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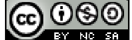

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β-Lactam Estrogen Receptor Antagonists and a Dual-Targeting Estrogen Receptor/Tubulin Ligand

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Abstract

Twelve novel β-lactams were synthesised and their antiproliferative effects and binding affinity for the predominant isoforms of the estrogen receptor (ER), ERα and ERβ, were determined. β-Lactams **23** and **26** had the strongest binding affinities for ERα (IC_{50} values: 40 and 8 nM respectively) and ERβ (IC_{50} values: 19 and 15 nM). β-Lactam **26** was the most potent in antiproliferative assays using MCF-7 breast cancer cells, and further biochemical analysis showed that it caused accumulation of cells in G_2/M phase (mitotic blockade) and depolymerisation of tubulin in MCF-7 cells. Compound **26** also induced apoptosis and downregulation of the pro-survival proteins Bcl-2 and Mcl-1. Computational modeling predicted binding preferences for the dual ER/tubulin ligand **26**. This series is an important addition to the known pool of ER antagonists and β-lactam **26** is the first reported compound that has dual-targeting properties for both the ER and tubulin.

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Key words

Antiproliferative, azetidinone, designed multiple ligand, estrogen receptor, β-lactam, polypharmacy, SERM, tubulin

Introduction

Estrogen receptors (ERs) belong to the superfamily of nuclear receptors, which includes steroid receptors, thyroid receptors and vitamin D receptors.[1] The ER is a ligand-activated transcriptional regulator that mediates the effects of the endogenous hormone 17β-estradiol (**1**, Figure 1). ERs possess two activation domains (AF1 and AF2) that promote interactions with co-regulator proteins and facilitate transcriptional activation of target gene expression.[2] Approximately 75% of breast tumours express ER[3] and treatment of breast cancer with ER antagonists contributes to a dramatic reduction in breast cancer mortality.[4-5] Tamoxifen (**2**, Figure 1) is a synthetic, non-steroidal anti-estrogenic drug used clinically for the treatment of breast cancer.[1] It is metabolically activated *in vivo* into 4-hydroxytamoxifen and *N*-desmethyltamoxifen followed by secondary metabolism to 4-hydroxy-*N*-desmethyltamoxifen (endoxifen).[6] Compound **2** has high binding affinity for the ER and it is termed a selective estrogen receptor modulator (SERM) as it exhibits tissue-dependent effects, imitating the action of estrogens in certain tissues while opposing their action in others.[2] Compound **2** was the first clinically successful SERM that acted as an antagonist at the ER in breast tissue and prevented estrogen-stimulated breast tumour growth.[7] Recent clinical guidelines recommend the prophylactic use of **2** for particular groups of women with a family history of cancer.[8] The main concerns regarding usage of **2**, namely increased incidences of blood clots and endometrial cancer, are linked to its estrogen-like properties in postmenopausal women.[7] Raloxifene (**3**, Figure 1) was the first of the 2,3-disubstituted benzothiophene group of SERMs to be introduced into clinical use for the treatment of osteoporosis in postmenopausal women.[9] It has the additional benefits of preventing coronary heart disease and breast cancer.[4] At a

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molecular level, the basic side-chain of **3** interferes with exposure of the AF1 and AF2 sites and prevents interaction with nuclear receptor co-activators.[4] Many alternative non-isomerisable scaffolds for ER modulators have been reported, e.g. tetrahydronaphthalene (lasofoxifene),[10] pyrazole,[11] tetrahydroisoquinoline,[12] benzopyran (EM-652)[13] and dihydrobenzoxathiin.[14] Carbocyclic and heterocyclic core scaffolds have been reported as ER agonist ligands e.g. the isoflavone genistein (**4**),[15] the propylpyrazole triol (PPT) **5**,[16] the benzoxazole ERB041 (**6**)[15] and the 7-thiabicyclo[2.2.1]hept-2-ene-7-oxide type ligand **7** (Figure 1).[17]

