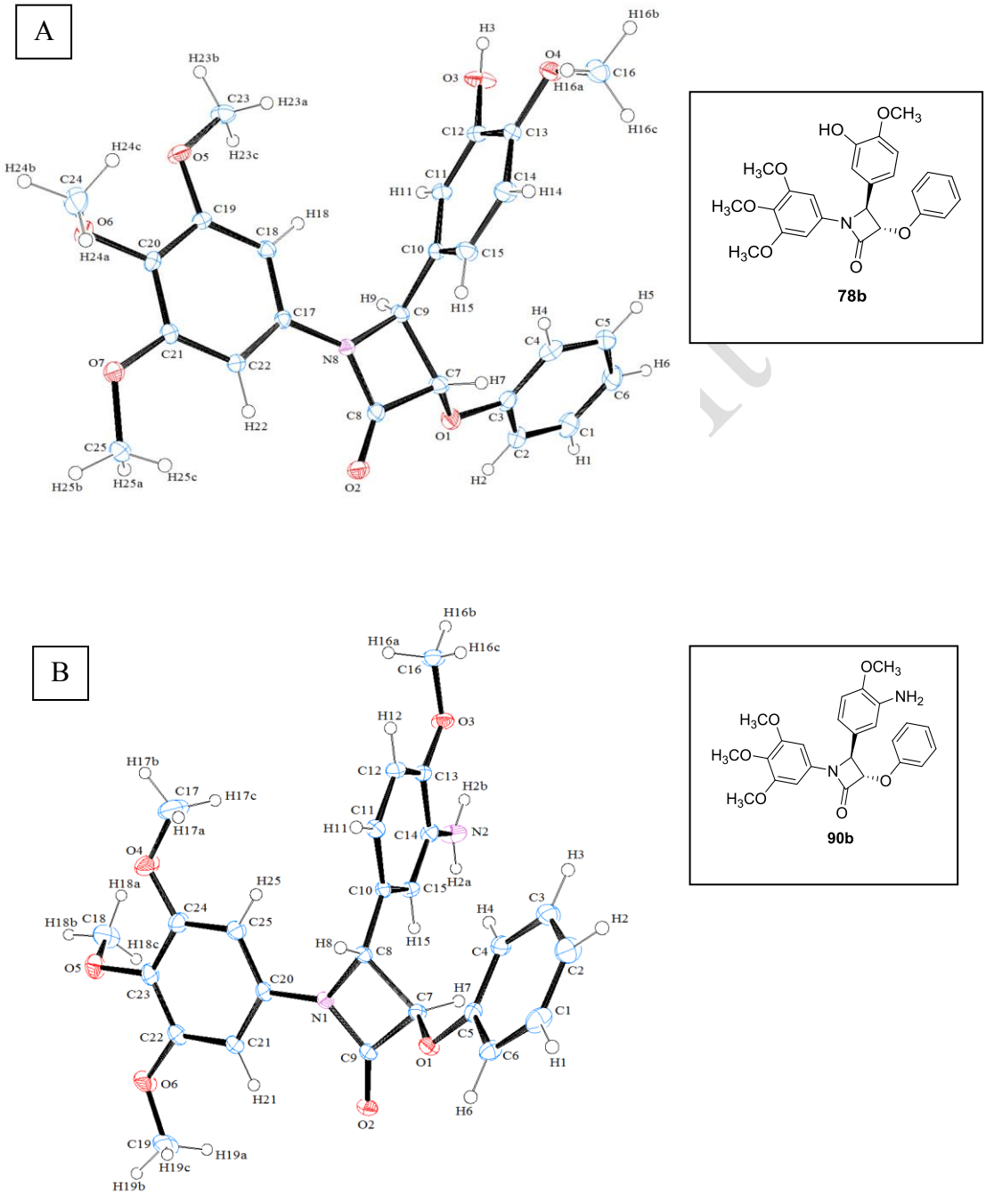


Figure 5. ORTEP representations of the X-ray crystal structures of azetidinones (A) **78b** and (B) **90b** with 50% thermal ellipsoids.

