Accepted Manuscript

Title: Lead identification of conformationally restricted β-lactam type combretastatin analogues: synthesis, antiproliferative activity and tubulin targeting effects

Authors: Miriam Carr, Lisa M. Greene, Andrew J.S. Knox, David G Lloyd, Daniela M. Zisterer, Mary J. Meegan

PII: S0223-5234(10)00681-1

DOI: 10.1016/j.ejmech.2010.09.033

Reference: EJMECH 4453

To appear in: *European Journal of Medicinal Chemistry*

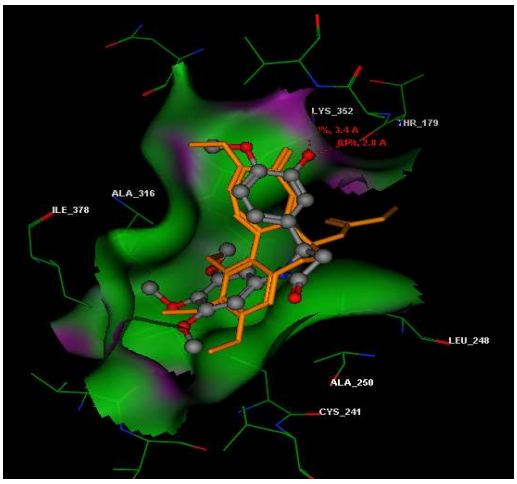
Received Date: 13 April 2010

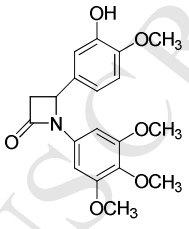
Revised Date: 9 September 2010

Accepted Date: 14 September 2010

Please cite this article as: M. Carr, L.M. Greene, A.J.S. Knox, D.G. Lloyd, D.M. Zisterer, M.J. Meegan. Lead identification of conformationally restricted β-lactam type combretastatin analogues: synthesis, antiproliferative activity and tubulin targeting effects, European Journal of Medicinal Chemistry (2010), doi: 10.1016/j.ejmech.2010.09.033

Graphical abstract





12d

Docked pose of β-lactam **12d** overlayed with N-deacetyl-N-(2-mercaptoacetyl)colchicine (DAMA-colchicine) in the tubulin binding site

Lead identification of conformationally restricted β-lactam type combretastatin analogues: synthesis, antiproliferative activity and tubulin targeting effects

MIRIAM CARR[1], LISA M. GREENE[2], ANDREW J.S. KNOX[3], DAVID G LLOYD[3], DANIELA M. ZISTERER[2] and MARY J. MEEGAN[1*]

[1]*School of Pharmacy and Pharmaceutical Sciences, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin 2, Ireland.*

[2]*School of Biochemistry & Immunology, Trinity College Dublin, Dublin 2, Ireland*

[3]*Molecular Design Group, School of Biochemistry & Immunology, Trinity College Dublin, Dublin 2, Ireland.*

*To whom correspondence should be addressed

Correspondence should be addressed to:

Mary J Meegan, School of Pharmacy and Pharmaceutical Sciences, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin 2, Ireland.

Tel: +353-1-8962798 ; Fax: +353-1-8962793 ; E-mail: mmeegan@tcd.ie

Abstract

The synthesis and study of the structure-activity relationships of a series of rigid analogues of combretastatin A-4 are described which contain the 1,4-diaryl-2-azetidinone (β-lactam) ring system in place of the usual ethylene bridge present in the natural combretastatin stilbene products. The 1,4-diaryl-2-azetidinones are unsubstituted at C-3, or contain methyl substituent(s) at C-3. The most potent compounds **12d** and **12e** display antiproliferative activity at nanomolar concentrations when evaluated against the MCF-7 and MDA-MB-231 human breast carcinoma cell lines. **12d** exerts antimitotic effects through an inhibition of tubulin polymerisation and subsequent G_2/M arrest of the cell cycle in human MDA-MB-231 breast cancer cells**,** with similar activity to that of CA-4. These novel β-lactam compounds are identified as potentially useful scaffolds for the further development of antitumour agents which target tubulin.

Key words: Combretastatin A-4 analogues, β-lactam, 2-azetidinone, cytotoxicity, tubulin, structure-activity.

Abbreviations

CA-4	Combretastatin A-4
DEPT	Distortionless Enhancement by Polarization Transfer
EGTA	Ethylene glycol tetraacetic acid
EI	Electron Impact
ER	Estrogen receptor
FACS	Fluorescence-activated cell sorting
FBS	Fetal Bovine Serum
GTP	Guanidine triphosphate
HMQC	Heteronuclear Multiple Quantum Coherence
HRMS	High Resolution Molecular Ion Determination
IR	Infra Red
LRMS	Low Resolution Mass Spectra
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect
PBS	Phosphate buffer saline
SAR	Structure-Activity Relationship
THF	Tetrahydrofuran
TMS	Tetramethylsilane

1. Introduction

Microtubules are cytoskeletal structures that are formed by the self-assembly of α and β tubulin heterodimers and are involved in many cellular functions[1]. Their most important role is in the formation of the mitotic spindle, which is intimately involved in cell division. Antimitotic agents are one of the major classes of cytotoxic drugs for cancer treatment and microtubules are a significant target for many natural product anticancer agents[2]. The three characterised binding sites of tubulin are the taxane domain, the vinca domain and the colchicine domain and many compounds interact with tubulin at these known sites.

Paclitaxel binds to tubulin at the taxane site and was the first compound found to stabilise tubulin by promoting the assembly of tubulin heterodimers into microtubules[3]. The vinca alkaloids, vinblastine and vincristine bind at the vinca domain resulting in depolymerisation of microtubules and destruction of mitotic spindles at high concentrations[4]. Colchicine (**1**, Figure 1) also depolymerises microtubules at high concentrations and stabilizes microtubules dynamics at low concentrations; however it binds at a separate site to the vinca alkaloids, the colchicine domain[5, 6].

The combretastatins were obtained from the African willow tree *Combretum caffrum*. Pettit *et al* [7] showed that combretastatin A-4 (**2**, Figure 1) (CA-4) potently inhibited microtubule activity by binding to tubulin at the colchicine site, thereby interfering with cell growth and proliferation. CA-4 acts as a vascular targeting agent and induces blood flow reduction and subsequent tumour cell death.[4, 8-10] In contrast to colchicine, the anti-vascular effects of CA-4 *in vivo* are apparent well below the maximum tolerated dose, offering a wide therapeutic window. The disodium phosphate salt of CA-4 **3** has been developed with improved solubility[11, 12] and is currently in phase II clinical trials for the treatment of thyroid cancer[13-15]. Hydrolysis *in vivo* by endogenous non-specific phosphatases under physiological conditions affords CA-4[11, 12]. The *cis* double bond in CA-4 is readily

converted to the more stable *trans* geometry during storage or use, resulting in a dramatic decrease in antitumour activity,[16, 17], [18].

Insert Figure 1

Many structural modifications to CA-4 have been reported including variation of the A and B-rings substituents (Figure 1)[19-21]. Most modifications of the B-ring result in decreased bioactivity; the exception is the removal of the 3'-hydroxy group, which results in a compound with almost the same potency as CA-4 in both tubulin polymerisation and colchicine binding assays[18, 22]. Substitution of the 3'-OH with an amino group results in a compound with equipotent bioactivity as CA-4 and good water solubility [23]. The 3,4,5-trimethoxy substituted pattern in ring A is optimal for bioactivity of CA-4 as it coincides with the trimethoxyaryl ring of colchicine [20]. A large number of analogues with a modified bridge have also been reported. These include saturation of the olefinic bridge, and replacement with $–NHCH_2$-, $-CH_2NH$-, -CONH-, $-OCH_2$-, $-CH_2O$-, -O- and many others.

Isomerisation of the *cis*-double bond in CA-4 has led to intensive investigations of rigid ring modifications[10, 19, 20, 24], including imidazole **4** [25], 1,3-dioxolane **5**[26], furazan (1,2,5-oxadiazole) **6**[27], heteroarylcoumarin **7**[28] and 3-aroyl-6-methoxyindole **8**[29] analogues (Figure 1). In addition to tumour cell growth inhibition, most of these non-isomerisable compounds have been shown to be capable of binding to and depolymerising tubulin. The anticancer activity of some β-lactam-containing compounds has been reported[30], including the non-isomerisable combretastatin analogues **9** and **10** [31]. We have previously reported antiproliferative activity of compounds containing the β-lactam scaffold which exhibited antiestrogenic effects in MCF-7 cells[32]. It was decided to examine a range of compounds containing the β-lactam ring as potential tubulin targeting agents. These novel compounds are unsubstituted at C-3, or contain methyl substituent(s) at C-3. The

β-lactam ring provides a useful scaffold structure with a similar spatial arrangement between the two phenyl rings as observed in the *cis* conformation of CA-4.

2. Results and discussion

2.1 Chemistry

The general procedure for the synthesis of the required β-lactams is illustrated in Scheme 1. The compounds which were initially chosen for synthesis contained the 3,4,5-trimethoxyphenyl (Ring A) and 3-hydroxy-4-methoxyphenyl (Ring B) substituents as the β-lactam N-1 and C-4 substituents, which are present in Combretastatin A-4. The Reformatsky β-lactam synthesis requires the appropriately substituted Schiff bases **11a** and **11c** which were obtained by condensation of the appropriate amine and aldehyde. Schiff bases **11a** and **11c** were then protected by treatment with *tert*-butyldimethylchlorosilane to yield the respective silyl ether products **11b** and **11d**. The racemic β-lactam products **12a** and **12g** were obtained in moderate yields by reaction of imines **11b** and **11d** with ethylbromoacetate in the presence of zinc and trimethylchlorosilane using the conditions similar to those reported by Palomo et al [33].

The ^{1}H-NMR spectrum of **12a,** the C-3 proton at δ 2.91(dd, J=2.0 Hz, 15.0 Hz) is in a *trans* orientation to H-4 which appears as a double doublet at δ 4.86 (J=2.0 Hz, 5.4 Hz) [34]. The second C-3 proton is observed at δ 3.49 (dd, J=5.4 Hz, 15.4Hz) and is *cis* to H-4. The silyl protecting group was removed from both β-lactams **12a** and **12g** using TBAF to afford **12d** (62 %) and **12j** (39 %) respectively, the β-lactam analogues of CA-4. The related 3-methylazetidinones **12b** and **12h** were similarly obtained by Reformatsky reaction of Schiff bases **11b** and **11d** using ethyl 2-bromopropionate as the α-bromoester, (Scheme 1). These azetidinones were obtained as mixtures of *cis/trans* isomers (3:2) which were not separated.

The 3,3-dimethyl analogues **12c** and **12i** were prepared in a similar manner by reaction of the Schiff bases **11b** and **11d** with ethyl-2-bromoisobutyrate.

The *tert*-butyldimethylsilyl protecting group is removed from compounds **12b,c,h,i** using TBAF to yield the phenolic products **12e,f,k,l** respectively. The diastereomeric composition of compound **12e** was demonstrated by chiral HPLC, (rt 3.39min and 4.11min for *trans* isomers and 4.33min and 4.66min for *cis* isomers, assigned from integration). The effect of product stereochemistry on the biochemical activity of the β-lactams reported in this study will be considered in the future development of these compounds. (The stereoselective activity of the cholesterol lowering β-lactam drug ezetimibe(1-(4-fluorophenyl)-(3*R*)-[-3-(4-fluorophenyl)-(3*S*)-hydroxypropyl]-4*S*-(4-hydroxyphenyl)-2-azetidinone) has been reported[35, 36]).

Because of the observation that the 3,4,5-trimethoxy ring substitution (Ring A) is optimal for bioactivity in all analogues of CA-4 in literature, compounds **12d-f** and **12j-l** were examined for their antiproliferative effects in both MCF-7 and MDA-MB-231 cell lines with the aim of discovering if the placement of this critical 3,4,5-trimethoxyphenyl ring on the N-1 or C-4 positions resulted in significantly different antiproliferative activities, (see results and discussion section, Table 1). Results in both cell lines concurred, showing the compounds with the 3,4,5-trimethoxyphenyl ring (Ring A) on the N-1 position being significantly more potent than the compound with the 3,4,5-trimethoxyphenyl substituent (Ring A) on the C-4 position, as previously reported by Sun et al. with other β-lactam compounds[31]. From our initial investigations we established that positioning of the 3,4.5-trimethoxyphenyl ring on the rigid β-lactam ring system for these compounds, was an important consideration and required that any further β-lactams synthesized would contain the 3,4,5-trimethoxyphenyl ring at the N-1 position.

Structural modification of the initially synthesised β-lactam products **12d-f** was then investigated with a view to further optimization of the CA-4 analogue β-lactam template for antiproliferative activity. The synthesis of products **12m- x** with modification of the C-4 aryl ring substituents is shown in Scheme 2. The 3-unsubstituted β-lactam **12d** contains the 3-hydroxy-4-methoxy phenyl ring at the C-4 position; which is also present in CA-4 (Ring B). A variety of different β-lactams **12m-v**, were obtained by using different substitution patterns on the C-4 phenyl ring (Ring B), e.g. 4-F, 4-OCH_3, 2,3,4-trimethoxy, 3,4,5-trimethoxy and 3,4-dimethoxy. The aryl ring at C-4 was also replaced with the alternative 6-methoxy-2-naphthyl and 2-thiophene ring systems (compounds **12w-x**). The required imines **11e-11l** were prepared by condensation of 3,4,5-trimethoxyaniline with the appropriate aldehyde. The β-lactam products **12m-o**, **12r-t** and **12w-x** were obtained in moderate yields by reaction of imines **11e-11l** with ethylbromoacetate in the presence of zinc and trimethylchlorosilane. Enantiomeric separation of the racemic product **12o** was achieved by chiral HPLC (rt 7.88min and 8.92min).

The 3-methyl and 3,3-dimethyl compounds **12p, 12q, 12u** and **12v** were obtained in a similar manner using ethyl-2-bromopropionate and ethyl-2-bromoisobutyrate as the α-bromoesters respectively. The nature of the Schiff base influences the yields obtained in these Reformatsky reactions. While good yields were obtained (e.g. up to 83% for compound **12p**), we have found that after optimization, lower yields of β-lactam products e.g. **12n** (28%) and **12s** (16%) are consistently obtained from the Schiff bases **11f** and **11i** both having *ortho* methoxy substitutents, which may be causing some steric hindrance for the imine in forming the β-lactam ring.

To further investigate the contribution of the β-lactam carbonyl group to the activity of this compound class, the novel thione analogues **13a-c** were prepared in moderate yield by reaction of the compounds **12o-q** with Lawesson's reagent (2,4-bis-(4-methoxyphenyl)-

1,2,3,4-dithiaphosphetane-2,4-disulfide), (Scheme 2). The characteristic C=S absorption band was observed in the region 1590-1595cm^{-1} for these products.

Insert Scheme 1

Insert Scheme 2

2.2 Biochemical studies

2.2.1 Antiproliferative activity in MCF-7 and MDA-MB 231 breast cancer cells.

The first aim of the biochemical evaluation of the compounds was to determine if the position of the 3,4,5-trimethoxyphenyl ring on the β-lactam scaffold at N-1 or C-4, which is critical in the activity of colchicines and combretastatin type compounds, effected the potency of these β-lactam compounds. Compounds **12d-f** and **12j-l** were screened for their antiproliferative activity using two breast cancer cell lines, the ER expressing (ER dependent) MCF-7 human breast cancer and the ER lacking (ER independent) MDA-MB-231 human breast cancer cell lines, using the MTT (tetrazolium) based viability assay. The drug concentration required to inhibit the cell growth by 50% (IC_{50}) following incubation of the cells in the culture medium for 72h was determined and the results are displayed in Table 1. The IC_{50} values obtained for Combretastatin CA-4 in this assay are 31 nM for MCF-7 and 43 nM for MDA-MB 231 and are in good agreement with the reported values for Combretastatin CA-4 using the MTT assay on human MCF-7 and MDA-MB 231 breast cancer cell lines [7, 37, 38].