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We have previously reported the antiproliferative activity of SERM-type compounds containing the β-lactam (azetidinone) scaffold with a basic side-chain which demonstrated antiestrogenic effects in MCF-7 cells (e.g. β-lactam **8**, Figure 1).[18] As part of our ongoing interest in the development of ER ligands having novel scaffold structures we therefore decided to investigate a related series of β-lactams to identify potential lead compounds for further development as ERα or ERβ ligands. We were also interested in finding a common scaffold for the development of designed multiple ligands targeting both the ER and tubulin. Tubulin is an αβ heterodimeric protein and is the main constituent of microtubules, which are essential to the mitotic division of cells. Many tubulin binding compounds, such as paclitaxel and vinblastine, are in clinical use for various types of cancer.[19] There are a number of potential advantages for designed multiple ligands, for example, improved efficacy, less incidence of side effects and a lower risk of drug-drug interactions.[20-22] A large number of dual-ligands are progressing in clinical trials, including the novel drug conjugate trastuzumab emtansine (T-DM1)

which is in phase III clinical trials for Her-2 positive breast cancer. It combines the distinct mechanism of action of both DM1 (a microtubule inhibitor) and trastuzumab.[23] A designed multiple ligand targeting both the ER and tubulin is potentially of clinical use in cases where a microtubule inhibitor is combined with a SERM, e.g. the combination of paclitaxel and tamoxifen.[24] Our novel compounds were assessed for their binding affinities for ERα and ERβ and their antiproliferative activities in MCF-7 cells. Selected compounds were further assessed for their effects on the cell cycle and tubulin polymerisation. Finally, a computational study was undertaken to investigate the binding orientation of the most potent and selective compounds.

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Chemistry

The β-lactam ring scaffold is the template for a variety of drugs and preclinical compounds including antibiotics, tubulin-targeting agents,[25-29] SERMs,[18] cholesterol-absorption inhibitors[30] and anti-asthmatics.[31] The two most common routes for synthesis of the β-lactam core are the Staudinger[32-33] and Reformatsky[34] reactions and both of these reactions were utilised in the synthesis of the required ER targeting β-lactams. The β-lactams reported herein incorporate the characteristic pharmacophore for ER binding, namely a hydrophobic core with two phenolic groups approximately 11 Å apart (Schemes 2 and 3).[35] An unsubstituted β-lactam ring is not inherently hydrophobic, but the presence of aromatic rings at C-1 and C-4 increases the hydrophobicity of this series substantially. The newly reported compounds have similar spatial arrangements to many SERMs, but the majority lack the basic, nitrogen-containing ether substituent characteristic of many SERMs such as **3**. The preparation of precursors to the β-lactam ring-forming reactions

involved synthesis of appropriately protected imines. *Tert*-butyldimethylsilane (TBDMS) and benzyl (Bn) groups were used to protect phenol groups and were chosen due to their ease of introduction, stability under the conditions of both the Staudinger and Reformatsky reactions, and ease of removal without decomposition of the β-lactam ring. Imines **10**-**14** were obtained by condensation of the appropriate amines and aldehydes under reflux conditions in ethanol (Scheme 1).

β-Lactams **15**-**18** were obtained by a Reformatsky-type reaction of a series of appropriately substituted imines with ethyl bromoacetate under microwave conditions to afford the 3-unsubstituted products (Scheme 2). β-Lactams **19**-**24** were synthesised from imine **10**, and β-lactam **25** from imine **11**, by the Staudinger route using triethylamine as tertiary base (Scheme 2). The β-lactams were deprotected *in situ* before final characterisations were carried out (reaction monitored by IR and TLC). Both TBDMS and Bn protecting groups were successfully removed without decomposition of the β-lactam ring (Scheme 2). For compounds **19**-**22, 24** and **25** the ^{1}H NMR spectrum showed formation of the product exclusively as the *trans* isomer as evidenced by the coupling constant between H-3 and H-4 of the β-lactam ring [e.g. for compound **25**: δ 4.20 (d, 1H, J = 2.5 Hz, H_3), 4.78 (d, 1H, J = 2.5 Hz, H_4)].