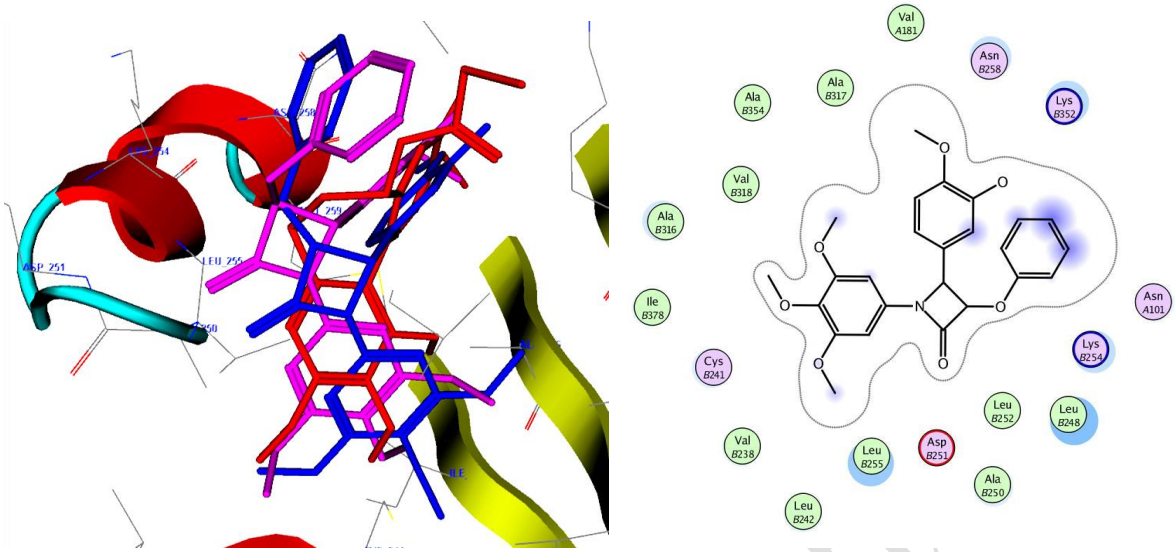


Figure 6. (Left) Docked solution of **78a** (pink) and **78b** (blue) with DAMA-colchicine (red)[6]; (Right) 2D representation of the ligand-protein interactions of **78b** with the colchicine-binding site, rendered using LigX module of MOE.

Schemes

Scheme 1. Synthesis of benzaldehydes 8, 11, 13 and imines 14-48[a]

[a]Reagents and conditions: (a) concd HNO_3, acetone, rt, 30 min, 42%, 31%; (b) $(CH_3CO)_2O$, KOH, diethyl ether, 0 °C, 30 min, 93%; (c) concd HNO_3, -18 to -5 °C, 20 min, 82%; (d) 5% aq. NaOH, rt, 8%; (e) $NaNO_2$, H_2O, 2M H_2SO_4, 0 °C, 15 min; NaN_3, H_2O, rt, 1 h, 90%; (f) PCC, CH_2Cl_2, rt, 1 h, 78%; (g) EtOH, H_2SO_4, reflux, 5 h, 10-95%; (h) t-$BuMe_2SiCl$, DBU, CH_2Cl_2, rt, until complete as indicated by TLC, 75-92%. R=H unless otherwise indicated.

Scheme 2. Synthesis of azetidinones 49-77[a]

[a]Reagents and conditions: (a) $C_6H_5OCH_2COCl$, Et_3N, anhyd CH_2Cl_2, 40 °C, 5 h then rt, 16 h, 21-98%; (b) (b) $Pd(OAc)_2$, $(C_6H_5)_2PC_3H_6P(C_6H_5)_2$, Et_3N, anhyd DMF, 100ºC, 18 h, 16%; (c) mCPBA (1 equiv.), anhyd CH_2Cl_2, rt, 2 h, 63%; (d) mCPBA (2.5 equiv.), anhyd CH_2Cl_2, rt, 30 min, 66%. Products obtained as a mixture of enantiomers; one enantiomer represented. R=H unless otherwise indicated.

Scheme 3. Synthesis of phenolic azetidinones 78-83[a]

[a]Reagents and conditions: (a) TBAF, THF, 0 °C, 15 min, 26-57%. Products obtained as a mixture of enantiomers; one enantiomer represented.

Scheme 4. Synthesis of amino azetidinones 84-92[a]

[a]Reagents and conditions: (a) TBAF, THF, 0 °C, 15 min, 22-39%; (b) Zn dust, CH_3CO_2H, rt, 7 days, 73-94%. Products obtained as a mixture of enantiomers; one enantiomer represented. R=H unless otherwise indicated.