From the results in Table 1 it is evident that the 3,4,5-trimethoxyphenyl ring located at the N-1 position of the β-lactam ring is the structural arrangement for optimum activity as previously observed by Sun et al. with other β-lactam compounds[31]. All three compounds **12d-f** (in which the 3,4,5-trimethoxyphenyl ring is located at the N-1 position) are much more potent in both cell lines than the corresponding compounds **12k** and **12l** (where the

3,4,5-trimethoxyphenyl ring is located at the C-4 position) and therefore this template was chosen for the further compounds examined. The 3-unsubstituted compound **12j** was the most active of the products with 3,4,5-trimethoxy ring is located at the C-4 position, (IC_{50}value = 0.130μM in MCF-7 cells). It is interesting to note that the most active compounds described here (**12d**, **12e**) are considerably more potent as antiproliferative agents than the previously reported 3-hydroxy, 3-methoxy and 3-acetoxy substituted β-lactams.[31] There is little difference in antiproliferative activity between the 3-unsubstituted compound **12d** and 3-methyl compound **12e**, both demonstrating low nanomolar activity in both MCF-7 cell line (IC_{50} = 17nM and 10nM respectively) and MDA-MB-231 cell line (IC_{50} =54 and 47nM respectively). These values are similar to that obtained for CA-4 (IC_{50} = 3nM for MCF-7 and IC_{50} = 43nM for MDA-MB-231 cell line). However, the introduction of the 3,3-dimethyl substitution in compound **12f** results in significant loss of activity with IC_{50} = 0.25 μM for MCF-7 cells and IC_{50} = 0.27 μM for the MDA-MB-231 cell line.

The cytotoxic effect of these β-lactam compounds on proliferating cells was determined in the lactose dehydrogenase (LDH) assay.[39] Cytotoxicity values in the range 2-15% were obtained for compounds **12d-f, j** at a concentration of 10μM in MCF -7 cells and 6-25% for the same compounds in MDA-MB-231 cells at a concentration of 10μM. CA-4 was determined to have cytotoxicity value of 5.6% in MCF-7 cells and 4.3% in MDA-MB-231 cells at 10μM concentration.

Insert Table 1

The antiproliferative results in MCF-7 and MDA-MB 231 breast cancer cell lines for the second series of compounds synthesised **12m-x**, **13a-c** are displayed in Table 2. All compounds contain the 3,4,5-trimethoxyphenyl substituent located at N-1 with the C-3 position unsubstituted, or mono or dimethyl substituted. The effects on antiproliferative activity of a number of aryl substitution patterns are investigated for the aryl group at C-4,

including 2,3,4-trimethoxy, 3,4,5-trimethoxy, 3,4 dimethoxy, 4-methoxy and 4-fluoro are displayed in Table 2. There is no significant loss in activity observed on removal of the C-3 phenolic group from the most potent compounds **12d** and **12e** as shown in the results obtained for **12o** and **12p** with IC_{50} values of 39nM and 47nM respectively. These compounds also showed reduced cytoxicity values in the LDH assay of 1.5% and 5.7% respectively at 10μM concentration. The related 3,3-dimethyl-2-azetidinones **12q** and **12v** show good antiproliferative effects on MCF-7 cells (IC_{50} = 265nM and 344nM respectively). Introduction of the 3,4-dimethoxyphenyl substituent at C-4 results in intermediate antiproliferative activity for compound **12t** (IC_{50} value = 1.64μM), with moderate activity also obtained for the 3-methyl substituted analogue **12u** (IC_{50} value = 2.96μM) and also for compound **12x** (containing the 6-methoxy-2-naphthyl substituent at C-4), with IC_{50} value of 1.51μM. The introduction of the fluoro substituent in **12m** resulted in loss of activity, (IC_{50} value =25.9μM). The presence of two trimethoxyphenyl ring systems in the β-lactam products e.g. compound **12s** resulted in a moderate reduction in activity (IC_{50} = 0.31μM). However for the related 1,4-bis(trimethoxyphenyl) substituted compounds **12n** and **12r** a significant reduction in the antiproliferative activity was observed IC_{50} values in the high μM range. The replacement of aryl ring at C-4 position with the heterocyclic 2-thiophene ring in **12w** also resulted in much reduced antiproliferative activity with an IC_{50} value of 21.6 μM and notably higher cytotoxicity of 20 %. A similar trend was observed for antiproliferative activity of these compounds in the MDA cell line, with compounds **12o** and **12p** again displaying the most potent antiproliferative activity (IC_{50} = 210 and 127nM respectively) with low cytotoxicity (1.1% and 4.5% respectively, at 10μM concentration).

The replacement of the β-lactam carbonyl group with the thione in compounds **13a-c** resulted in a reduction in the observed antiproliferative activity; the most active thione compound **13c** was found to be IC_{50} = 0.35μM for MCF-7 cells and IC_{50} = 1.33μM for MDA-MB 231 breast

cancer cells. This could be related to the difference in lipophilicity (e.g. cLogP for **12q** = 3.36 compared with cLogP for **13c** = 2.86.[40]

The influence of the rigid β-lactam ring scaffold structure on the activity of the above compounds was investigated. The antiproliferative effect of the two Schiff bases **11a** and **11c** (related in structure to the most potent β-lactam **12d** and the related **12j**) was determined in MCF-7 breast cancer cells. Neither of the Schiff bases shows any significant antiproliferative activity at concentrations up to 50 μM, which is in sharp contrast to the corresponding β-lactams **12d** and **12j**. As already observed by Cushman et al. the lack of activity of these Schiff bases probably derives from their *trans* geometry[41]

Insert Table 2

2.2.2 Evaluation of G_2/M arrest in MDA-MB-231 cells exposed to compound 12d

The extent of G_2/M arrest and apoptosis (sub-G_1 peak) induced by compound **12d** in MDA-MB-231 cells was statistically quantified by flow cytometric analysis. The fluorescent dye, propidium iodide (PI) intercalates with the DNA and hence, the amount of fluorescence measured per cell is proportional to the DNA content. Cells were harvested after 24, 48 or 72 hours and analysed for DNA content by flow cytometry. Tables 3 shows the percentage of cells in each phase of the cell cycle over the three different time scales.

The results obtained for compound **12d** show a large increase of cells in the G_2/M phase at concentrations 100 nM, 1 μM and 10 μM after 24 hours of exposure,(highlighted in Table 3). This increase is accompanied by a corresponding reduction in the G_1 phase. At a concentration of 10 nM, there appears to be little effect on the cells giving results similar to the vehicle value. After 48 hours a significant increase in G_2/M peak (highlighted) is again seen at 100 nM and above but not to the same degree as seen after 24 hours as highlighted in

Table 3. This is due to the parallel increase in sub-G_1 phase indicating induction of apoptosis. After 72 hours a decrease in G_2/M and in G_1 phase is accompanied by a large increase in sub-G_1 peak. These results indicate that this compound's mechanism of action may indeed be by targeting the microtubules. The ability of these β-lactam compounds to bind to and depolymerise tubulin was then investigated.

Insert Table 3

2.2.3 Tubulin polymerisation study

The effects of representative β-lactam combretastatin A-4 analogues on the assembly of purified bovine tubulin were evaluated. The assay was optimised using a polymerisation enhancer (paclitaxel) and polymerisation suppressor (nocodazole) (data not shown). Compound **12d** which demonstrated potent antiproliferative effects (low nanomolar) *in vitro* and compound **12h** which demonstrated poor antiproliferative effects (high micromolar) were assessed. The ability of combretastain A-4 to effectively inhibit the assembly of tubulin was assessed as a positive control. Tubulin polymerisation was determined by measuring the increase in absorbance over time at 340nm. The Vmax value offers the most sensitive indicator of tubulin/ligand interactions, hence Vmax values were calculated for each test compound. Fold changes in Vmax values for polymerisation curves of each test compound with reference to ethanol control were calculated and detailed in Table 6. As anticipated the active ß-lactam combretastatin A-4 analogue **12d** inhibited the polymerisation of tubulin whilst the relatively inactive counterpart **12h** did not significantly affect the rate of tubulin polymerisation (Table 4). In more detail, the active ß-lactam **12d** when evaluated at 10 µM concentration, reduced the Vmax value for the rate of tubulin polymerisation from 6 to 10-fold. This value is comparable if not superior to the rate of inhibition of tubulin assembly (6-

fold) observed with combretastatin A-4. These results suggest that the molecular target of the active ß-lactam combretastatin A-4 analogues is indeed tubulin.

Insert Table 4

2.3 Molecular modeling studies

To investigate potential binding modes of these β-lactam compounds, a docking study was carried out using the most potent compound in the series, the 3-unsubstituted β-lactam **12d**. Using the reported X-ray structure of tubulin co-crystallised with the colchicine derivative, N -deacetyl-N-(2-mercaptoacetyl)colchicine (DAMA-colchicine, PDB entry – 1SA0) [42], possible binding orientations for **12d** were probed with the docking program FREDv2.2.3 (Openeye Scientific Software) [43]. 3-D conformations were enumerated using CORINAv3.4 (Molecular Networks GMBH) [44] for **12d** and Combretastatin A-4 followed by multiple conformations using OMEGAv2.2.1 (Openeye Scientific Software) [45]. Each conformation was subsequently docked and scored with Chemgauss3 as outlined previously by our laboratory [46] The top binding poses were refined using the LigX procedure (MOE - Chemical Computing Group) [47]. Postdock analysis (SVL script; MOE) of the docked poses for **12d** revealed that approximately 10% of the population appeared to dock in a similar orientation to colchicine as illustrated in Figure 2.

For comparison, the docked pose of CA4 overlayed with N-deacetyl-N-(2-mercaptoacetyl)-colchicine (DAMA-colchicine) in the tubulin binding site (PDB entry 1SA0) is illustrated in Figure 3. The strong hydrogen-bonding to Thr179 observed for the 3-hydroxy substituent of **12d** is also observed with Combretastatin-A4 as depicted in Figure 3. (The X-ray crystal structure of the disodium phosphate salt of combretastatin A-4 (**3**) suggests that the conformation of this stilbene is not planar. The crystal structure reveals that the planes of the

two phenyl rings are inclined to each other, suggesting a low-energy conformation that may be the one involved in binding at the tubulin receptor site [48]). Importantly, an additional H-bond is seen to occur with residue Lys352 of the active site for **12d**, colchicine and CA-4. Superimposition of **12d**, CA4 and colchicine as they docked within the active site of 1SA0, illustrates the similar nature of positioning of the trimethoxy substituents (ring A) and also 3-hydroxy-4-methoxy substituents of the B-ring. These binding parallels may rationalise the potency observed for **12d** in its tubulin effects which is seen to be close to that reported for CA4.

Insert Figure 2 & Figure 3.

4. Conclusion

We have synthesized a series of β-lactam compounds which show potent antiproliferative activity in breast cancer cells. The 3-unsubstituted compound **12d** and 3-methylsubstituted compound **12e** (each with 3-hydroxy-4-methoxyaryl substitution pattern at C-4) were identified as the most potent compounds of the series having low nanomolar activity in both MCF-7 and MDA-MB 231 breast cancer cells. The most effective compound **12d** was shown to arrest cells in the G2/M phase of the cell cycle. **12d** was also shown to inhibit the polymerisation of tubulin with improved efficacy when compared with combretastatin CA-4. However, as these compounds were evaluated in this study as racemates, the effect of product stereochemistry on the biochemical activity will be relevant in the future development of these compounds. The β-lactam ring provides a scaffold structure with a similar spatial arrangement between the two phenyl rings as observed in *cis* conformation of combretastatin CA-4. These conformationally restricted β-lactam structures, which unlike the *cis*-stilbene CA-4 are not easily isomerised, are promising lead compounds in the development of new

anticancer agents. Further studies will determine the possible antiangiogenic effects of these compounds.

5. Experimental

5.1 Chemistry

All reagents were commercially available and were used without further purification unless otherwise indicated. Tetrahydrofuran (THF) was distilled immediately prior to use from Na/Benzophenone under a slight positive pressure of nitrogen, and toluene was dried by distillation from sodium and stored on activated molecular sieves (4 A). 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, 400.13MHz for ^{1}H spectra, 100.61MHz for ^{13}C spectra, in either $CDCl_3$, CD_3COCD_3 or CD_3OD (internal standard tetramethylsilane). Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD GC–MS system in an electron impact mode, while high resolution accurate mass determinations for all final target compounds were obtained on a Micromass Time of Flight mass spectrometer (TOF) equipped with electrospray ionization (ES) interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory by Dr. Martin Feeney in the Department of Chemistry, Trinity College Dublin. Thin layer chromatography 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. Chiral liquid chromatography was carried out on selected compounds using a Chiral-AGP^{TM} 150x4.0mm column supplied by ChromTech Ltd. (now supplied by Chiral Technologies Europe) with a Chiral- AGP^{TM} guard column. The HPLC system consisted of the following components: a Waters 1525 binary HPLC pump, a Waters

2487 Dual Wavelength Absorbance Detector, a Waters In-Line Degasser AF and a Waters 717 plus Autosampler. Gradient elution was used beginning with 10% of organic phase and finishing with 90% of organic phase over a period of 20 minutes. The organic mobile phase was 2-propanol and the aqueous phase was a sodium phosphate buffer. The sodium phosphate buffer, consisting of 10mM sodium dihydrogen orthophosphate dihydrate (NaH_2PO_4) in HPLC-grade water, was made up to pH 7.0 using sodium hydroxide. The flow rate was 0.5ml/minute and detection was carried out at 225 nm. Compounds **11c**[49], **11g**[31] and **11h** [50]were prepared as previously reported.

5.1.1. 2-Methoxy-5-[(3,4,5-trimethoxyphenylamino)methyl]phenol (11a). A solution of 3-hydroxy-4-methoxybenzaldehyde (10 mmol, 1.36 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g) in ethanol (50 mL) was heated to reflux for three hours. The reaction mixture was reduced to 25 mL under vacuum, and the solution transferred to a beaker. The mixture was left to stand for 3h and the Schiff base product crystallized out of the solution. The crude product was then re-crystallized from ethanol and filtered to yield the purified product. Yield 84 %, pale yellow crystals, m.p. 176-178°C. IR ν_{max} (KBr) cm^{-1}: 1602.6 cm^{-1} (C=N), 3069.2 cm^{-1} (OH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 3H, O-CH_3), 3.81 (s, 3H, O-CH_3), 3.85 (s, 6H, 2xOCH_3), 6.57 (s, 2H, Ar-H), 7.03 (d, 1H, J=8.2 Hz, Ar-H), 7.29 (dd, 1H, J=2.0, 8.2 Hz, Ar-H), 7.42 (d, 1H, J=2.0 Hz, Ar-H), 8.48 (s, 1H, CH=N), 9.33 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO): δ 55.83 (O-CH_3), 56.09 (O-CH_3), 60.09 (O-CH_3), 98.50, 111.57, 113.57, 122.26, 129.27, 135.50, 146.78, 147.59, 150.79, 153.21 (Aromatic C), 159.42 (CH=N). HRMS: Found: 318.1345; $C_{17}H_{20}NO_5$ requires 318.1341(M^++H).

5.1.2. (4-Fluorobenzylidene)-(3,4,5-trimethoxyphenyl)amine (11e). Preparation was as above from 4-fluorobenzaldehyde (10 mmol, 1.24 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 72 %, pale yellow crystals, m.p. 87-90°C. IR ν_{max} (KBr) cm^{-1}: 1626.1 cm^{-1} (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, O-CH_3), 3.90 (s, 6H, 3xO-CH_3), 6.48 (s,

2H, Ar-H), 7.14-7.29 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 8.44 (s, 1H, -CH=N). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.98 (O-CH_3), 60.88 (O-CH_3), 98.00, 106.60, 115.73, 115.98, 130.56, 130.65, 131.51, 136.30, 147.58, 153.46, (Aromatic C), 158.04 (CH=N). Elemental Analysis: Found: C, 66.28; H, 5.54; N, 4.73; F, 16.87. $C_{16}H_{16}FNO_3$ requires C, 66.43; H, 5.57; N, 4.84; F, 16.59 %.