A structurally related group of compounds containing the common β-lactam core structure substituted with aryl rings at the N-1 and C-4 positions, together with the arylhydroxymethyl substituent at C-3, were also prepared to provide further insight on the structural requirements of the β-lactam scaffold for ER binding activity. The

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introduction of the required substituent at the C-3 position was achieved with an aldol type reaction of a suitable phenolic aldehyde to produce the α-(hydroxyaryl)methyl group at the C-3 position of the β-lactam.[36] Reaction of **16**, **17** and **18** with 4-hydroxybenzaldehyde afforded the products **26**, **27** and **29** respectively (Scheme 3). Debenzylation of **27** yielded the required triphenolic product **28**. Alkylation of **26** with 1-(2-chloroethyl)pyrrolidine hydrochloride resulted the isolation of the ether product **30**, which contains a basic side-chain similar to known SERMs (Scheme 3). This substituent was previously identified as the optimal basic side-chain for SERM activity in β-lactams.[18] The products were obtained as diastereomeric mixtures evident from the ^{1}H NMR spectra, e.g. compound **26** was obtained in a 3:1 ratio, compound **29** in a 3:2 ratio and compounds **28** and **30** in a 1:1 ratio.

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Biochemical Evaluation

The binding affinities of the novel β-lactams for ERα and ERβ was assessed using a competitive binding assay with a fluorescent estrogen ligand. Compound **1** was included as a positive control and had an IC_{50} value of 5.7 nM in ERα and 5.6 nM in ERβ consistent with reported literature values.[15, 37-38] SERM **2** was also evaluated and had an IC_{50} value of 61 nM in ERα and 190 nM in ERβ. Many SERMs contain two phenolic groups to mimic the hydroxyl groups of **1**. Initially a series of six compounds (**15** and **19**-**23**, Scheme 2) with phenolic substitutions at N-1 and C-4 but with a variety of substituents at C-3 was evaluated. A clear pattern of activity for these compounds can be observed, with smaller substituents at C-3 leading to decreased affinity in the ER binding assay compared to larger substituents (Table 1). The potency increases in the order of

unsubstituted **15**<phenyl **19**<phenoxy **21**<2-naphthyl **22**<diphenyl **23**. The least potent compound of this mini-series, **15**, is unsubstituted at position 3 and has an IC_{50} value in ERα of 13 μM. 3, 3-Diphenyl compound **23** binds strongly with IC_{50} values of 0.04 μM (ERα) and 0.019 μM (ERβ). The calculated distance between the oxygen atoms of the phenolic groups at positions 1 and 4 of the β-lactam ring of **23** is 9.4 Å,[39] which compares with a value of 10.8 Å for the distance between the hydroxyl groups in **1**. It is possible that the bulkier C-3 substituents interfere with exposure of the AF1 and AF2 sites in a similar manner to the basic side-chain of **3**. However, with the exception of previously reported compound **16**, compound **23** was the only β-lactam in Scheme 2 to display antiproliferative activity in MCF-7 cells at concentrations < 50 μM (IC_{50}=45 μM).

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The relative lack of antiproliferative activity for the majority of compounds in the series prompted us to examine their binding mode to determine if the compounds were ER agonists. The structures of β-lactams **15**-**23** lack the conventional basic side chain present in most ER antagonists such as **2** and **3**, and therefore could be expected to resemble the ER agonist core structures of **4** (Figure 1, ERα agonist/partial ERβ agonist),[15] THC (5R,11R-diethyl,5,6,11,12-tetrahydrochrysene-2,8-diol) (ERα agonist),[40] propyl pyrazole triol (PPT) (ERα agonist)[16] and the benzothiophene raloxifene core (ER agonist).[41] As a representative example of the compounds with no antiproliferative activity, β-lactam **20** was assessed in a TR-FRET assay to determine if the compound was acting as an agonist or antagonists for ERα. The IC_{50} value for **20** in the TR-FRET assay (1.4 μM) was in agreement with the value of 1.1 μM obtained in the fluorescence polarisation assay.