Scheme 5. Synthesis of azetidinone 95[a]

[a]Reagents and conditions: (a) $ClCH_2CO_2H$, anhyd DMF, NaH, 0 °C then rt, 2 days, 48%; (b) Triphosgene, Et_3N, anhyd CH_2Cl_2, reflux, 6 h; (c) TBAF, THF, 0 °C, 15 min, 22-39%, 36%. Product obtained as a mixture of enantiomers; one enantiomer represented.

Scheme 6. Synthesis of azetidinones 96-99[a]

[a]Reagents and conditions: (a) DIBALH, anhyd THF, reflux, 2 h, 27%; (b) Lawesson's reagent, anhyd toluene, reflux, 12 h, rt, overnight, 63%; (c) Chlorambucil, DMAP, EDCI, anhyd CH_2Cl_2, rt, 24 h, 38-53%. Products obtained as a mixture of enantiomers; one enantiomer represented.

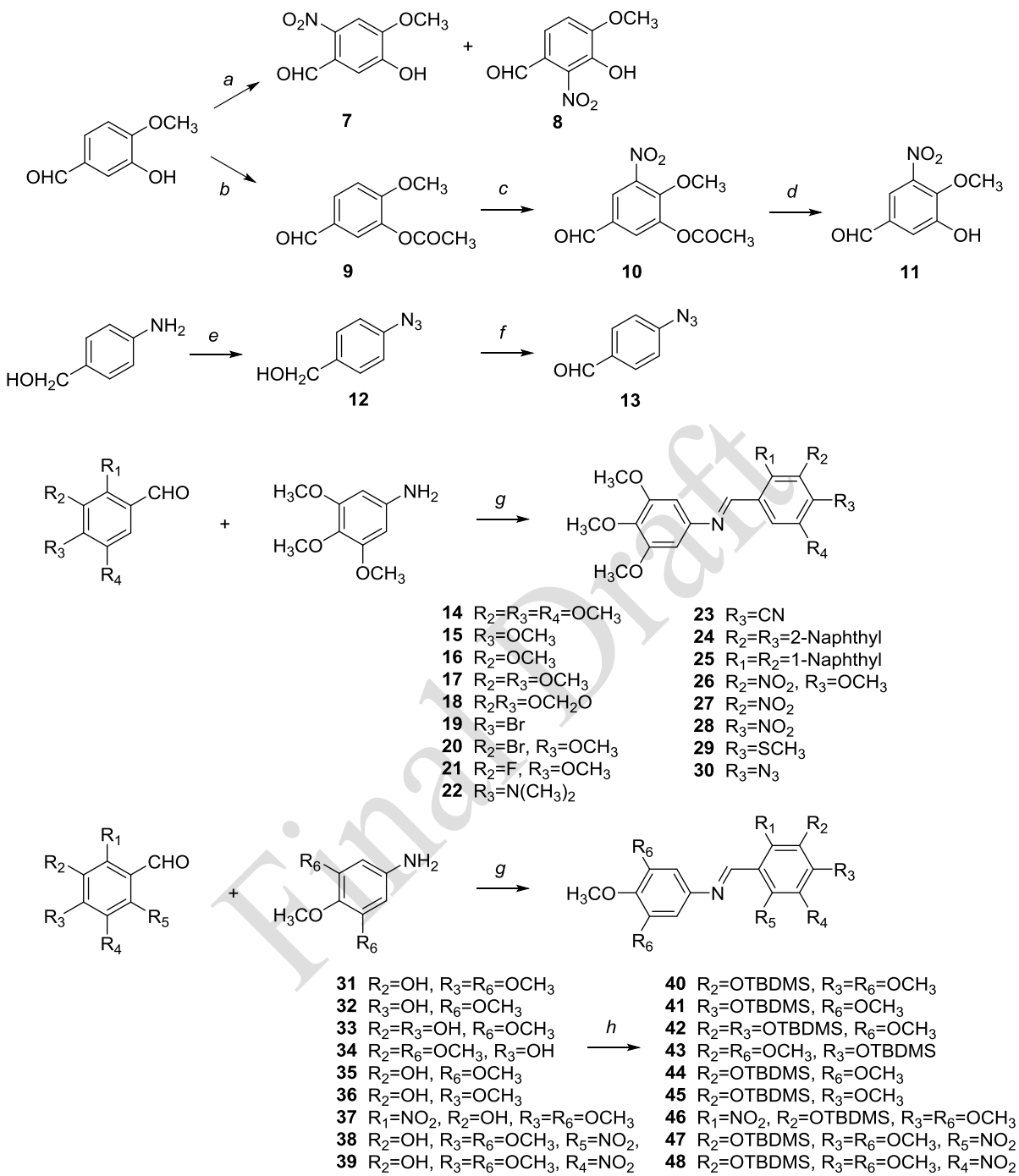
Scheme 7. Synthesis of amino acid prodrugs 102a, 102b and 103[a]