5.1.3. (2,4,5-Trimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (11f). Preparation as described above from 2,4,5-trimethoxybenzaldehyde (10 mmol, 1.48 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 78 %, Yellow crystals, m.p. 123°C. IR ν_{max} (KBr) cm^{-1}: 1619.5 cm^{-1} (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H, O-CH_3), 3.89 (s, 6H, 2xO-CH_3), 3.90(s, 3H, O-CH_3), 3.94 (s, 3H, O-CH_3), 3.95 (s, 3H, O-CH_3), 6.50 (s, 2H, Ar-H), 6.53 (s, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 8.80 (s, 1H, -CH=N). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.42 (O-CH_3), 55.81(O-CH_3), 56.06(O-CH_3), 57.93(O-CH_3), 60.62 (O-CH_3), 95.71, 97.87, 108.47, 122.48, 116.78, 125.43, 143.06, 143.39, 153.08, 154.52, (Aromatic C), 155.35 (CH=N). Elemental Analysis: Found: C, 62.68; H, 6.31; N, 3.05. $C_{19}H_{23}NO_6$, requires C, 63.16; H, 6.37; N, 3.88 %.

5.1.4. (2,3,4-Trimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (11i). Preparation as described above from 2,3,4-trimethoxybenzaldehyde (10 mmol, 1.48 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 74 %, Yellow crystals, m.p. 178°C. IR ν_{max} (KBr) cm^{-1}: 1619.6 cm^{-1} (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, O-CH_3), 3.91 (s, 9H, 3xO-CH_3), 3.93 (s, 3H, O-CH_3), 3.98 (s, 3H, O-CH_3), 6.48 (s, 2H, Ar-H), 6.80 (d, 1H, J=8.8 Hz, Ar-H), 7.85 (d, 1H, J=9.0 Hz, Ar-H), 8.74 (s, 1H, CH=N). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.96 (O-CH_3), 60.82 (O-CH_3), 60.87 (O-CH_3), 61.96 (O-CH_3), 98.15, 107.71, 122.45, 123.57, 135.96, 141.64, 148.61, 153.38, 154.47, 155.49 (Aromatic C), 155.25 (CH=N),. Elemental Analysis: Found: C, 63.06; H, 6.44; N, 3.82. $C_{19}H_{23}NO_6$ requires C, 63.10; H, 6.40; N, 3.90 %.

5.1.5. (3,4-Dimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (11j). Preparation as described above from 3,4-dimethoxybenzaldehyde (10 mmol, 1.34 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 73 %, Yellow crystals, m.p. 102-104°C. IR ν_{max} (KBr) cm^{-1}: 1606.9 cm^{-1} (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, O-CH_3), 3.90 (s, 6H, 2xO-CH_3), 3.95 (s, 3H, O-CH_3), 3.99 (s, 3H, O-CH_3), 6.48 (s, 2H, Ar-H), 6.93 (d, 1H, J=8.4 Hz, Ar-H), 7.31-7.33 (m, 1H, Ar-H), 7.60 (s(br), 1H, Ar-H), 8.39 (s, 1H, -CH=N). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.89 (O-CH_3), 55.97 (O-CH_3), 60.90 (O-CH_3), 97.97, 108.35, 110.35, 124.31, 129.25, 135.97, 148.05, 151.92, 153.42 (Aromatic C) 159.13 (CH=N). HRMS: Calculated for $C_{21}H_{22}NO_5$: 332.1498; Found: 332.1485, (M^++H).

5.1.6. Thiophen-2-ylmethylene-(3,4,5-trimethoxyphenyl)amine (11k). Preparation as described above from thiophene-2-carbaldehyde (10 mmol, 1.12 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 81 %, pale yellow crystals, m.p. 169°C. IR ν_{max} (KBr) cm^{-1}: 1617.8 (C=N), 1584.53 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.92 (s, 6H, 2xOCH_3), 6.52 (s, 2H, ArH), 7.16 – 7.18 (m, 1H, ArH), 7.52 – 7.55 (m, 2H, ArH), 8.61 (s, 1H, HC=N). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.65 (O-CH_3), 60.56 (O-CH_3), 97.82, 127.38, 129.90, 131.83, 135.96, 142.17, 146.81, 153.30 (Aromatic C), 151.95 (CH=N). HRMS Calculated for $C_{14}H_{16}NO_3S$: 278.0851; Found: 278.0852, (M^++H). Elemental analysis: Found: C, 60.62; H, 5.44; N, 5.01; $C_{14}H_{15}NO_3S$ requires C, 60.63; H, 5.45; N, 5.05%.

5.1.7. (6-Methoxynaphthalen-2-ylmethylene)-(3,4,5-trimethoxyphenyl)amine (11l). Preparation as described above from 6-methoxynaphthaldehyde (10 mmol, 1.86 g) and 3,4,5-trimethoxyaniline (10 mmol, 1.37 g). Yield 80 %, Yellow crystals, m.p. 40°C. IR ν_{max} (KBr) cm^{-1}: 1616.4 cm^{-1} (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, O-CH_3), 3.91 (s, 6H, 2xO-CH_3), 3.93 (s, 3H, O-CH_3), 6.53 (s, 2H, Ar-H), 7.17 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 8.08-8.12 (m, 2H, Ar-H), 8.58 (s, 1H, N=CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.95 (O-

CH_3), 55.67 (O-CH_3), 60.60 (O-CH_3), 97.72, 105.61, 119.02, 124.08, 127.09, 128.00, 129.89, 130.57, 131.32, 135.82, 136.09, 147.72, 153.14, 158.63 (Aromatic C), 159.36 (N=CH). HRMS: Calculated for $C_{21}H_{22}NO_4$: 352.1549; Found: 352.1549 (M^++H).

5.1.8. [3-(*tert*-Butyldimethylsilanyloxy)(4-methoxybenzylidene)-(3,4,5-trimethoxy phenyl)amine (11b). To a suspension of 2-methoxy-5-[(3,4,5-trimethoxyphenylamino)methyl]phenol (**11a**) (0.02 mol, 6.34 g) and dimethyl-*tert*-butylchlorosilane (0.024 mol) in dry DCM (60 mL) was added 1,8-diazobicyclo[5.4.0] undec-7-ene (DBU) (0.032 mol). The resulting mixture was stirred at room temperature until complete on thin layer chromatography. The solution was then diluted with DCM (80 mL) and washed with water (60 mL), 0.1M HCl (60 mL) and finally with saturated aqueous $NaHCO_3$ (60 mL). The organic layer was removed and dried using anhydrous sodium sulphate. Evaporation of the solvent afforded the product which was recrystallised from ethanol. Yield 91 %, Yellow crystals, m.p. 88-92°C, IR ν_{max} (KBr) cm^{-1}: 1618.8cm^{-1} (C=N). ^{1}H NMR (400 MHz, DMSO): δ 0.21 (s, 6H, CH_3-Si-CH_3), 1.04 (s, 9H, Si-C-$(CH_3)_3$), 3.74 (s, 3H, O-CH_3), 3.87 (s, 6H, 2xO-CH_3), 3.93 (s, 3H, O-CH_3), 6.58 (s, 2H, Ar-H), 7.12 (d, 1H, J=8.2 Hz, Ar-H), 7.48-7.55 (m, 2H, Ar-H), 8.51 (s, 1H, CH=N). ^{13}C NMR (100 MHz, DMSO): δ -5.78 (CH_3-Si-CH_3), 17.71 (CH_3-C-CH_3), 24.74 (C-(CH_3)$_3$), 55.00 (O-CH_3), 59.25 (O-CH_3), 98.04, 111.22, 121.27, 125.43, 129.50, 135.95, 144.68, 147.73, 153.32 (Aromatic C), 158.27 (CH=N). HRMS: Calculated for $C_{23}H_{34}O_5Si$: 432.2216; Found: 432.2213 (M^++H).

5.1.9. [3-(*tert*-Butyldimethylsilanyloxy)-(4-methoxyphenyl]–(3,4,5-trimethoxybenzylidene)amine (11d). Preparation as described above from 2-methoxy-5-[(3,4,5-trimethoxybenzylidene)amino]phenol (**11c**) (0.02 mol, 6.34 g). Yield 71 %, yellow crystals, m.p.108-109°C. IR ν_{max} (KBr) cm^{-1}: 1614.7 cm^{-1} (C=N). ^{1}H NMR (400 MHz, DMSO): δ 0.20 (s, 6H, CH_3-Si-CH_3), 1.03 (s, 9H, Si-C-$(CH_3)_3$), 3.84 (s, 3H, O-CH_3), 3.93 (s,

3H, O-CH_3), 3.96 (s, 6H, 2xO-CH_3), 6.84-6.87 (m, 3H, Ar-H), 7.15 (2H, s, ArH), 8.36 (s, 1H, CH=N). ^{13}C NMR (100 MHz, DMSO): δ -5.05 (CH_3-Si-CH_3), 18.02 (CH_3-C-CH_3), 25.30 (C-CH_3)$_3$), 55.24 (O-CH_3), 55.78 (O-CH_3), 60.53 (O-CH_3), 105.04, 111.72, 113.62, 113.82, 131.52, 140.16, 144.65, 144.95, 149.33, 153.02 (Aromatic C), 157.53 (CH=N). HRMS: Calculated for $C_{23}H_{34}NO_5Si$: 432.2206; Found: 432.2216, (M^++H).

5.1.10. 4-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (12a). To a suspension of zinc dust (0.9 g, 13.8 mmol) in benzene (20 mL) under nitrogen was added trimethylchlorosilane (0.65 mL, 5 mmol) and the resulting mixture was stirred at room temperature for 15 minutes and then under reflux for a further 2 minutes. The suspension was cooled and ([3-(*tert*-butyldimethylsilanyloxy)-4-methoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11b**) (7 mmol, 3.017 g) and ethylbromoacetate (1.33 mL, 12 mmol) were successively added. The reaction mixture was refluxed under nitrogen for 8 hours and then cooled in an ice-water bath. It was then poured over 20 mL of saturated NH_4Cl and 20 mL of 25 % NH_4OH. The mixture was extracted with dichloromethane (20 mL) and the organic layer was further washed with HCl (20 mL, 0.1 N) and water (20 mL). The organic layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure, and the β-lactam was purified by flash chromatography over silica gel (eluent: dichloromethane-ethyl acetate; 19:1) Yield 21 %, yellow crystals, m.p. 90-91°C. IR ν_{max} (KBr) cm^{-1}: 1748.1 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.15 (s, 3H, CH_3-Si-CH_3), 0.16(s, 3H, CH_3-Si-CH_3), 0.88 (s, 9H, C-(CH_3)$_3$), 2.91 (dd, 1H, J= 2.0 Hz, 15.0 Hz, H-3), 3.49 (dd, 1H, J=5.4 Hz, 15.4 Hz, H-3), 3.70 (s, 6H, 2xO-CH_3), 3.75 (s, 3H, O-CH_3), 3.79 (s, 3H, O-CH_3), 4.86, (dd, 1H, J=2.0Hz, J = 5.4 Hz, H-4), 6.54 (s, 2H, Ar-H), 6.81-6.83 (m, 2H, Ar-H), 6.92-6.95 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ -5.13 (CH_3-Si-CH_3), 13.65 (CH_3-C-CH_3), 25.20 (C-(CH_3)$_3$), 46.39 (CH_2), 53.57 (CH), 54.91 (O-CH_3), 55.41 (O-CH_3), 55.69 (O-CH_3), 94.04, 111.20, 111.81, 115.26, 119.05, 129.99, 133.57,

143.98, 150.72, 152.72 (Aromatic C), 164.08 (C=O). HRMS: Calculated for $C_{25}H_{36}NO_6Si$: 474.2311; Found: 474.2312 (M^++H).

5.1.11. 4-([3-*tert*-Butyldimethylsilanyloxy]-4-methoxyphenyl)-3-methyl–1-(3,4,5-trimethoxy phenyl)azetidin-2-one (12b). Preparation as described above from 3-(*tert*-butyldimethylsilanyloxy)-(4-methoxyphenyl]-(3,4,5-trimethoxybenzylidene)amine (**11a**) (10 mmol, 1.8312 g) and ethyl-2-bromopropionate (12 mmol, 1.55 mL) and isolated as mixture of diastereomers. Yield 61 %, brown solid which was used without further purification. IR ν_{max} (film) cm^{-1}: 1745.6 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (s, 3H, Si-CH_3), 0.05 (s, 3H, Si-CH_3), 0.91 (s, 9H, C-$(CH_3)_3$), 1.17-1.21 (m, 2H, -CH_3), 1.26-1.30 (m, 1H, -CH_3), 3.05-3.12 (m, 0.4H, H-3), 3.55-3.58 (m, 0.6H, H-3), 3.68 (s, 6H, 2xO-CH_3), 3.73 (s, 3H, O-CH_3), 3.78 (s, 3H, O-CH_3), 4.44 (bs, 0.4H, H-4), 5.05 (d, 0.6H, J=5.5 Hz, H-4), 6.51 (s, 1H, Ar-H), 6.52 (s, 1H, Ar-H), 6.54 (s(br), 1H, Ar-H), 6.67-6.82 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ -5.38- -5.30 (CH_3-Si-CH_3), 9.05 (CH_3), 12.52 (CH_3), 17.87 (C-($\underline{C}H_3)_3$), 28.73 ($\underline{C}$-$(CH_3)_3$), 48.61 (CH), 54.50 (O-CH_3), 54.87 (O-CH_3), 54.93 (O-CH_3), 55.36 (CH), δ 55.39 (O-CH_3), 60.32 (CH), 62.10 (CH), 94.10, 94.32, 111.48, 111.76, 110.17, 120.08, 127.78, 133.53, 133.61, 144.55, 150.39, 152.90 (Aromatic C), 167.80 (C=O), 167.94 (C=O).

5.1.12. 4-([3-*tert*-Butyldimethylsilanyloxy]-4-methoxyphenyl)-3,3-dimethyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12c). Preparation as described above from [3-(*tert*-butyldimethylsilanyloxy)-(4-methoxyphenyl]-(3,4,5-trimethoxybenzylidene)amine (**11a**) (10 mmol, 1.831 g) and ethyl-2-bromoisobutyrate (12 mmol, 1.77 mL). Yield 80 %, orange solid, which was used without further purification. IR ν_{max} (film) cm^{-1}: 1751.2 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.03 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3), 0.88 (s, 3H, -CH_3), 0.93 (s, 9H, C-$(CH_3)_3$), 1.50 (s, 3H, -CH_3), 3.72 (s, 6H, 2xO-CH_3), 3.77 (s, 3H, O-CH_3), 3.88 (s, 3H, O-CH_3), 4.69 (s, 1H, H-4), 6.57 (s, 2H, Ar-H), 6.67 (d, 1H, J=8.5Hz, Ar-H), 6.77-6.80 (m, 1H, Ar-H), 6.83 (d, 1H, J=2.5 Hz, Ar-H). ^{13}C NMR (100

MHz, $CDCl_3$): δ -4.97 (CH_3-Si-CH_3), 17.60 (C-$\underline{C}H_3)_3$), 18.28 (C-($\underline{C}$)-C), 22.64 (-CH_3), 25.46 (-CH_3), 55.19 (CH_3-$\underline{C}$-CH_3, C_3), 55.30 (O-CH_3), 55.82 (O-CH_3), 60.78 (O-CH_3), 66.25 (CH), 94.84, 111.85, 119.28, 120.10, 127.47, 133.87, 134.01, 144.97, 150.74, 153.30 (Aromatic C), 171.34 (C=O).