Compound **20** was inactive in the agonist mode but active in the antagonist mode, confirming that β-lactam **20** is acting as an ERα antagonist. This is not unprecedented, as both 1,1,2-tris(4-hydroxyphenyl)alkenes and 7-oxabicyclo[2.2.1]hept-5-enes (OBHS) are known as ER antagonists, despite lacking the basic side chain substituent typically required for these effects.[42-43] Hence, the lack of antiproliferative activity is due to other factors. The ER assay uses isolated estrogen receptor and is not a cell-based assay, and β-lactam degradation is unlikely to occur in these conditions. However, the presence of cellular enzymes in the cellular viability assay in MCF-7 cells could cause metabolic deactivation by opening the β-lactam ring, e.g. by hydrolysis, leading to a decrease in the *in vitro* antiproliferative effect.

We focused our further efforts on the development of a dual ligand for ER and tubulin. It is known that a trimethoxyaryl ring, found in the natural products colchicine and combretastatin A-4 as well as in tubulin-targeting β-lactams, contributes to strong interactions with the colchicine-binding site of tubulin.[26, 44] We incorporated this group into an ER pharmacophore containing two phenolic groups in the molecule. Comparison of the binding affinities of compounds **24** and **25** indicated that positioning of phenolic rings at N-1 and C-4 positions gave more potent antiproliferative activity than when they were located at N-1 and C-3. β-Lactam **24** had 10-fold greater binding affinity than **25** in ERα binding (IC_{50}=1.47 μM and 17.5 μM respectively). The same pattern was determined for ERβ [IC_{50}=23 μM (**24**) and 144 μM (**25**)]. However, neither β-lactam **24** nor **25** displayed antiproliferative activity in MCF-7 cells at concentrations up to 50 μM.

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Compound **16**, previously reported as a tubulin-targeting β-lactam and included here for comparative purposes, showed no ER binding affinity.

In an attempt to improve the antiproliferative activity, whilst maintaining the ER binding affinity, compounds with a α-(hydroxyaryl)methyl substituent at C-3 were prepared (**26**, **28** and **29**, Scheme 3). Compound **26** was identified as a potent compound in the series with IC_{50} values of 0.008 μM (ERα) and 0.015 μM (ERβ). Compounds **28** and **29** did not bind strongly to either ERα or ERβ in comparison to compound **26** (Table 1). Compounds **28** and **29** were also not potent antiproliferative compounds in MCF-7 cells whereas the antiproliferative activity of β-lactam **26** was the best of all the ER binding compounds, with an IC_{50} of 0.21 μM. The subsequent introduction of the basic pyrrolidine SERM-type ether substituent in **30** resulted in elimination of the ER binding activity from the core β-lactam structure of **26**. This compound shows no affinity for either ERα or ERβ in the competitive binding assay so it can be assumed that this is not the mechanism of action of **30**. Compound **30** also has a reduced antiproliferative effect compared to compound **26** (IC_{50}=3.80 μM). Compound **30** is structurally similar to our previously reported ER binding β-lactam **8** [Figure 1, IC_{50}=4.63 μM (MCF-7)[18]], and the loss of activity indicates that the trimethoxy-substitution at N-1 is detrimental to ER binding affinity for this particular compound. Although compound **30** incorporates a basic-side chain, it does not contain two phenolic groups and this may explain its lack of ER binding affinity.