[a]Reagents and conditions: (a) N-Fmoc-L-amino acid, anhyd DMF, DCC, HOBt.H_2O, rt, 24h, 22-79%; (b) TBAF, 1-octanethiol, CH_2Cl_2, rt, until complete as indicated by TLC, 34-69%. Products obtained as a mixture of enantiomers; one enantiomer represented.

Scheme 8. Synthesis of phosphate ester prodrugs 105a and 105b[a]

[a]Reagents and conditions: (a) CCl_4, CH_3CN, DIPEA, DMAP, dibenzyl phosphite, 0 °C, until complete as indicated by TLC, 9%, 86%; (b) H_2, Pd/C, EtOH:EtOAc (1:1), rt, until complete as indicated by TLC, 21%, 47%. Products obtained as a mixture of enantiomers; one enantiomer represented.

Scheme 1

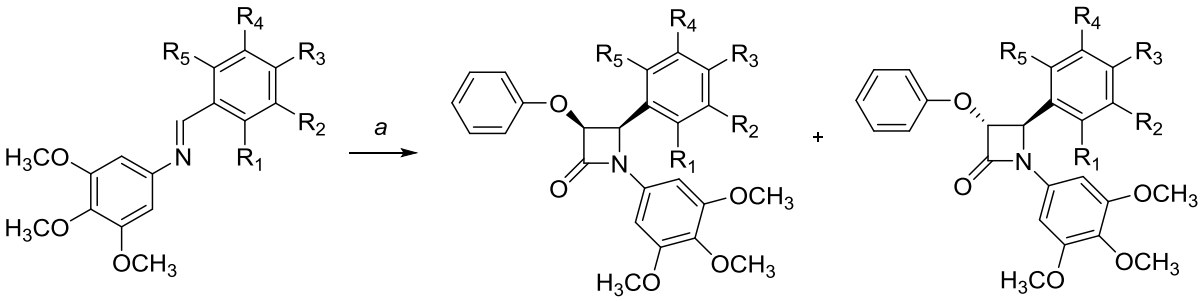


14 R_2=R_3=R_4=OCH_3
15 R_3=OCH_3
16 R_2=OCH_3
17 R_2=R_3=OCH_3
18 R_2R_3=OCH_2O
19 R_3=Br
20 R_2=Br, R_3=OCH_3
21 R_2=F, R_3=OCH_3
22 R_3=$N(CH_3)_2$
23 R_3=CN
24 R_2=R_3=2-Naphthyl
25 R_1=R_2=1-Naphthyl
26 R_2=NO_2, R_3=OCH_3
27 R_2=NO_2
28 R_3=NO_2
29 R_3=SCH_3
30 R_3=N_3

31 R_2=OH, R_3=R_6=OCH_3
32 R_3=OH, R_6=OCH_3
33 R_2=R_3=OH, R_6=OCH_3
34 R_2=R_6=OCH_3, R_3=OH
35 R_2=OH, R_6=OCH_3
36 R_2=OH, R_3=OCH_3
37 R_1=NO_2, R_2=OH, R_3=R_6=OCH_3
38 R_2=OH, R_3=R_6=OCH_3, R_5=NO_2,
39 R_2=OH, R_3=R_6=OCH_3, R_4=NO_2

40 R_2=OTBDMS, R_3=R_6=OCH_3
41 R_3=OTBDMS, R_6=OCH_3
42 R_2=R_3=OTBDMS, R_6=OCH_3
43 R_2=R_6=OCH_3, R_3=OTBDMS
44 R_2=OTBDMS, R_6=OCH_3
45 R_2=OTBDMS, R_3=OCH_3
46 R_1=NO_2, R_2=OTBDMS, R_3=R_6=OCH_3
47 R_2=OTBDMS, R_3=R_6=OCH_3, R_5=NO_2
48 R_2=OTBDMS, R_3=R_6=OCH_3, R_4=NO_2