5.1.13. 1-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12g) Preparation as described above from [3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-(3,4,5-trimethoxybenzylidene)amine (**11d**) (10 mmol, 4.310 g). Yield 47 %, Orange crystals, m.p. 112°C. IR ν_{max} (KBr) cm^{-1}: 1747.6 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.21 (s, 6H, CH_3-Si-CH_3), 0.87 (s, 9H, C-$(CH_3)_3$), 2.84 (dd, 1H, J=2.0 Hz, 15.0 Hz, H-3), 3.40 (dd, 1H, J=5.4 Hz, 15.0 Hz, H-3), 3.82 (s, 3H, O-CH_3), 3.85 (s, 9H, 3xO-CH_3), 4.79 (dd, 1H, J=2.4 Hz, J = 5.0 Hz H-4), 6.53 (s, 2H, Ar-H), 6.57 (d, 1H, J=3.2 Hz, Ar-H), 6.62 (d, 1H, J=3.4 Hz, Ar-H), 6.72 (d, 1H, J=8.8 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ -5.14 (CH_3-Si-CH_3), 17.97 (CH_3-$\underline{C}$-CH_3), 25.24 (C-$(CH_3)_3$), 43.10 (C_3, CH_2), 55.34 (O-CH_3), 60.35 (O-CH_3), 70.04 (CH), 102.03, 111.82, 113.36, 136.76, 138.00, 152.80 (Aromatic C), 167.08 (C=O). HRMS: Calculated for $C_{25}H_{35}NO_6SiNa$: 496.2131; Found 496.2131 (M^++Na).

5.1.14. 1-[(3-*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl)]-3-methyl-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12h). Preparation as described above from [(3-*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-(3,4,5-trimethoxybenzylidene)amine (**11d**) (5 mmol, 2.158 g) and ethyl-2-bromopropionate (6 mmol, 0.78 mL) and isolated as mixture of diastereomers. Yield 10 %, orange gel. IR ν_{max} (film) cm^{-1}: 1749.1 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.01-0.03 (m, 3H, Si-CH_3), 0.05-0.06 (m, 3H, Si-CH_3), 0.90 (s, 9H, C-$(CH_3)_3$), 0.96 (d, 2H, J=7.6 Hz, CH_3), 1.46 (d, 1H, J=7.0 Hz, CH_3), 3.14 (m, 0.4H, H-3), 3.65-3.68 (m, 0.6H, H-3), 3.75-3.78 (m, 6H, 2xO-CH_3), 3.81-3.84 (m, 6H, 2xO-CH_3), 4.42 (bs, 0.4H, H-4), 5.03 (d, 0.6H, J=5.5 Hz, H-4), 6.42 (s, 1H, Ar-H), 6.55 (s, 1H, Ar-H), 6.65-6.66 (m, 1H, Ar-H), 6.74-6.76 (m, 1H, Ar-H), 6.99-7.02 (m, 1H, Ar-H). ^{13}C NMR (100

MHz, $CDCl_3$):δ -5.32 (CH_3-Si-CH_3), 9.17 (-CH_3), 12.63 (-CH_3), 17.93 (C-(CH_3)$_3$), 25.14 (C-(CH_3)$_3$), 25.28 (C-(CH_3)$_3$), 48.80 (C_3,CH), 54.73 (C_3,CH), 55.38 (O-CH_3), 55.42 (O-CH_3), 55.59 (O-CH_3), 55.64 (O-CH_3), 55.69 (O-CH_3), 60.33 (CH), 60.37 (O-CH_3), 62.81 (CH), 102.13, 103.44, 109.71, 110.71, 110.15, 110.18, 112.13, 112.23, 130.27, 130.88, 131.04, 131.04, 137.05, 137.38, 147.19, 147.23, 153.03, 153.42 (Aromatic C), 167.38 (C=O), 167.51 (C=O). HRMS: Calculated for $C_{26}H_{37}NO_6SiNa$: 510.2288; Found: 510.2281 (M^++Na).

5.1.15. 1-[(3-*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl)]-3,3-dimethyl-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12i). Preparation as described above from [(3-*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-(3,4,5-trimethoxybenzylidene)amine (**11d**) (5 mmol, 2.158 g) and ethyl-2-bromoisobutyrate (6 mmol, 0.88 mL). Yield 18 %, orange solid, m.p. 95°C. IR ν_{max} (film) cm^{-1}: 1747.5 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ -0.17 (s, 3H, Si-CH_3), -0.12 (s, 3H, Si-CH_3), 0.72 (s 9H, C-(CH_3)$_3$), 0.74 (s, 3H, -CH_3), 1.32 (s, 3H, -CH_3), 3.57 (s, 3H, O-CH_3), 3.58 (s, 6H, 2xO-CH_3), 3.65 (s, 3H, O-CH_3), 4.46 (s, 1H, H-4), 6.21 (s, 2H, Ar-H), 6.57 (m, 2H, Ar-H), 6.81 (dd, 1H, J=2.5 Hz, 8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ -5.37 (CH_3-Si-CH_3), 17.23 (C-(C)-C), 22.36 (C-CH_3)$_3$), 25.11 (-CH_3), 25.25 (-CH_3), 54.92 (CH_3-C-CH_3), 55.60 (O-CH_3), 55.69 (O-CH_3), 60.32 (O-CH_3), 66.47 (CH), 94.84, 111.85, 119.28, 120.10, 127.47, 133.87, 134.01, 144.96, 150.74, 153.30 (Aromatic C), 171.33 (C=O). HRMS: Calculated for $C_{27}H_{39}NO_6SiNa$: 524.2444; Found 524.2427 (M^++Na).

5.1.16. 4-(4-Fluorophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12m). Preparation as described above from (4-fluorobenzylidene)-3,4,5-trimethoxyphenylamine (**11e**) (10 mmol, 2.89 g). Yield 53 %, pale orange crystals, m.p. 96°C. IR ν_{max} (KBr) cm^{-1}: 1739.8 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.72 (dd, 1H, J= 2.5 Hz, 15.5 Hz, H-3), 3.34 (dd, 1H, J=5.5 Hz, 15.0 Hz, H-3), 3.48 (s, 6H, 2xO-CH_3), 3.54 (s, 3H, O-CH_3), 4.78 (dd, 1H, J= 2.0 Hz, 5.5 Hz, H-4), 6.34 (s, 2H, Ar-H), 6.83-6.87 (m, 2H, Ar-H), 7.17-7.21 (m, 2H,

Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.42 (CH_2), 53.23 (CH), 55.45 (O-CH_3), 60.44 (O-CH_3), 90.90, 115.58, 127.22, 133.36, 133.52, 133.84, 153.00 (Aromatic C), 160.94 (C-F), 163.40 (C=O). HRMS: Calculated for $C_{18}H_{18}FNO_4Na$: 354.1118; Found 354.1132 (M^++Na).

5.1.17. 4-(2,4,5-Trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12n). Preparation as described above from (2,4,5-trimethoxybenzylidene)-3,4,5-trimethoxyphenylamine (**11f**) (10 mmol, 3.614 g). Yield 28 %, orange gel, IR ν_{max} (film) cm^{-1}: 1746.9 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.01 (dd, 1H, J= 2.0 Hz, 15.0 Hz, H-3), 3.51 (dd, 1H, J=6.0 Hz, 15.1 Hz, H-3), 3.73 (s, 6H, 2xO-CH_3), 3.74 (s, 3H, O-CH_3), 3.75 (s, 3H, O-CH_3), 3.77 (s, 6H, 2xO-CH_3), 5.24 (dd, 1H, J = 2.5, 5.7 Hz, H-4), 5.91 (s, 2H, Ar-H), 6.59 (s, 1H, Ar-H), 6.78 (s,1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.88 (CH_2), 48.02 (CH), 51.70 (O-CH_3), 55.49 (O-CH_3), 56.24 (O-CH_3), 60.45 (O-CH_3), 60.58 (O-CH_3), 92.05, 109.79, 118.39, 133.64, 133.71, 142.68, 145.07, 149.39, 151.32, 152.96, (Aromatic C), 164.68 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_7Na$: 426.1529; Found 426.1534 (M^++Na).

5.1.18.4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12o). Preparation as described above from (4-methoxybenzylidene)-3,4,5-trimethoxyphenylamine (**11g**) (5 mmol, 1.506 g). Yield 43 %, green crystals, m.p. 70-71°C. IR ν_{max} (film): 1747.5 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.85 (dd, 1H, J= 2.5 Hz, 15.0 Hz, H-3), 3.48 (dd, 1H, J=5.5 Hz, J=15.1 Hz, H-3), 3.65 (s, 6H, 2xO-CH_3), 3.70 (s, 3H, O-CH_3), 3.73 (s, 3H, O-CH_3), 4.88 (dd, J= 2.6Hz, 5.5Hz, 1H, H-4), 6.50 (s, 2H, Ar-H), 6.86 (d, 2H, J=8.6 Hz, Ar-H), 7.26 (d, 2H, J=8.6 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.36 (CH_2), 53.56 (CH), 54.78 (O-CH_3), 55.49 (O-CH_3), 60.36 (O-CH_3), 93.92, 113.58, 126.83, 129.48, 133.62, 133.68, 152.94, 159.29 (Aromatic C), 164.14 (C=O). HRMS: Calculated for $C_{19}H_{21}NO_5Na$: 366.1317; Found: 366.1330 (M^++Na).

5.1.19. 4-(4-Methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12p). Preparation as described above from (4-methoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11g**) (5 mmol, 1.506 g) and ethyl-2-bromopropionate (6 mmol, 0.78 mL) and isolated as mixture of diastereomers. Yield 83 %, dark orange gel. IR ν_{max} (film): 1725.5 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (d, 2H, J=7.5 Hz, CH_3), 1.33 (d, 1H, J=7.5 Hz, CH_3), 3.54 (q(br), 0.66H, J=7.5 Hz, H-3), 3.94 (q(br), 0.34H, J=7.5 Hz, H-3), 3.57 (3H, s, O-CH_3), 3.58 (3H, s, O-CH_3), 3.62 (s, 1.2H, O-CH_3), 3.64 (s, 2.8H, O-CH_3), 4.42 (bs, 0.34H, H-4), 5.01 (d, 0.66H, J=6.0 Hz, H-4), 6.44 (s, 1.3H, Ar-H), 6.46 (s, 0.8H, Ar-H), 6.76 (m, 2H, Ar-H), 7.06 (d, 1.33H, J=8.0 Hz, Ar-H), 7.20 (d, 0.67H, J=8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ9.16 (-CH_3), 12.43(CH_3),48.65 (CH), 55.38 (CH), 54.52 (O-CH_3), 54.58 (O-CH_3), 54.65 (O-CH_3), 55.32 (O-CH_3), 57.76 (CH), 60.25 (CH), 62.09 (O-CH_3), 93.98, 94.24, 113.51, 113.93, 126.05, 126.77, 127.67, 129.14, 133.47, 152.90, 158.90 (Aromatic C), 167.78 (C=O), 167.93 (C=O). HRMS: Calculated for $C_{20}H_{23}NO_5Na$: 380.1473; Found 380.1473 (M^++Na).

5.1.20. 4-(4-Methoxyphenyl)-3,3-dimethyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12q). Preparation as described above from (4-methoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11g**) (5 mmol, 1.507 g) and ethyl-2-bromoisobutyrate (6 mmol, 0.88 mL). Yield 68 %, yellow powder, m.p. 110°C. IR ν_{max} (KBr): 1747.6 cm^{-1} (C=O, β-lactam). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, -CH_3), 1.52 (s, 3H, -CH_3), 3.72 (s, 6H, 2xO-CH_3), 3.78 (s, 3H, O-CH_3), 3.81 (s, 3H, O-CH_3), 4.73 (s, 1H, H-4), 6.57 (s, 2H, Ar-H), 6.87 (d, 2H, J=8.5 Hz, Ar-H), 7.14 (d, 2H, J=8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.43 (-CH_3), 22.31 (-CH_3), 54.79 (O-CH_3), 54.93 (C-3), 55.57 (O-CH_3), 60.51 (O-CH_3), 66.11 (CH), 94.50, 113.61, 126.79, 127.39, 133.68, 153.00, 158.92 (Aromatic C), 171.11 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_5Na$: 394.1631; Found: 394.1620 (M^++Na).

5.1.21. 1-(3,4,5-Trimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12r). Preparation as described above from (3,4,5-trimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11h**) (5 mmol, 1.807 g). Yield 68 %, orange gel. IR ν_{max} (film): 1746.9 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.88 (dd, 1H, J= 2.5 Hz, 15.5 Hz, H-3), 3.49 (dd, 1H, J=5.5 Hz, J=15.1 Hz, H-3), 3.64 (s, 3H, O-CH_3), 3.66 (s, 6H, 2xO-CH_3), 3.76 (s, 9H, 3xO-CH_3), 4.82 (dd, J = 5.5 Hz, 2.5 Hz, 1H, H-4), 6.55 (s, 2H, Ar-H), 6.59 (s, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.25 (CH_2), 55.19 (CH), 55.53 (O-CH_3), 55.68 (O-CH_3), 60.22 (O-CH_3), 60.41 (O-CH_3), 90.86, 102.22, 129.58, 133.41, 137.46, 138.00, 152.93, 153.36 (Aromatic C), 164.14 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_7Na$: 426.1529; Found 426.1530 (M^++Na).

5.1.22. 4-(2,3,4-Trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12s). Preparation as described above from (2,3,4-trimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11i**) (5 mmol, 1.807 g). Yield 16 %, yellow gel. IR ν_{max} (film): 1751.7 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.99 (dd, 1H, J= 2.5 Hz, 15.1 Hz, H-3), 3.50 (dd, 1H, J=5.5 Hz, J=15.1 Hz, H-3), 3.73 (s, 6H, 2xO-CH_3), 3.75 (s, 3H, O-CH_3), 3.83 (s, 3H, O-CH_3), 3.86 (s, 3H, O-CH_3), 3.92 (s, 3H, O-CH_3), 5.21 (dd, 1H, J = 2.5 Hz, 6.0 Hz, H-4), 6.57 (s, 2H, Ar-H), 6.64 (d, 1H, J=8.5 Hz, Ar-H), 6.97 (d, 1H, J=8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.34 (CH_2), 48.66 (CH), 55.50 (O-CH_3), 55.56 (O-CH_3), 59.80 (O-CH_3), 60.33 (O-CH_3), 60.95 (O-CH_3), 93.84, 107.10, 120.99, 122.69, 133.64, 139.03, 141.66, 151.42, 153.38 (Aromatic C), 164.38 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_7Na$: 426.1529; Found: 426.1549 (M^++Na).

5.1.23. 4-(3,4-Dimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12t). Preparation as described above from (3,4-dimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11j**) (5 mmol, 1.655 g). Yield 15 %, orange crystals, m.p. 99°C. IR ν_{max} (KBr): 1745.9 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.86 (dd, 1H, J= 2.5 Hz, 15.1

Hz, H-3), 3.48 (dd, 1H, J=5.5 Hz, J=15.1 Hz, H-3), 3.64 (s, 6H, 2xO-CH_3), 3.67 (s, 3H, O-CH_3), 3.69 (s, 6H, 2xO-CH_3), 4.83 (dd, 1H, J= 2.5 Hz, 5.5 Hz, H-4), 5.84 (s(br), 2H, Ar-H), 6.50 (s, 2H, Ar-H), 6.91 (dd, 1H, J=2 Hz, 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.37 (CH_2), 54.16 (O-CH_3), 55.27 (O-CH_3), 55.48 (O-CH_3), 60.52 (CH), 90.91, 105.19, 118.60, 124.46, 127.70, 128.28, 128.63, 133.59, 153.25, 157.90 (Aromatic C), 164.16 (C=O). HRMS: Calculated for $C_{20}H_{23}NO_6Na$: 396.1423; Found: 396.1408 (M^++Na).