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Further biochemical characterization was carried out on β-lactams **16**, **26** and **30**. Compound **26** was amongst the compounds with the highest affinity for ERα and ERβ in the competitive binding assays and the most potent antiproliferative activity in MCF-7 cells. Cytotoxic effects were evaluated using the lactate dehydrogenase assay. 1.5%, 10% and 7% cell death was observed in MCF-7 cells for compounds **16**, **26** and **30** respectively at 10 μM (compound **2** as a control demonstrated 13% cell death at 10 μM; β-lactam **8** has been reported to cause 14.8% cell death in MCF-7 cells at 10 μM[18]). Flow cytometric analysis was performed to observe the effects of **16**, **26** and **30** on the cell cycle distribution of MCF-7 cells after 24 hours and to quantify the extent of G_2/M arrest and sub-G_1 arrest induced by these compounds (Figure 2.A). Compound **16**, structurally similar to known tubulin inhibitors[25], caused accumulation of cells in G_2/M arrest at concentrations of 100 nM and above. There is also a significant increase in sub-G_1 cells at concentrations of 1 μM and 10 μM indicating induction of apoptosis even after 24 hours. There were increased numbers of cells in sub-G_1 at concentrations of 10 nM and above of compound **30**, indicative of apoptosis. There was also an increase in the percentage of cells in G_2/M phase for compound **30** (10 μM). Compound **26** (1 μM and 10 μM) also caused accumulation of cells in G_2/M phase indicating mitotic blockage at 24 hr. Additional flow cytometry experiments were performed at 48 and 72 hr for compound **26** in order to investigate if the percentage of cells in sub-G_1 increased at extended time-points (Figure 2.B). No significant differences compared to vehicle control were observed at 24 hr. There was an increase in % of sub-G_1 cells for compound **26** at 48 hr (9.3% compared to 1.9% for control) and a statistically significant difference was

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observed at 72 hr (17% compared to 3.7% for control). This indicates that compound **26** causes prolonged G_2/M arrest followed by apoptosis.

The potential tubulin-targeting properties of β-lactam **26** were investigated using a sedimentation assay and Western blotting (Figure 3). Paclitaxel and nocodazole served as controls; paclitaxel promotes the polymerization of tubulin and nocodazole is a tubulin depolymerizer. Polymerized and depolymerized microtubules have different solubilities and localize in either the pellet or supernatant of lysed, centrifuged cells, respectively. Tubulin from nocodazole-treated cells was depolymerized and detected almost wholly in the supernatant, whereas tubulin from paclitaxel-treated cells was polymerized and detected solely in the pellet (Figure 3). Tubulin from β-lactam **26**-treated cells (1 μM) was found exclusively in the supernatant, indicating complete depolymerization of tubulin (Figure 3). This confirmed that compound **26** is acting as a tubulin depolymerizer in addition to its ER targeting properties.

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The effects of other tubulin-targeting agents mediating signal transduction pathways of apoptosis have been previously described.[45-47] Anti-apoptotic proteins of the Bcl-2 family contribute to an increased apoptotic threshold in cancer cells and allow cells to survive in stressful environments.[48] We examined the effects of compound **26** on two anti-apoptotic members of the Bcl-2 family, Bcl-2 and Mcl-1, in MCF-7 cells. Western blotting of the pro-survival proteins Bcl-2 and Mcl-1 showed decreases in both at 72 hrs (Figure 4), indicating downregulation of expression of these proteins upon treatment with compound **2**6. Decreases in the level of Bcl-2 were also seen at 48 hrs (data not shown). In

combination with cell cycle analysis (above), this result indicates that compound **26** induces apoptosis in MCF-7 cells.

An increasing number of designed multiple ligands for cancer therapy are known.[22] Combretastatin A-4/steroid hybrids that inhibit the polymerisation of tubulin have been reported but the ER binding affinity of these compounds is unknown.[49] To the best of our knowledge, β-lactam **26** is the first reported compound that is a designed multiple ligand targeting both the estrogen receptor and tubulin. Lead optimization will be required to determine the optimal ratio of activity that enables both targets to be modulated to an appropriate degree in vivo. This is an exciting discovery that provides a lead compound targeting two clinically relevant targets in breast cancer therapy.