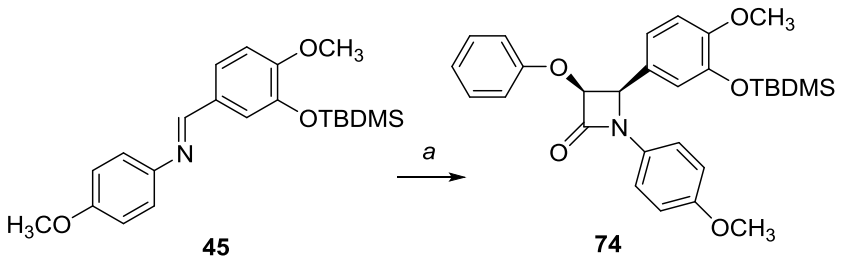
Scheme 2



14-30, **40-44**, **46-48**

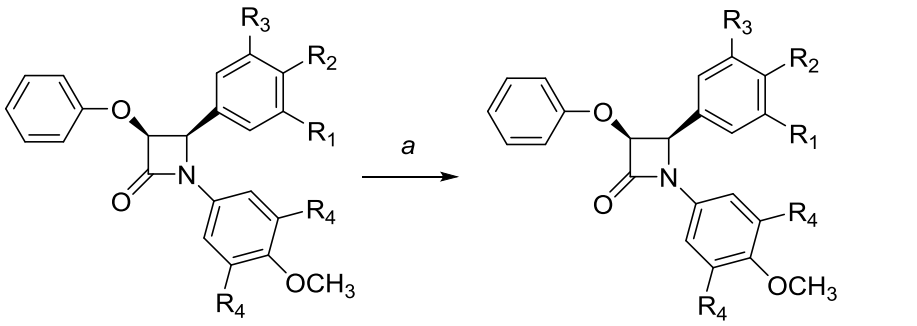
49 $R_2=R_3=R_4=OCH_3$
50a $R_3=OCH_3$
51 $R_2=OCH_3$
52 $R_2=R_3=OCH_3$
53 $R_2R_3=OCH_2O$
54 $R_3=Br$
75 $R_3=CH=CHCO_2C_2H_5$
55a $R_2=Br$, $R_3=OCH_3$
56 $R_2=F$, $R_3=OCH_3$
57 $R_3=N(CH_3)_2$
58a $R_3=CN$
59 R_2R_3=2-Naphthyl
60 R_1R_2=1-Naphthyl
61a $R_2=NO_2$, $R_3=OCH_3$
62 $R_2=NO_2$
63 $R_3=NO_2$
64 $R_3=SCH_3$
76 $R_3=SOCH_3$
77 $R_3=SO_2CH_3$
65 $R_3=N_3$
66a R_2=OTBDMS, $R_3=OCH_3$
67 $R_2=OCH_3$, R_3=OTBDMS
68 R_3=OTBDMS,
69 R_2=OTBDMS
70 $R_2=R_3$=OTBDMS
71 $R_1=NO_2$, R_2=OTBDMS, $R_3=OCH_3$
72 R_2=OTBDMS, $R_3=OCH_3$, $R_4=NO_2$
73 R_2=OTBDMS, $R_3=OCH_3$, $R_5=NO_2$