5.1.24. 4-(3,4-Dimethoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12u). Preparation as described above from (3,4-dimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11j**) (5 mmol, 1.657 g) and ethyl-2-bromopropionate (6 mmol, 0.78 mL) and isolated as mixture of diastereomers. Yield 10 %, brown gel. IR ν_{max} (film): 1744.6 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (d, 2H, J=7.5 Hz, -CH_3), 1.48 (d, 1H, J=7.5 Hz, -CH_3), 3.16 (m, 0.34H, H-3), 3.69 (m, 0.66H, H-3), 3.72 (s, 3H, O-CH_3), 3.76 (s, 3H, O-CH_3), 3.80 (s, 9H, 3xO-CH_3), 4.50 (d, 0.34H, J=2.0 Hz, H-4), 5.11 (d, 0.66H, J=6.0 Hz, H-4), 6.56 (s, 0.6H, Ar-H), 6.59 (s, 1.4H, Ar-H), 6.72 (d, 1H, J=1.5 Hz, Ar-H), 6.72-6.85 (dd, 1H, J=1.65 Hz, 8.1 Hz, Ar-H), 6.88 (d, 1H, J=8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.18 (-CH_3), 12.62 (-CH_3), 48.84 (CH), 54.59 (CH), 55.40 (O-CH_3), 55.49 (O-CH_3), 55.51 (O-CH_3), 55.54 (O-CH_3), 55.58 (O-CH_3), 58.18 (CH), 60.49(O-CH_3), 60.60 (O-CH_3), 62.65 (CH), 92.06, 94.64, 107.90, 109.31, 110.67, 110.89, 118.28, 119.04, 126.68, 130.08, 133.55, 142.61, 152.98, 153.36 (Aromatic C), 168.00 (C=O), 168.13 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_6Na$: 410.1580; Found: 410.1567 (M^++Na).

5.1.25. 4-(3,4-Dimethoxyphenyl)-3,3-dimethyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12v). Preparation as described above using method 2 from (3,4-dimethoxybenzylidene)-(3,4,5-trimethoxyphenyl)amine (**11j**) (5 mmol, 1.657 g) and ethyl-2-bromoisobutyrate (6 mmol, 0.88 mL) Yield 10 %, green powder, m.p. 102°C. IR ν_{max} (film): 1745.9 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H, -CH_3), 1.52 (s, 3H, -CH_3), 3.73 (s, 6H, 2xO-

CH_3), 3.79 (s, 3H, O-CH_3), 3.84 (s, 3H, O-CH_3), 3.89 (s, 3H, O-CH_3), 4.73 (s, 1H, H-4), 6.60 (s, 2H, Ar-H), 6.70 (d, 1H, J=2.0 Hz, Ar-H), 6.81 (dd, 1H, J=2.0 Hz, 8.5 Hz, Ar-H), 6.88 (d, 1H, J=8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.29 (-CH_3), 22.35 (-CH_3), 30.48 (C-3), 54.99 (O-CH_3), 55.39 (O-CH_3), 55.56 (O-CH_3), 55.58 (O-CH_3), 66.39 (CH), 94.47, 109.01, 110.67, 118.69, 127.33, 133.71, 133.77, 148.32, 148.65, 152.99 (Aromatic C), 171.12 (C=O). HRMS: Calculated for $C_{22}H_{27}NO_6Na$: 424.1736; Found 424.1726 (M^++Na).

5.1.26. 4-Thiophen-2-yl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12w). Preparation as described above from thiophen-2-ylmethylene-(3,4,5-trimethoxyphenyl)amine (**11k**) (5 mmol, 1.387 g). Yield 27 %, dark brown gel. IR ν_{max} (film): 1735.5 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.95 (dd, 1H, J= 15.1 Hz, J=2.9 Hz, H-3), 3.61 (dd, 1H, J=15.1 Hz, J=5.8 Hz, H-3), 3.74 (s, 6H, 2xO-CH_3), 3.76 (s, 3H, O-CH_3), 5.05, (dd, 1H, J=2.9 Hz, 5.5Hz, H-4), 6.61 (s, 2H, Ar-H), 7.00 (m, 1H, thiophene H), 7.17 (d, 1H, J=4.1 Hz, thiophene H), 7.31 (d, 1H, J=5.2 Hz, thiophene H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 47.52 (CH_2), 51.40 (CH), 55.68 (O-CH_3), 55.84 (O-CH_3), 60.85 (O-CH_3), 94.26, 123.82, 125.81, 127.05, 133.67, 134.24, 141.93, 153.31 (Aromatic C), 163.86 (C=O). HRMS: Calculated for $C_{16}H_{18}NO_4S$: 320.0957; Found 320.1003 (M^++H).

5.1.27. 4-(7-Methoxynaphthalen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12x). Preparation as described above from (6-methoxynaphthalen-2-ylmethylene)-(3,4,5-trimethoxyphenyl)amine (**11l**) (5 mmol, 1.757 g). Yield 20 %, orange gel. IR ν_{max} (film): 1736.4 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.02 (dd, 1H, J= 2.5 Hz, 12.1 Hz, H-3), 3.61 (dd, 1H, J=5.5 Hz, J=15.3 Hz, H-3), 3.69 (s, 6H, 2xO-CH_3), 3.76 (s, 3H, O-CH_3), 3.89 (s, 3H, O-CH_3), 5.09, (dd, 1H, J=2.5 Hz, J=5.5 Hz, H-4), 5.89 (s, 2H, Ar-H), 6.62 (s, 1H, Ar-H), 7.10-7.14 (m, 2H, Ar-H), 7.46-7.48 (dd, 1H, Ar-H), 7.69- 7.78 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 47.52 (CH_2), 51.40 (CH), 55.68 (O-CH_3), 55.84 (O-CH_3), 60.85 (O-

CH_3), 90.90, 94.26, 105.31, 118.60, 123.82, 125.81, 127.05, 128.43, 128.85, 129.62, 133.67, 134.24, 141.93, 153.31(Aromatic C), 163.86 (C=O).

5.1.28. 4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12d). To a suspension of 4-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12a**) (0.634 mmol, 0.30 g) in THF (20 mL) was added of 1M tetra-*n*-butylammonium fluoride (1.5 equiv). The solution was stirred in an ice bath for 15 minutes to avoid decomposition of the β-lactam ring. The reaction mixture was then diluted with ethyl acetate (100 mL) and quenched with 10 % HCl (100 mL). The layers are separated and the aqueous layer was extracted with Ethyl acetate (2 x 50 mL). The organic layer was then washed with water (100 mL) and brine (100 mL) and dried with sodium sulphate. The crude product was purified by flash chromatography over silica gel (eluent: dichloromethane). Yield 62 %, yellow gel. IR ν_{max} (film): 1746.0 (C=O), 3404.2 cm^{-1} (OH). ^1H NMR (400 MHz, $CDCl_3$): δ 2.91 (dd, 1H, J=2.4 Hz, 15.6 Hz, H-3), 3.48 (dd, 1H, J=5.5 Hz, 15.0 Hz, H-3), 3.70 (s, 6H, 2xO-CH_3), 3.74 (s, 3H, O-CH_3), 3.85 (s, 3H, O-CH_3), 4.86 (dd, 1H, J=2.5 Hz, J=5.5 Hz, H-4), 6.54 (s, 2H, Ar-H), 6.81-6.94 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.47 (CH_2), 46.31 (CH), 53.66 (O-CH_3), 55.53 (O-CH_3), 60.44 (O-CH_3), 94.01, 110.13, 111.60, 117.35, 130.64, 133.57, 143.21, 145.88, 146.44, 152.94, 164.21 (C=O). HRMS: Calculated for $C_{19}H_{22}NO_6$: 360.1447; Found 360.1454 (M^++H).

5.1.29. 4-(3-Hydroxy-4-methoxyphenyl)-3-methyl–1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12e). Preparation as described above from 4-([3-*tert*-butyldimethylsilanyloxy]-4-methoxyphenyl)-3-methyl–1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12b**) (8 mmol, 3.901 g) and isolated as mixture of diastereomers. Yield 45 %, brown gel. IR ν_{max} (KBr): 1724.2, (C=O), 3240.2 cm^{-1} (OH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (d, 2H, J=7.5 Hz, -CH_3), 1.44 (d, 1H, J=7.5 Hz, -CH_3), 3.09-3.14 (dq, 0.33H, J=2.2 Hz, 7.5 Hz, H-3), 3.59-3.67 (dq, 0.67H, J=7.5 Hz, 6.0 Hz, H-3), 3.72 (s(br), 6H, 2x O-CH_3), 3.76 (s, 1H, O-CH_3), 3.78 (s, 2H, O-

CH_3), 3.89 (s, 3H, O-CH_3), 4.44 (d, 0.33H, J=2.0 Hz, H-4), 5.07 (d, 0.67H, J=5.6 Hz, H-4), 5.81 (bs, 1H, OH), 6.55 (s, 0.67H, Ar-H), 6.57 (s, 1.33H, Ar-H), 6.70-6-85 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.15 (-CH_3), 12.62 (-CH_3), 45.51 (CH), 54.61 (CH), 55.47 (O-CH_3), 55.56 (O-CH_3), 56.62 (O-CH_3), 57.90 (CH), 62.31 (CH), 94.14, 94.37, 110.17, 110.49, 112.71, 118.24, 127.36, 130.48, 133.64, 145.33, 146.29, 152.98 (Aromatic C), 167.94 (C=O), 168.10 (C=O). HRMS: Calculated for $C_{20}H_{23}NO_6Na$: 396.1423; Found: 396.1419 (M^++Na).

5.1.30. 4-(3-Hydroxy-4-methoxyphenyl)-3,3-dimethyl–1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12f). Preparation as described above from 4-([3-*tert*-butyldimethylsilanyloxy]-4-methoxyphenyl)-3,3-dimethyl–1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12c**) (8 mmol, 4.01 g). Yield 53 %, pale brown powder. IR ν_{max} (KBr): 1747.4 (C=O), 3460.1 cm^{-1} (OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (s, 3H, -CH_3), 1.40 (s, 3H, -CH_3), 3.63 (s, 6H, 2xO-CH_3), 3.69 (s, 3H, O-CH_3), 3.79 (s, 3H, O-CH_3), 4.60 (s, 1H, H-4), 6.50 (s, 2H, Ar-H), 6.61 (dd, 1H, J=2.0 Hz, 8.5 Hz, Ar-H), 6.71 (d, 1H, J=2.0 Hz, Ar-H), 6.76 (d, 1H, J=8.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.24 (-CH_3), 22.27 (-CH_3), 54.89 (C-3), 55.53 (O-CH_3), 55.56 (O-CH_3), 60.46 (O-CH_3), 66.06 (CH), 94.54, 110.24, 112.45, 117.80, 127.87, 133.63, 133.73, 145.38, 146.12, 152.96-153.17(Aromatic C), 171.56 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_6Na$: 410.1580; Found: 410.1588 (M^++Na).

5.1.31. 1-(3-Hydroxy-4-methoxyphenyl)]-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12j). Preparation as described above from 1-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12g**) (1.05 mmol, 0.497 g). Yield 39 %, orange gel. IR ν_{max} (film): 1731.9 (C=O), 3500.0 cm^{-1} (OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.90 (dd, 1H, J= 2.7 Hz, 15.0 Hz, H-3), 3.50 (dd, 1H, J=5.5 Hz, 15.3 Hz, H-3), 3.82 (s, 3H, O-CH_3), 3.85 (s, 9H, 3xO-CH_3), 4.86 (dd, 1H, J=2.7 Hz, 5.4 Hz, H-4), 6.55 (s, 2H, Ar-H), 6.73

(d, 1H, J=8.9 Hz, Ar-H), 6.82 (dd, 1H, J=2.1 Hz, 8.8 Hz, Ar-H), 6.95 (d, 1H, J=2.7 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.43 (CH_2), 54.07 (CH), 55.56 (O-CH_3), 55.63 (O-CH_3), 60.34 (O-CH_3), 102.05, 103.92, 107.98, 110.64, 131.38, 133.49, 143.05, 145.56, 152.76, 153.33 (Aromatic C), 164.01 (C=O). HRMS: Calculated for $C_{19}H_{22}NO_6$: 360.1447; Found: 360.1449 (M^++H).

5.1.32. 1-(3-Hydroxy-4-methoxyphenyl)]-3-methyl-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (12k). Preparation as described above from 1-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-3-methyl-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12h**) (0.149 mmol, 0.072 g) and isolated as mixture of diastereomers. Yield 82 %, brown solid, m.p. 165°C; ν_{max} (film): 1739.6 (C=O), 3409.0 cm^{-1} (OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (d, 2H, J=7.6 Hz, -CH_3), 1.40 (d, 1H, J=7.8 Hz, -CH_3), 3.15 (dq, 0.36H, J=2.0 Hz, 7.6 Hz, H-3), 3.64 (dq, 0.64H, J=7.5 Hz, 5.2 Hz, H-3), 3.79 (s, 3H, O-CH_3), 3.83 (m, 9H, 3xO-CH_3), 4.43 (d, 0.36H, J=1.9 Hz, H-4), 5.05 (d, 0.64H, J=5.5 Hz, H-4), 5.81 (bs, 1H, OH), 6.41 (s, 0.89H, Ar-H), 6.53 (s, 1.10H, Ar-H), 6.74 (2xd, overlapping, 1H, J=8.5 Hz, 8.6 Hz, Ar-H), 6.84-6.90 (2xdd overlapping, 1H, J=2.5 Hz, 8.8 Hz, Ar-H), 6.92 (d, 0.5H, J = 2.5Hz), 6.95 (d, 0.5H, J=2.3 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.16 (-CH_3), 12.66 (-CH_3), 48.86 (CH), 54.81 (CH), 55.70 (O-CH_3), 55.73 (O-CH_3), 58.41 (CH), 60.42 (O-CH_3), 62.80 (CH), 102.01, 103.34, 103.81, 103.90, 108.28, 108.39, 110.51, 110.51, 130.23, 131.46, 133.22, 142.73, 142.79, 145.41, 153.02-153.39 (Aromatic C), 167.69 (C=O). HRMS: Calculated for $C_{20}H_{23}NO_6Na$: 396.1423; Found: 396.1443 (M^++Na).

5.1.33. 1-(3-Hydroxy-4-methoxyphenyl)]-3,3-dimethyl-4-(3,4,5-trimethoxyphenyl)-azetidin-2-one (12l). Preparation as described above from 1-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-3,3-dimethyl-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12i**) (0.7 mmol, 0.352 g). Yield 59 %, colourless powder, m.p. 174-178°C. IR ν_{max} (film): 1724.5 (C=O), 3367.7 cm^{-1} (OH). ^{1}H NMR (400 MHz,

$CDCl_3$): δ 0.91 (s, 3H, -CH_3), 1.51 (s, 3H, -CH_3), 3.78 (s, 6H, 2xO-CH_3), 3.83 (s, 3H, O-CH_3), 3.85 (s, 3H, O-CH_3), 4.67 (s, 1H, H-4), 6.39 (s, 2H, Ar-H), 6.74 (d, 1H, J=8.5 Hz, Ar-H), 6.80 (dd, 1H, J=2.5 Hz, 8.8 Hz, Ar-H), 7.08 (d, 1H, J=2.5 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.20 (-CH_3), 22.34 (-CH_3), 54.91 (C-3), 55.66 (O-CH_3), 55.69 (O-CH_3), 60.38 (O-CH_3), 66.60 (C-4), 103.06, 104.37, 108.27, 110.62, 130.79, 131.44, 137.05, 143.00, 145.60, 153.00, 170.98 (C=O). HRMS: Calculated for $C_{21}H_{25}NO_6Na$: 410.1580; Found 410.1591 (M^++Na).

5.1.34 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-thione (13a) 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12o**) (0.206 g, 0.6 mmol) and Lawesson's reagent (0.4 mmol, 0.162 g) (0.68 equiv) were refluxed in toluene (5 mL) at 135°C under N_2 for 3 hours. The solvent was evaporated and the brown solid residue was purified by flash column chromatography (eluent: dichloromethane) to afford the product as a yellow solid. Yield 47.5 %, R_f 0.89 (CH_2Cl_2: MeOH; 19:1), m.p. 84°C. IR: NaCl film υ_{max}: 1595.7 cm^{-1} (C=S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.00 (dd, 1H, J=15.6Hz, J = 2.0Hz, H-3), 3.50 (dd, 1H, J=15.6Hz,, J = 5.0Hz, H-3), 3.72 (s, 6H, 2xO-CH_3), 3.78 (s, 3H, O-CH_3), 3.80 (s, 3H, O-CH_3), 5.43 (dd, J = 2.0Hz, J = 5.0Hz, 1H, H-4), 6.92 (d, 2H, J=8.5 Hz, Ar-H), 7.19 (s, 2H, Ar-H), 7.30-7.32 (d, 2H, J=9.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 49.30 (CH_2), 54.90 (O-CH_3), 55.58 (O-CH_3), 60.43 (O-CH_3), 62.13 (CH), 95.26, 114.21, 127.04, 128.29, 134.11, 135.17, 152.63, 159.65 (Aromatic C), 196.01 (C=S). HRMS, Calculated for $C_{19}H_{21}NO_4SNa$: 382.1089; Found: 382.1076 (M^+ + Na).

5.1.35. 4-(4-Methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-thione (13b) Preparation as described above from 4-(4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12p**) (0.214 g, 0.6 mmol) Evaporation of the solvent yielded a brown solid residue which was purified using by flash column chromatography over silica gel (eluent: dichloromethane) to afford the diastereomeric product as a yellow gel.

Yield 63.8 %, R_f 0.86 (CH_2Cl_2: MeOH; 19:1). IR: NaCl film υ_{max}: 1591.11 cm^{-1} (C=S). ^1H NMR (400 MHz, $CDCl_3$): δ 0.96 (d, 2H, J=7.5 Hz, CH_3), 1.47 (d, 1H, J=7.5 Hz, -CH_3), 2.99-3.04 (m, 0.33H, H-3), 3.46-3.49 (m, 0.67H, H-3), 3.71 (s, 6H, 2xO-CH_3), 3.77 (s, 1H, O-CH_3), 3.80 (s, 5H, O-CH_3), 5.02 (d, J = 1.8Hz, 0.33H, H-4), 5.67 (d, 0.67H, J=5.5 Hz, H-4), 6.88-6.90 (m, 2H, Ar-H), 6.92-7.31 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.31 (-CH_3), 14.59 (-CH_3), 50.66 (CH), 54.80 (O-CH_3), 54.89 (O-CH_3), 55.56 (O-CH_3), 55.61 (O-CH_3), 56.06 (CH), 60.43 (O-CH_3), 66.31 (CH), 70.38 (CH), 95.50, 95.61, 113.73, 114.16, 125.21, 127.06, 127.77, 133.93, 135.07, 152.61, 158.19, 159.59 (Aromatic C), 201.72, 202.21 (C=S). HRMS: Calculated for $C_{20}H_{23}NO_4SNa$: 396.1245; Found: 396.1235 (M^++Na).

5.1.36 4-(4-Methoxyphenyl)-3,3-dimethyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-thione (13c). Preparation as described above from 4-(4-methoxyphenyl)-3,3-dimethyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**12q**) (1.065 g, 2.868 mmol) Evaporation of the solvent yielded a brown solid residue which was purified using flash column chromatography over silica gel (eluent: dichloromethane) to afford the product as a yellow solid. Yield 46.7 %, R_f 0.85 (CH_2Cl_2: MeOH; 19:1), m.p. 94°C. IR: NaCl film υ_{max}: 1595.2 cm^{-1} (C=S). ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (s, 3H, -CH_3), 1.48 (s, 3H, -CH_3), 3.71 (s, 6H, 2xO-CH_3), 3.79 (s, 3H, O-CH_3), 3.795 (s, 3H, O-CH_3), 5.29 (s, 1H, H-4), 6.89 (d, 2H, J=8.6 Hz, Ar-H), 7.1 (d, 1H, J=8.5 Hz, Ar-H), 7.23 (s, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.37 (-CH_3), 24.34 (-CH_3), 54.79 (O-CH_3), 51.14 (C-3), 56.60 (O-CH_3), 60.42 (O-CH_3), δ 73.85 (C-4), 95.91, 113.69, 125.85, 127.46, 133.99, 135.03, 152.62, 159.11 (Aromatic C), 206.80 (C=S). HRMS: Calculated for $C_{21}H_{26}NO_4S$: 388.1583; Found: 388.1586 (M^++1).

5.2. Biochemical evaluation of activity:

5.2.1. Antiproliferation studies. All assays were performed in triplicate for the determination of mean values reported. Compounds were assayed as the free bases isolated

from reaction. 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, 2mM 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 trypsinised and 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 room temperature 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 and from these, graphs of percentage cell viability versus concentration of subject compound added were drawn.

5.2.2. Cell cycle analysis:

Flow cytometry. The MDA-MB-231 cells were seeded at a density of 18 x 10^4 cells/mL in 5 mL of medium (900,000 cells per flask). After 24 hours, cells were treated with either 50 µL of ethanol (1% v/v) as vehicle control or selected compound ranging from 10 nM-10 µM (final concentration). They were incubated for 72 hours. Following incubation, the cells were removed from the bottom of the flask by scraping and the medium placed in a 20 mL

sterilin. Cells were then centrifuged for 10 minutes at 600xg. The supernatant was decanted and the pellet resuspended in 1 mL of ice-cold phosphate buffer saline (PBS); cells were again centrifuged for 10 minutes at 600xg. The supernatant was decanted and the pellet resuspended in 200 μL of ice- cold (PBS). Subsequently ice-cold 70 % ethanol (2 mL) was slowly added to the tube as it was gently vortexed. The cells were kept at -20°C for at least one hour. After the fixation 5 μL of FBS was added to the samples. The cells were harvested by centrifugation at 600xg for 10 mins. The ethanol was carefully removed and the pellet resuspended in 400 μL of PBS and transferred to FACS microtubes. A 25 μL aliquot of RNase A (1 mg/mL) and 75 μL of propidium iodide (PI) 1mg/mL, a DNA binding fluorescent dye, was added to each tube. The samples were wrapped in aluminium foil and incubated for a minimum of 30 min at 37°C. The samples were read at 488 nM using FACscalibur flow cytometer from Becton Dickinson. The FACS data for 10,000 cells was analysed using the Macintosh–based application Cellquest and the data was stored as frequency histograms.

5.2.3. Tubulin polymerisation assay

The effect of compounds on the assembly of purified bovine brain tubulin was determined spectrophotometrically by monitoring the change in turbidity. This assay used a 96-well plate format with 300 μg of > 99 % purified bovine brain tubulin in each well. Lyophilised tubulin (1 mg, Cytoskeleton, Denver, CO) was resuspended on ice in 300 μL in ice-cold G-PEM buffer (80 mM PIPES pH 6.9, 0.5 mM $MgCl_2$, 1 mM EGTA, 1 mM guanidine triphosphate (GTP), 10.2 % (v/v glycerol)) and was left on ice for 1 minute to allow for complete resuspension. 10 μL of 10X strength of each compound tested was pipetted into a half area 96-well plate prewarmed to 37°C. A 100 μL volume of tubulin was then pipetted into the prewarmed plate. Samples were mixed well and tubulin assembly was monitored at

an absorbance of A340 nm at 30 second intervals for 60 minutes at 37°C in a Spectromax 340PC spectrophotometer (Molecular Devices).

5.3. Computational Procedure

For ligand preparation, all compounds were drawn using ACD/Chemsketch v10 and SMILES strings generated. A single conformer 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, the PDB entries 1SA0 and 1SA1 were downloaded from the Protein Data Bank (PDB). All waters were retained in both isoforms. Addition and optimisation of hydrogen positions for these waters was carried out using MOE 2007.09 ensuring all other atom positions remained fixed. Using the reported X-ray structure of tubulin co-crystallised with a colchicine derivative, DAMA-colchicine (PDB entry – 1SA0) [42], possible binding orientations ligands were probed with the docking program FREDv2.2.3 (Openeye Scientific Software) [43]. Docking was carried out using FREDv2.2.3 in conjunction with Chemgauss3. 3-D ligand conformations of compound **12d** were enumerated using CORINAv3.4 (Molecular Networks GMBH) [44] for ligands followed by generation of multiple conformations using OMEGAv2.2.1 (Openeye Scientific Software) [45]. Each conformation was subsequently docked and scored with Chemgauss3 as outlined previously. [46] The top binding poses were refined using the LigX procedure (MOE - Chemical Computing Group) [47] together with Postdock analysis (SVL script; MOE) of the docked ligand poses.

Acknowledgements

This work was supported through funding from the Trinity College IITAC research initiative (HEA PRTLI), Enterprise Ireland (EI), Science Foundation Ireland (SFI), and the

Health Research Board (HRB), with additional support for computational facilities from the Wellcome Trust. A postgraduate research award from Trinity College is gratefully acknowledged.

References

[1] K.H. Downing, E. Nogales, Tubulin and microtubule structure, Curr Opin Cell Biol, 10 (1998) 16-22.
[2] M.A. Jordan, Mechanism of action of antitumor drugs that interact with microtubules and tubulin, Curr Med Chem Anticancer Agents, 2 (2002) 1-17.
[3] M.A. Jordan, L. Wilson, Microtubules as a target for anticancer drugs, Nat Rev Cancer, 4 (2004) 253-265.
[4] J.A. Hadfield, S. Ducki, N. Hirst, A.T. McGown, Tubulin and microtubules as targets for anticancer drugs, Prog Cell Cycle Res, 5 (2003) 309-325.
[5] R.L. Margolis, C.T. Rauch, L. Wilson, Mechanism of colchicine-dimer addition to microtubule ends: implications for the microtubule polymerization mechanism, Biochemistry, 19 (1980) 5550-5557.
[6] B. Bhattacharyya, D. Panda, S. Gupta, M. Banerjee, Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin, Med Res Rev, 28 (2008) 155-183.
[7] G.R. Pettit, S.B. Singh, E. Hamel, C.M. Lin, D.S. Alberts, D. Garcia-Kendall, Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4, Experientia, 45 (1989) 209-211.
[8] J.W. Lippert, 3rd, Vascular disrupting agents, Bioorg Med Chem, 15 (2007) 605-615.
[9] P. Hinnen, F.A. Eskens, Vascular disrupting agents in clinical development, Br J Cancer, 96 (2007) 1159-1165.
[10] K.J. Pinney, C; Edvardsen,K; Chaplin,DJ; Pettit,GR, The Discovery and Development of Combretastatins, in: G.K. Cragg, DGI; Newman,DJ (Ed.) Anticancer Agents from Natural Products, Taylor & Francis, Boca Raton, FL, 2005, pp. 23-46.
[11] A. Cirla, J. Mann, Combretastatins: from natural products to drug discovery, Nat Prod Rep, 20 (2003) 558-564.
[12] G.R. Pettit, C. Temple, Jr., V.L. Narayanan, R. Varma, M.J. Simpson, M.R. Boyd, G.A. Rener, N. Bansal, Antineoplastic agents 322. synthesis of combretastatin A-4 prodrugs, Anticancer Drug Des, 10 (1995) 299-309.
[13] http://www.clinicaltrials.gov/ct/gui/show/NCT00060242;.
[14] M.M. Cooney, J. Ortiz, R.M. Bukowski, S.C. Remick, Novel vascular targeting/disrupting agents: combretastatin A4 phosphate and related compounds, Curr Oncol Rep, 7 (2005) 90-95.
[15] S.L. Young, D.J. Chaplin, Combretastatin A4 phosphate: background and current clinical status, Expert Opin Investig Drugs, 13 (2004) 1171-1182.

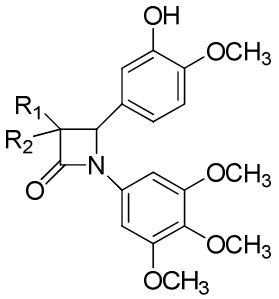
[16] G.R. Pettit, M.R. Rhodes, D.L. Herald, E. Hamel, J.M. Schmidt, R.K. Pettit, Antineoplastic agents. 445. Synthesis and evaluation of structural modifications of (Z)- and (E)-combretastatin A-41, J Med Chem, 48 (2005) 4087-4099.
[17] K. Ohsumi, T. Hatanaka, K. Fujita, R. Nakagawa, Y. Fukuda, Y. Nihei, Y. Suga, Y. Morinaga, Y. Akiyama, T. Tsuji, Syntheses and antitumor activity of cis-restricted combretastatins: 5-membered heterocyclic analogues, Bioorg Med Chem Lett, 8 (1998) 3153-3158.
[18] M. Cushman, D. Nagarathnam, D. Gopal, A.K. Chakraborti, C.M. Lin, E. Hamel, Synthesis and evaluation of stilbene and dihydrostilbene derivatives as potential anticancer agents that inhibit tubulin polymerization, J Med Chem, 34 (1991) 2579-2588.
[19] G.C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca, A.A. Genazzani, Medicinal chemistry of combretastatin A4: present and future directions, J Med Chem, 49 (2006) 3033-3044.
[20] N.H. Nam, Combretastatin A-4 analogues as antimitotic antitumor agents, Curr Med Chem, 10 (2003) 1697-1722.
[21] H.P. Hsieh, J.P. Liou, N. Mahindroo, Pharmaceutical design of antimitotic agents based on combretastatins, Curr Pharm Des, 11 (2005) 1655-1677.
[22] M. Cushman, D. Nagarathnam, D. Gopal, H.M. He, C.M. Lin, E. Hamel, Synthesis and evaluation of analogues of (Z)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents, J Med Chem, 35 (1992) 2293-2306.
[23] K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama, T. Tsuji, Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure-Activity Relationships J Med Chem, 41 (1998) 3022-3032.
[24] T.H. Brown, H,Jr; Lee, M Series: Topics in Heterocyclic Chemistry, Vol. 2 in: M.E. Lee (Ed.) Heterocyclic Antitumor Antibiotics, Springer, Berlin/Heidelberg, 2006, pp. 1-51.
[25] L. Wang, K.W. Woods, Q. Li, K.J. Barr, R.W. McCroskey, S.M. Hannick, L. Gherke, R.B. Credo, Y.H. Hui, K. Marsh, R. Warner, J.Y. Lee, N. Zielinski-Mozng, D. Frost, S.H. Rosenberg, H.L. Sham, Potent, orally active heterocycle-based combretastatin A-4 analogues: synthesis, structure-activity relationship, pharmacokinetics, and in vivo antitumor activity evaluation, J Med Chem, 45 (2002) 1697-1711.
[26] R. Shirai, H. Takayama, A. Nishikawa, Y. Koiso, Y. Hashimoto, Asymmetric synthesis of antimitotic combretadioxolane with potent antitumor activity against multi-drug resistant cells, Bioorg Med Chem Lett, 8 (1998) 1997-2000.
[27] G.C. Tron, F. Pagliai, E. Del Grosso, A.A. Genazzani, G. Sorba, Synthesis and cytotoxic evaluation of combretafurazans, J Med Chem, 48 (2005) 3260-3268.
[28] O.G. Ganina, E. Daras, V. Bourgarel-Rey, V. Peyrot, A.N. Andresyuk, J.P. Finet, A.Y. Fedorov, I.P. Beletskaya, S. Combes, Synthesis and biological evaluation of polymethoxylated 4-heteroarylcoumarins as tubulin assembly inhibitor, Bioorg Med Chem, 16 (2008) 8806-8812.
[29] J.P. Liou, Y.L. Chang, F.M. Kuo, C.W. Chang, H.Y. Tseng, C.C. Wang, Y.N. Yang, J.Y. Chang, S.J. Lee, H.P. Hsieh, Concise synthesis and structure-activity relationships of combretastatin A-4 analogues, 1-aroylindoles and 3-aroylindoles, as novel classes of potent antitubulin agents, J Med Chem, 47 (2004) 4247-4257.
[30] I. Banik, F.F. Becker, B.K. Banik, Stereoselective synthesis of beta-lactams with polyaromatic imines: entry to new and novel anticancer agents, J Med Chem, 46 (2003) 12-15.
[31] L. Sun, N.I. Vasilevich, J.A. Fuselier, S.J. Hocart, D.H. Coy, Examination of the 1,4-disubstituted azetidinone ring system as a template for combretastatin A-4 conformationally restricted analogue design, Bioorg Med Chem Lett, 14 (2004) 2041-2046.