50b $R_3=OCH_3$
55b $R_2=Br$, $R_3=OCH_3$
58b $R_3=CN$
61b $R_2=NO_2$, $R_3=OCH_3$
66b R_2=OTBDMS, $R_3=OCH_3$



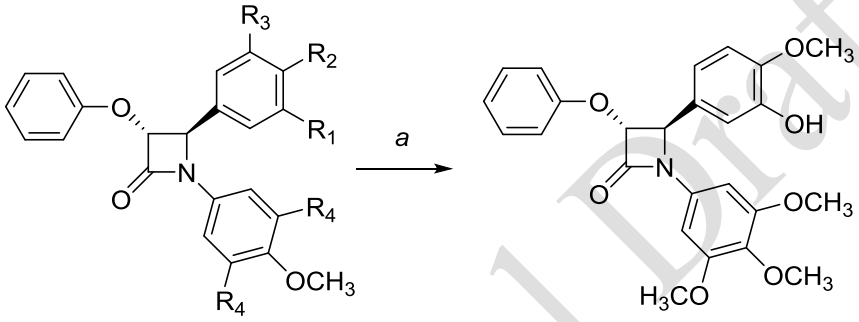
45 **74**

Scheme 3



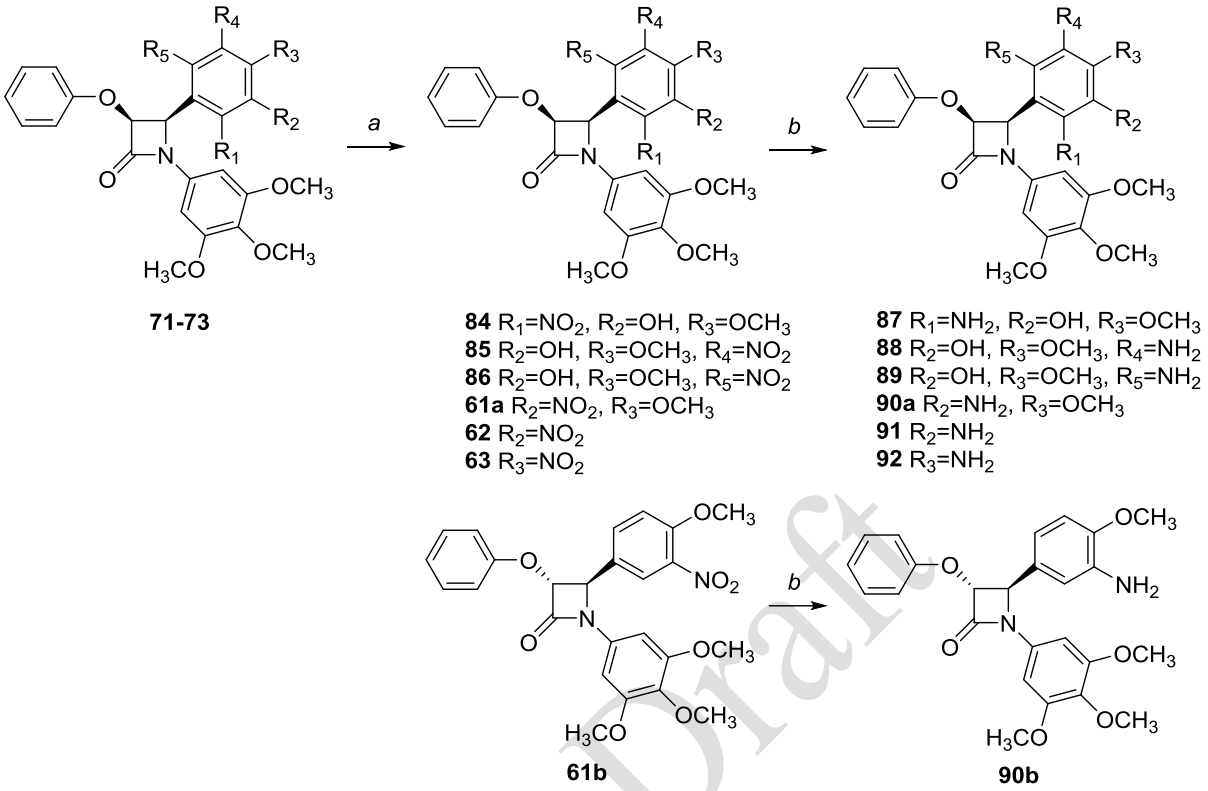
66a, 67-70, 74

78a R_1=OH, R_2=R_4=OCH_3, R_3=H
79 R_1=R_3=H, R_2=OH, R_4=OCH_3
80 R_1=R_4=OCH_3, R_2=OH, R_3=H
81 R_1=R_2=OH, R_3=H, R_4=OCH_3
82 R_1=OH, R_2=R_3=H, R_4=OCH_3
83 R_1=OH, R_2=OCH_3, R_3=R_4=H