[32] M.J. Meegan, M. Carr, A.J. Knox, D.M. Zisterer, D.G. Lloyd, Beta-lactam type molecular scaffolds for antiproliferative activity: synthesis and cytotoxic effects in breast cancer cells, J Enzyme Inhib Med Chem, 23 (2008) 668-685.
[33] C.C. Palomo, Fernando P.; Arrieta, Ana; Odriozola, Jose M.; Oiarbide, Mikel; Ontoria, Jesus M. , The Reformatskii type reaction of Gilman and Speeter in the preparation of valuable β-lactams in carbapenem synthesis: scope and synthetic utility Journal of Organic Chemistry 54 (1989) 5736-5745.
[34] A. Gaudemer, Determination of Configurations by NMR Spectroscopy, in: H.B. Kagan (Ed.) Stereochemistry: Fundamentals and Methods, Vol 1, Determination of Configurations by Spectroscopic Methods Georg Thieme Publishers, Stuttgart, 1977, pp. 87.
[35] S.B. Rosenblum, T. Huynh, A. Afonso, H.R. Davis, Jr., N. Yumibe, J.W. Clader, D.A. Burnett, Discovery of 1-(4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4 -hydroxyphenyl)-2-azetidinone (SCH 58235): a designed, potent, orally active inhibitor of cholesterol absorption, J Med Chem, 41 (1998) 973-980.
[36] J.W. Clader, The discovery of ezetimibe: a view from outside the receptor, J Med Chem, 47 (2004) 1-9.
[37] B.L. Flynn, G.P. Flynn, E. Hamel, M.K. Jung, The synthesis and tubulin binding activity of thiophene-based analogues of combretastatin A-4, Bioorg Med Chem Lett, 11 (2001) 2341-2343.
[38] G. De Martino, G. La Regina, A. Coluccia, M.C. Edler, M.C. Barbera, A. Brancale, E. Wilcox, E. Hamel, M. Artico, R. Silvestri, Arylthioindoles, potent inhibitors of tubulin polymerization, J Med Chem, 47 (2004) 6120-6123.
[39] J.M. Bidlack, R.A. Lockshin, Evolution of LDH isozymes during programmed cell death, Comp Biochem Physiol B, 55 (1976) 161-166.
[40] *W.t.S. C-QSAR; Biobyte Corp., Suite 204, Claremont, CA).*
[41] M. Cushman, H.M. He, C.M. Lin, E. Hamel, Synthesis and evaluation of a series of benzylaniline hydrochlorides as potential cytotoxic and antimitotic agents acting by inhibition of tubulin polymerization, J Med Chem, 36 (1993) 2817-2821.
[42] R.B. Ravelli, B. Gigant, P.A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, M. Knossow, Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, Nature, 428 (2004) 198-202.
[43] T. Schulz-Gasch, M. Stahl, Binding site characteristics in structure-based virtual screening: evaluation of current docking tools, J Mol Model, 9 (2003) 47-57.
[44] M. Feher, C.I. Williams, Effect of Input Differences on the Results of Docking Calculations, J Chem Inf Model, (2009).
[45] T. Takagi, M. Amano, M. Tomimoto, Novel method for the evaluation of 3D conformation generators, J Chem Inf Model, 49 (2009) 1377-1388.
[46] I. Barrett, M. Carr, N. O'Boyle, L.M. Greene, A.J. Knox, D.G. Lloyd, D.M. Zisterer, M.J. Meegan, Lead identification of conformationally restricted benzoxepin type combretastatin analogs: synthesis, antiproliferative activity, and tubulin effects, J Enzyme Inhib Med Chem, 25 (2010) 180-194.
[47] MOEv2008.10, developed and distributed by Chemical Computing Group, http://www.chemcomp.com.
[48] G.R. Pettit, M.R. Rhodes, D.L. Herald, E. Hamel, J.M. Schmidt, R.K. Pettit, Antineoplastic Agents. 445. Synthesis and Evaluation of Structural Modifications of (Z)- and (E)-Combretastatin A-4, J Med Chem, 48 (2005) 4087-4099.
[49] R.N. Shirai, H.; Okabe, T.; Koiso, K.; Iwasaki, S., , Preparation of azacombretastatin compounds as antitumor agents, office , e.p. Ed.: Japan, Vol. JP0806765619960312 (1996).

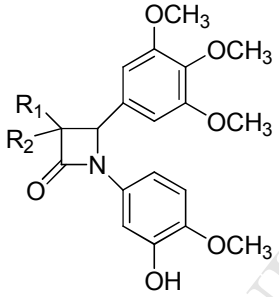
[50] M.C. Cariou, R.; Simonet, J., Enamines and endiamines: synthesis and anodic oxidation. Application in the formation of new heterocycles, Bull Soc Chim Fr, 5 (1986) 781-792.

List of Tables, Schemes, Figures & Legends

Table 1: Antiproliferative effects of β-lactam compounds **12d-f, 12j-l** in MCF-7 cells and MDA-MB-231 cells.



12d-f



12j-l

Compound Number	R_1	R_2	Antiproliferative activity [a,b] MCF-7 cells IC_{50} value (μM)	Cytotoxicity % death [c] 10 μM	Antiproliferative activity [a,b] MDA-MB-231 cells IC_{50} value (μM)	Cytotoxicity % death [c] 10 μM
12d	H	H	0.017 ± 0.002	9	0.054 ± 0.046	16
12e[d]	CH_3	H	0.010 ± 0.0032	15	0.047 ± 0.041	25
12f	CH_3	CH_3	0.25 ± 0.08	12	0.27 ± 0.15	20
12j	H	H	0.130 ± 0.054	2.3	1.67 ± 0.06	6.6
12k[d]	CH_3	H	2.96 ± 0.61	17	13.03 ± 4.36	0
12l	CH_3	CH_3	4.04 ± 2.35	11.5	3.19 ± 2.56	12.1
2 (CA-4)	-	-	0.0031	5.5	0.043	4.3

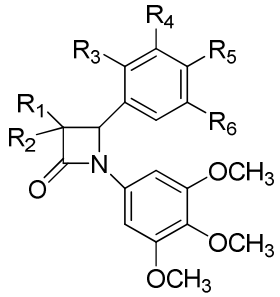
[a] IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 or MDA-MB-231 cells. Values represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

[b] The IC_{50} values obtained for Combretastatin CA-4 in this assay are 0.0031 μM for MCF-7 and 0.043 μM for MDA-MB 231 and are in good agreement with the reported values for Combretastatin CA-4 using the MTT assay on human MCF-7 and MDA-MB 231 breast cancer cell lines, (see references 7, 37 and 38).

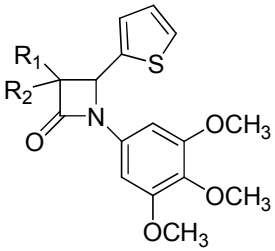
[c] Lactate Dehydrogenase assay: Following treatment of the cells, the amount of LDH was determined using LDH assay kit from Promega. Data is presented as % cell lysis at compound concentration of 10μM, (see reference 39).

[d] 3-Methyl substituted compounds **12e** and **12k** isolated and tested as *cis/trans* mixture

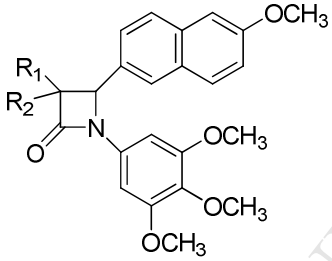
Table 2: Antiproliferative effects of β-lactam compounds **12m-x, 13a-c** in MCF-7 cells and MDA-MB-231 cells.



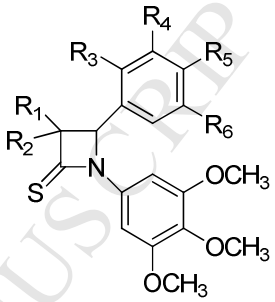
12m-v



12w



12x



13a-c

Compound Number	R_1	R_2	R_3	R_4	R_5	R_6	Antiproliferative activity MCF-7 cells IC_{50} value (μM)[a,b]	Cytotoxicity % death 10 μM[c]	Antiproliferative activity MDA-MB-231 cells IC_{50} value (μM) [a,b]	Cytotoxicity % death 10 μM[c]
12m	H	H	H	H	F	H	25.95 ± 11.95	0	25.95 ± 15.1	6.3
12n	H	H	OCH_3	H	OCH_3	OCH_3	63.74 ± 19.8	2.2	38.5 ± 6.43	0
12o	H	H	H	H	OCH_3	H	0.039 ± 0.013	1.5	0.21 ± 0.17	1.1
12p[d]	H	CH_3	H	H	OCH_3	H	0.047 ± 0.024	5.7	0.127 ± 0.0096	4.5
12q	CH_3	CH_3	H	H	OCH_3	H	0.265 ± 0.010	12	1.15 ± 0.16	16
12r	H	H	H	OCH_3	OCH_3	OCH_3	167.9 ± 121.1	1.9	94.39 ± 18.55	0
12s	H	H	OCH_3	OCH_3	OCH_3	H	0.31 ± 0.036	8.0	2.83 ± 1.98	9.0
12t	H	H	H	OCH_3	OCH_3	H	1.64 ± 0.02	5.0	0.97 ± 0.008	15
12u[d]	H	CH_3	H	OCH_3	OCH_3	H	2.96 ± 0.61	7.5	8.84 ± 0.50	6.1
12v	CH_3	CH_3	H	OCH_3	OCH_3	H	0.344 ± 0.116	14	1.46 ± 0.83	12.0
12w	H	H	-	-	-	-	21.6 ± 14.5	20	34.47 ± 1.50	15
12x	H	H	-	-	-	-	1.51 ± 0.56	13.5	13.18 ± 1.61	18
13a	H	H	H	H	OCH_3	H	1.12 ± 0.10	16	4.50 ± 2.08	3.0

13b[d]	CH_3	H	H	H	OCH_3	H	0.89 ± 0.21	3.2	9.26 ± 2.07	5.1
13c	CH_3	CH_3	H	H	OCH_3	H	0.35 ± 0.012	5.5	1.33 ± 0.11	8.0
2 (CA-4)	-	-	-	-	-	-	0.0031	5.6	0.043	4.3

[a]IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 or MDA-MB-231 cells. Values represent the mean ± S.E.M (error values x 10^{-6}) for three experiments performed in triplicate.

[b]The IC_{50} values obtained for Combretastatin CA-4 in this assay are 0.0031 µM for MCF-7 and 0.043 µM for MDA-MB 231 and are in good agreement with the reported values for Combretastatin CA-4 using the MTT assay on human MCF-7 and MDA-MB 231 breast cancer cell lines (see references 7, 37 and 38)

[c]Lactate Dehydrogenase assay: Following treatment of the cells, the amount of LDH was determined using LDH assay kit from Promega. Data is presented as % cell lysis at compound concentration of 10µM (see reference 39).

[d] 3-Methyl substituted compounds **12p**, **12u** and **13b** isolated and tested as *cis/trans* mixture

Table 3: Evaluation of G_2/M arrest in MDA-MB-231 cells exposed to compound **12d**[a]

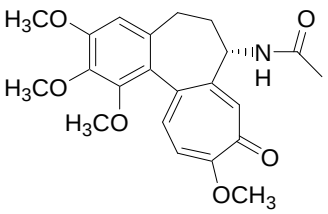
Concentration	Time	Sub-G_1 (%)	G_1 (%)	S (%)	G_2/M (%)	Polyploid (%)
Control	24 h	5.71 ± 3.47	51.20 ± 2.37	10.46 ± 4.19	20.18 ± 3.18	4.53 ± 1.66
10 nM	24 h	3.72 ± 1.97	48.33 ± 4.18	12.55 ± 2.54	25.61 ± 0.98	2.84 ± 0.59
100 nM	24 h	4.88 ± 0.85	18.95 ± 6.60	6.05 ± 0.38	59.43 ± 3.13	4.87 ± 4.39
1 µM	24 h	7.08 ± 4.20	19.22 ± 6.19	6.01 ± 2.90	63.16 ± 4.86	4.31 ± 3.17
10 µM	24 h	3.18 ± 0.25	29.30 ± 5.95	3.72 ± 0.71	55.36 ± 1.49	3.85 ± 3.83
Control	48h	9.80 ± 1.55	50.34 ±11.09	10.50 ±0.77	18.81 ±1.04	5.62 ±3.06
10 nM	48h	11.57 ± 3.06	45.25 ± 7.37	13.36 ±2.58	23.71 ±7.81	6.10 ±0.62
100 nM	48h	37.50 ± 0.71	10.76 ± 0.34	8.90 ± 0.85	36.42 ±3.42	4.61 ±0.86
10 µM	48h	51.27 ± 6.31	14.44 ± 2.28	4.47 ± 0.06	27.25 ±4.45	2.28 ±0.06
Control	72h	12.92 ± 2.61	44.13 ± 2.31	9.10 ± 2.75	21.51 ± 4.86	5.48 ± 3.26
10 nM	72h	15.25 ± 2.03	48.24 ± 2.79	9.08 ± 2.32	17.68 ± 4.58	3.55 ± 1.57
100 nM	72h	63.10 ± 20.06	13.90 ± 3.62	5.34 ± 4.32	12.62 ± 9.22	2.99 ± 1.89
1 µM	72h	50.53 ± 14.78	14.29 ± 2.20	7.76 ± 0.58	21.24 ± 12.05	5.97 ± 4.71
10 µM	72h	46.49 ± 17.49	17.14 ± 3.31	9.66 ± 1.84	21.81 ± 12.40	3.38 ± 2.84

[a]Cell cycle analysis of MDA-MB-231 cells treated with vehicle control (1 % (v/v) ethanol), or 10 nM, 100 nM, 1 µM, and 10 µM(final concentrations) of compound **12d** at 24 and 72 hours, and at 10 nM, 100 nM and 10 µM(final concentrations) of compound **12d** at 48h . % MDA-MB-231 cells in each cell cycle phase are shown after exposure to compound **12d**. Cells were analysed with the FACScan flow cytometry. Cells in the sub-G1 peak are indicative of apoptotic cells. Results show a typical experiment which has been repeated three times. Values represent the mean ± standard deviation for three experiments.

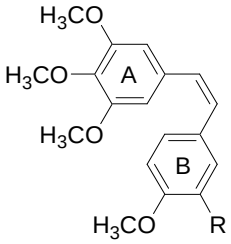
Table 4: Inhibition of tubulin polymerisation in response to β-lactam combretastastin analogues

Compound	Concentration	Fold inhibition of tubulin polymerisation[a] (Vmax ±SEM)
Ethanol (Control)	[1% v/v]	1
CA-4	10 μM	6.0 ± 1.4
12d	10 μM	10.2 ± 2.3
12n	10 μM	1.2 ± 0.2

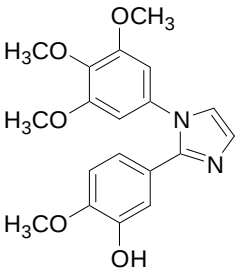
[a]Effects of **12d** and **12n** on *in vitro* tubulin polymerisation. Purified bovine tubulin and GTP were mixed in a 96-well plate. The reaction was started by warming the solution from 4 ^{o}C to 37^{o}C. CA-4 (10μM) was used as a reference, while ethanol (1%v/v) was used as a vehicle control. The effect on tubulin assembly was monitored in a Spectramax 340PC spectrophotometer at 340nm at 30 second intervals for 60 minutes at 37 ^{o}C. Fold inhibition of tubulin polymerisation was calculated using the Vmax value for each reaction. The results represent the mean ± standard error of the mean for three separate experiments.



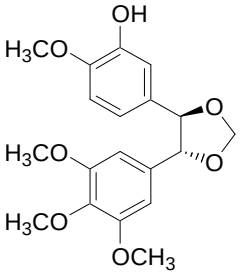
1 Colchicine



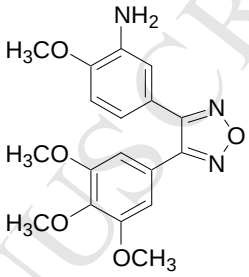
2 R = OH, Combretastatin A-4
3 R = OPO_3Na_2



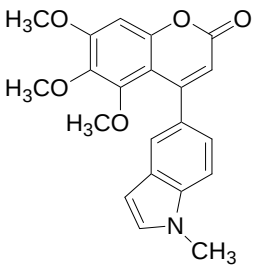
4



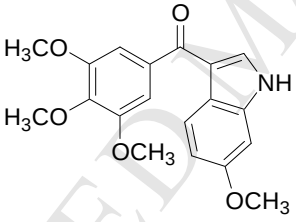
5



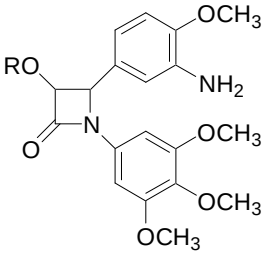
6



7



8



9 R = CH_3
10 R = H

Figure 1: Colchicine (**1**), Combretastatin A4 (**2**), Combretastatin A4 phosphate (**3**) and related antitubulin compounds **4-10**

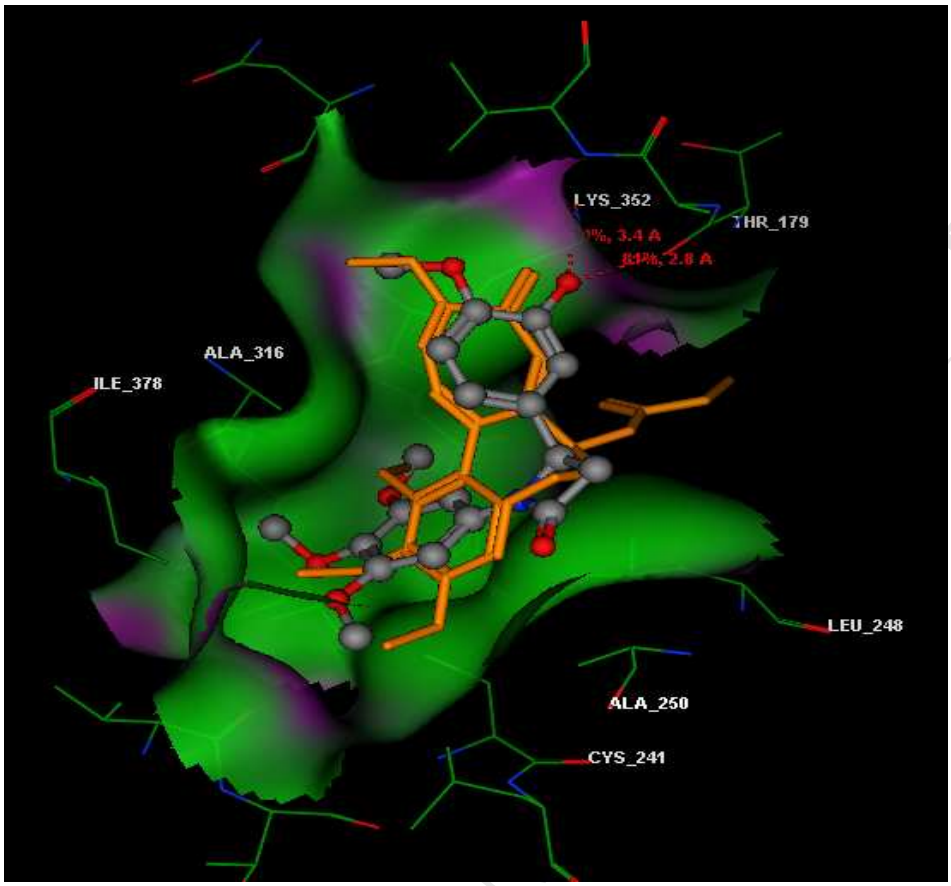


Figure 2. Docked pose of β-lactam **12d** overlayed with N-deacetyl-N-(2-mercaptoacetyl)colchicine (DAMA-colchicine) in the tubulin binding site (PDB entry 1SA0). Hydrogen-bonds were created with an svl script through MOE.

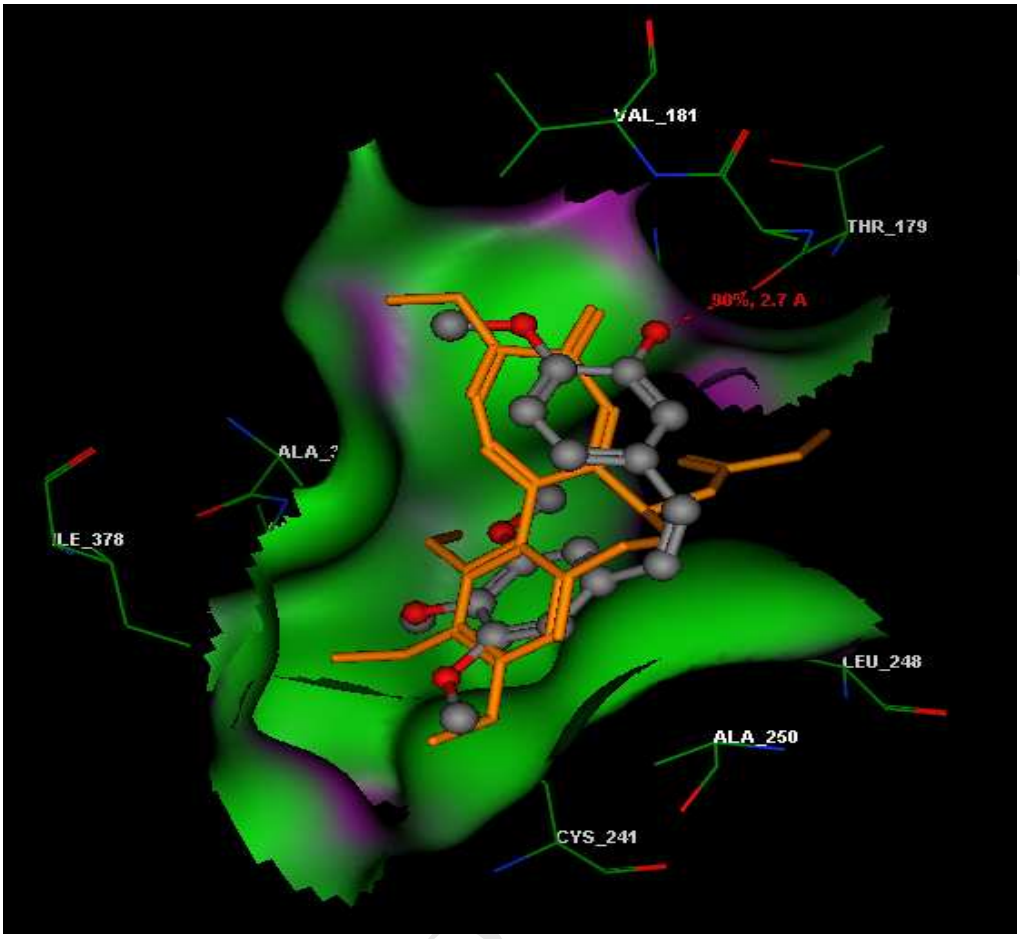
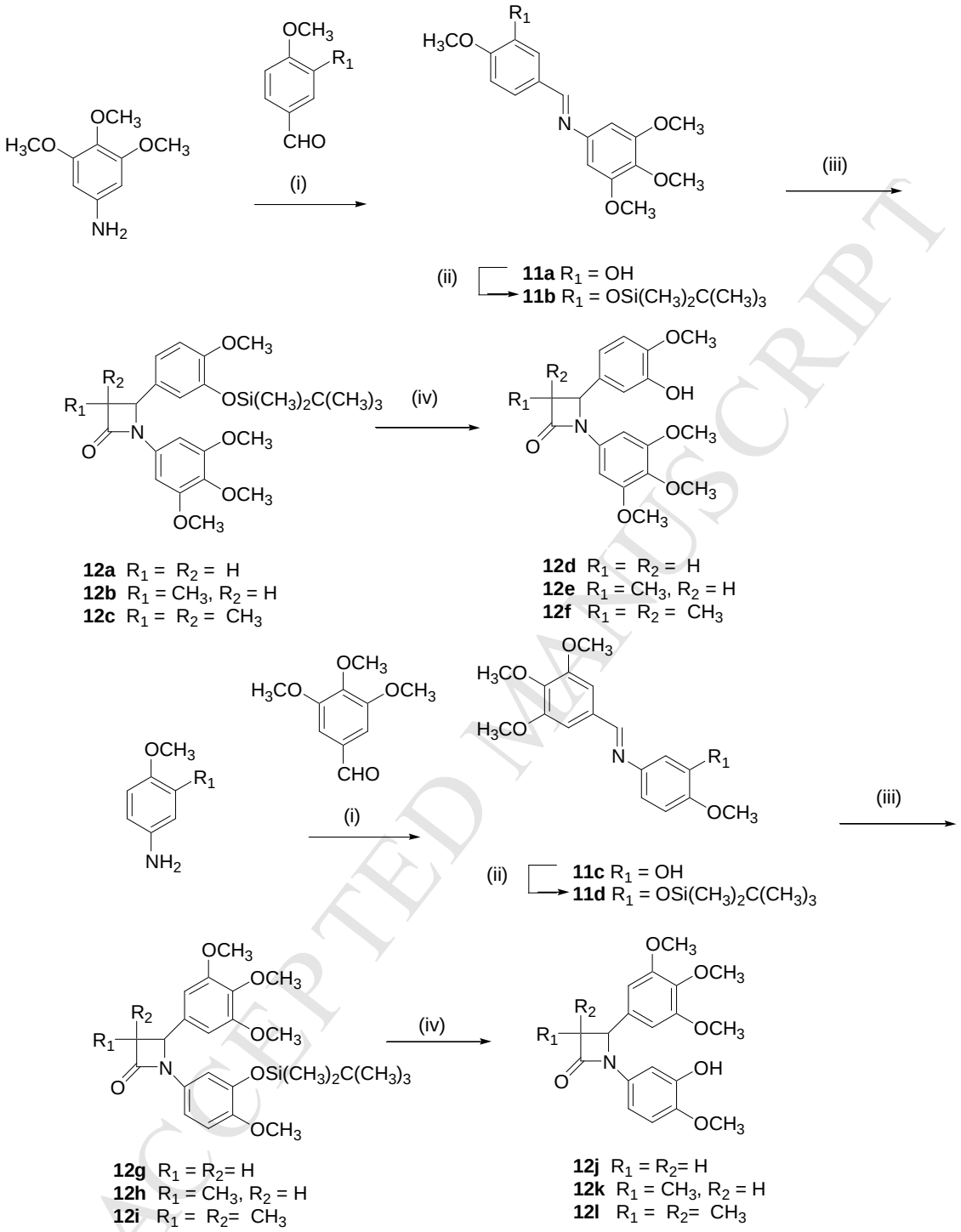
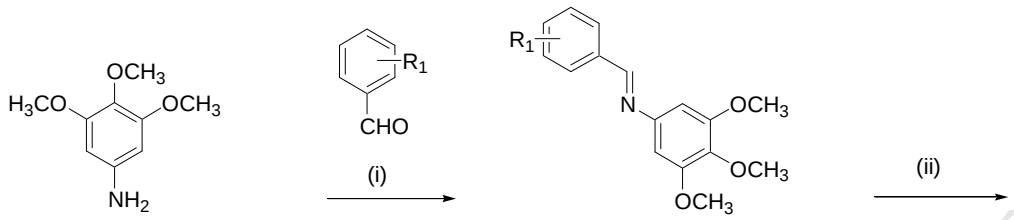


Figure 3. Docked pose of CA4 overlayed with N-deacetyl-N-(2-mercaptoacetyl)colchicine (DAMA-colchicine) in the tubulin binding site (PDB entry 1SA0).

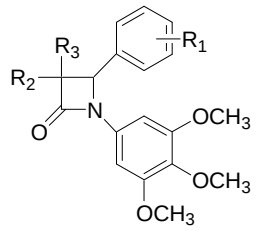


Scheme 1: Synthesis of compounds **12a-l**

Scheme reagents and conditions: (i) EtOH, reflux, 2.5h.(ii) $(CH_3)_3C(CH_3)_2SiCl$, K_2CO_3, CH_2Cl_2, DBU, 20^oC. (iii) $BrCH_2CO_2Et$, $BrCH(CH_3)CO_2Et$ or $Br(CH_3)_2CCO_2Et$, Zn, $(CH_3)_3SiCl$, C_6H_6, reflux.(iv) $(CH_3CH_2CH_2CH_2)_4NF$, THF, 0^oC.

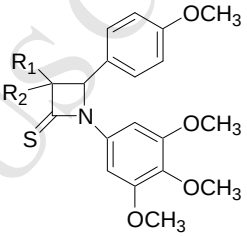


11e R_1 = 4-F
11f R_1 = 2,4,5-OCH_3
11g R_1 = 4- OCH_3
11h R_1 = 3,4,5-OCH_3
11i R_1 = 2,3,4-OCH_3
11j R_1 = 3,4-OCH_3

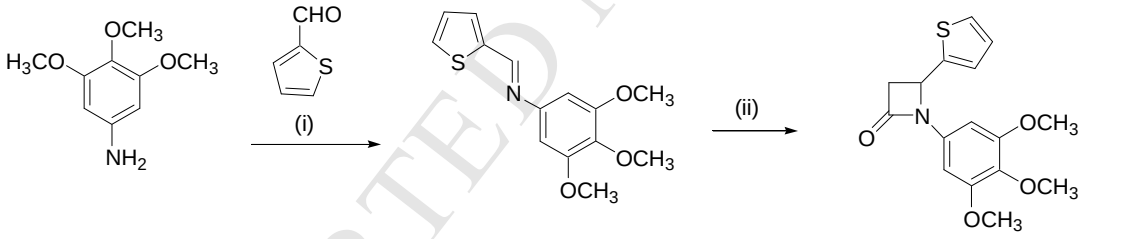


12m R_1 = 4-F, R_2 = R_3 = H
12n R_1 = 2,4,5-OCH_3, R_2 = R_3 = H
12o R_1 = 4- OCH_3, R_2 = R_3 = H
12p R_1 = 4- OCH_3, R_2 = CH_3, R_3 = H
12q R_1 = 4- OCH_3, R_2 = R_3 = CH_3
12r R_1 = 3,4,5-OCH_3, R_2 = R_3 = H
12s R_1 = 2,3,4-OCH_3, R_2 = R_3 = H
12t R_1 = 3,4-OCH_3, R_2 = R_3 = H
12u R_1 = 3,4-OCH_3, R_2 = CH_3, R_3 = H
12v R_1 = 3,4-OCH_3, R_2 = R_3 = CH_3

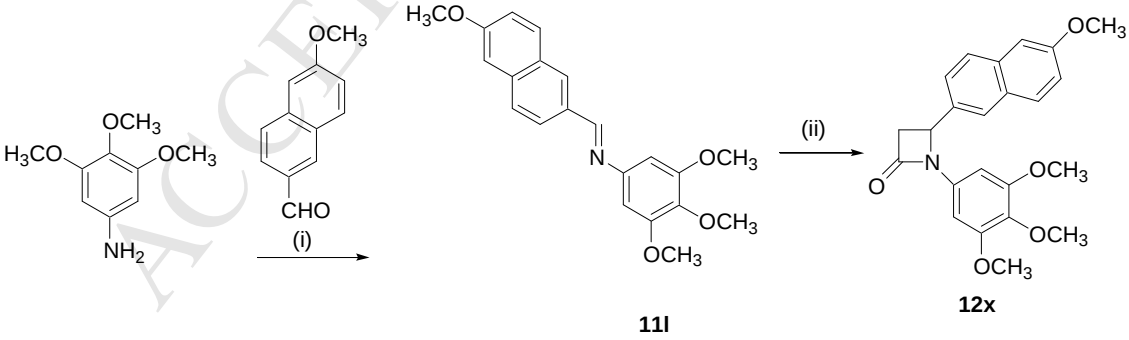
12o-q (iii)



13a R_1 = R_2 = H
13b R_1 = CH_3, R_2 = H
13c R_1 = R_2 =CH_3



11k **12w**



11l **12x**

Scheme 2: Synthesis of compounds **12m-x**
Scheme reagents and conditions: (i) EtOH, reflux, 2.5h. (ii) $BrCH_2CO_2Et$, $BrCH(CH_3)CO_2Et$ or $Br(CH_3)_2CCO_2Et$, Zn, $(CH_3)_3SiCl$, C_6H_6, reflux.(iii) Lawesson's reagent, toluene, reflux, 3h