66b **78b**

Scheme 4



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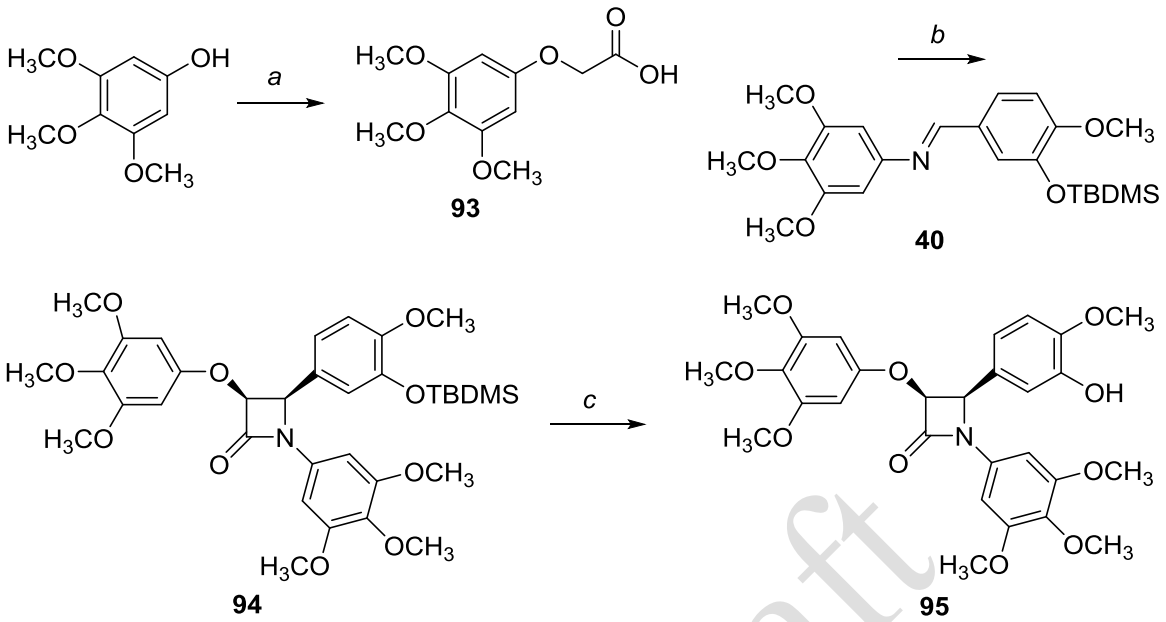

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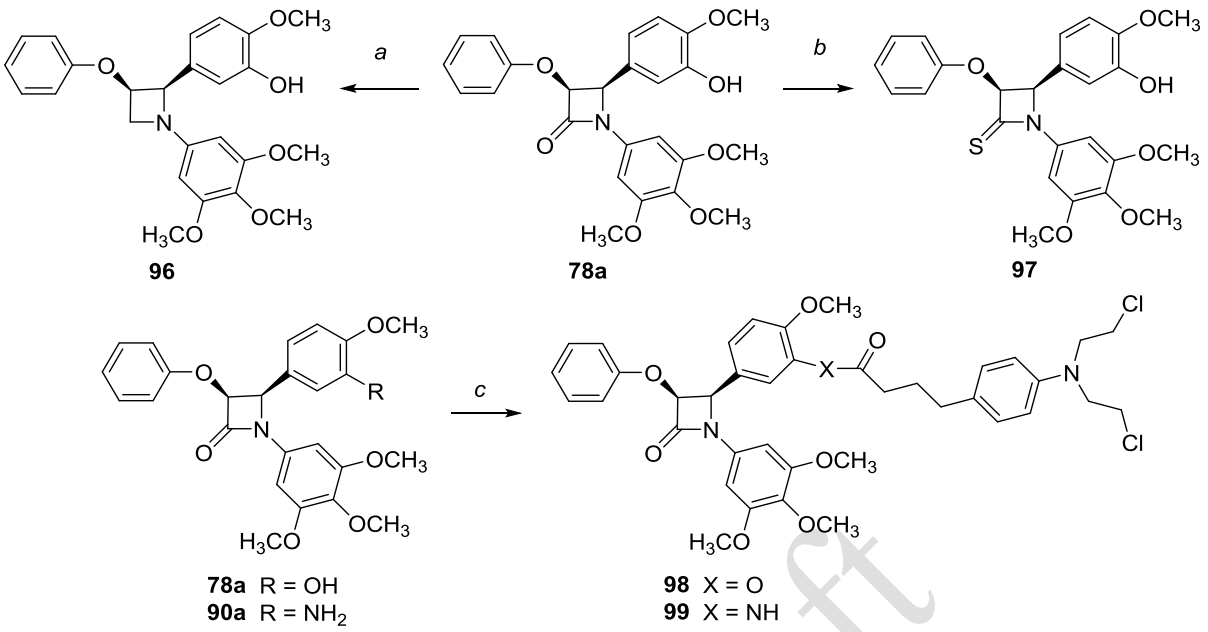

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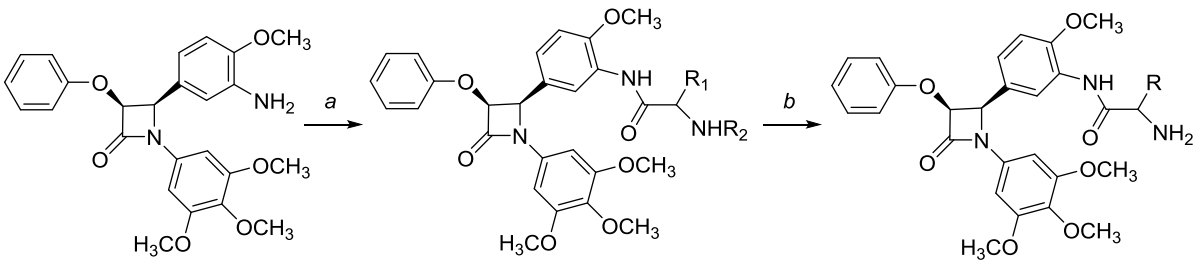
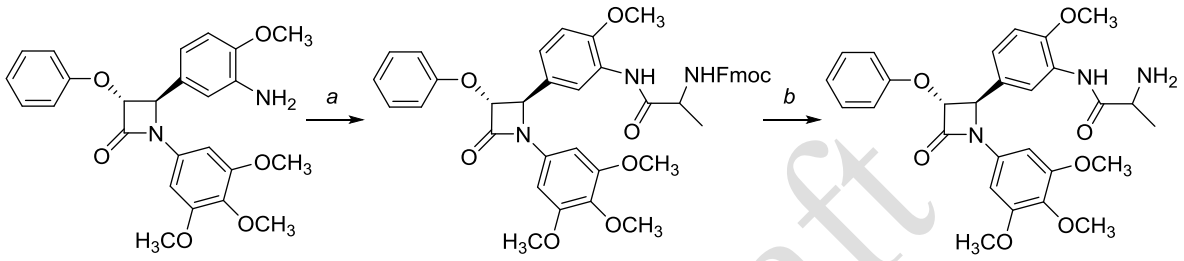


90a

100a R_1=CH_3, R_2 = Fmoc
101 R_1=H, R_2 = Fmoc

102a R=CH_3
103 R=H

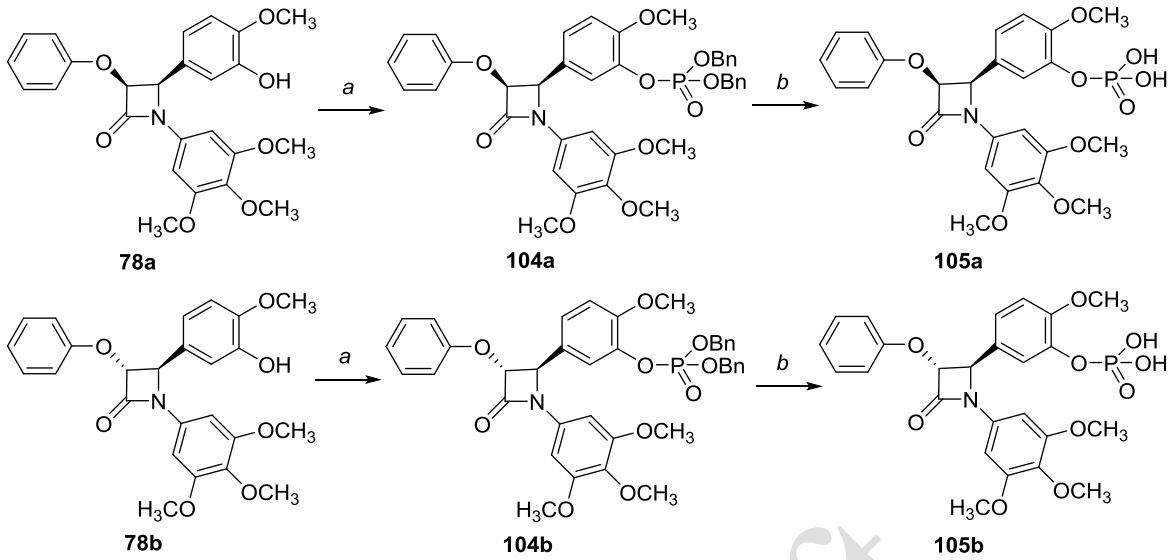


90b

100b

102b

Scheme 8



Graphical Abstract (for Table of Contents Only)