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Computational Modelling

The majority of structural studies on the ER focus on the ligand-binding domain (LBD). The LBD crystal structures for ERα with the natural hormone agonist **1** and ER antagonists **2** and **3** have been reported, amongst others.[40, 50] High resolution x-ray crystal structures of the ER LBD bound to a range of steroidal and non-steroidal ligands allow some rationalisation of the SARs observed for various ligand classes at the steroid receptor.[2]

We examined computational docking in ERα and tubulin for the dual-targeting β-lactam **26**. Figure 5 illustrates the binding pose of compound **26** docked in the binding site of ERα and importantly H-bonding with Glu353 and a π-stacking interaction with Phe404 are present as are commonly observed with many ER binding ligands.[2] These interactions

occur between the receptor and the arylhydroxymethyl substituent at C-3 of the β-lactam. Current ‘state-of-the-art’ docking and scoring algorithms have difficulty in accurately prioritising a series of analogs and by inference one can assume similar problems may occur in effectively ranking stereoisomers and their enantiomers of a compound. Figure S1 (supporting information) illustrates the orientation of the top five docking poses and indicates that all adopt a highly similar geometry in the binding site of ERα. It is also important to note that only two of all possible stereoisomers of compound **26** are present in the top 5 docked and scored solutions generated by HYBRID (Table S1, Supporting Information).

Our biochemical data indicates that compound **26** causes depolymerisation of tubulin, most probably through binding in the colchicine site. Figure 6 illustrates the binding orientation of **26** overlaid with the binding orientation of colchicine, whereby similar interactions with the important trimethoxyphenyl moiety and solvent bridging Cys239 are made for compound **26** as for colchicine.[44, 51] Other interactions such as σ-π bonding to Leu253 occur to stabilise compound **26** in the tubulin binding site. Interestingly, the top docked solution as represented in Figure S1 which depicts compound **26** in the colchicine binding site is the following: (3R,4R)-3-[(R)-hydroxy-(4-hydroxyphenyl)methyl]-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one. This is the same form that is represented in 3 of the 5 top docked solutions bound to ERα (Table S2, Supporting Information).

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Conclusion

A series of ER ligands were synthesised and evaluated for ER binding and antiproliferative activity. The most potent β-lactam in the antiproliferative assay, **26**, was demonstrated to be a designed multiple ligand targeting both the ER and tubulin, causing complete depolymerisation of tubulin in MCF-7 cells. It was also shown to induce apoptosis and downregulated the levels of anti-apoptotic proteins Bcl-2 and Mcl-1 in MCF-7 cells. These compounds represent an interesting and novel class of ER antagonist, and may have future potential applications as medicinal agents for anti-cancer use or mediation of inflammatory response. β-Lactam **26** has been identified as a lead compound for further investigations in the design of new compounds with enhanced affinity for both the ER and tubulin.

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Experimental Section

General Chemical Synthesis and Analysis

All reagents were commercially available and were used without further purification unless otherwise indicated. DCM was dried by distillation from calcium hydride prior to use. 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 ºC, 400.13 MHz for ^{1}H spectra, 100.61 MHz for ^{13}C spectra, in either $CDCl_3$ or CD_3OD (internal standard tetramethylsilane). High resolution accurate mass determinations for all final target compounds were obtained on a Micromass Time of Flight mass spectrometer equipped with electrospray ionization interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory by Dr. Martin Feeney in the School of Chemistry, Trinity College Dublin. TLC was performed using Merck Silica gel 60 TLC aluminium sheets with fluorescent indicator visualizing with UV light at 254 nm. Flash chromatography was carried out using standard silica gel 60 (230-400 mesh) obtained from Merck. All products isolated were homogenous on TLC. The purity of the tested compounds was determined by HPLC and unless otherwise stated, the purity level was $\geq$95%. HPLC was performed using a Waters 2487 Dual Wavelength Absorbance detector, a Waters 1525 binary HPLC pump and a Waters 717plus Autosampler. The column used was a Varian Pursuit XRs C18 reverse phase 150 x 4.6 mm chromatography column. Samples were detected using a wavelength of 254 nm. All samples were analyzed using acetonitrile (70%): water (30%) over 10 min and a flow rate of 1 mL/min. Imine **12** and β-lactam **16** (43% yield) were synthesised as previously described.[25]

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4-(*tert*-Butyldimethylsilanyloxy)phenylamine (9). To a solution of 4-aminophenol (20 mmol) and dimethyl-*tert*-butylchlorosilane (24 mmol) in anhydrous CH_2Cl_2 (60 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (32 mmol). The resulting mixture was stirred at room temperature under a nitrogen atmosphere until complete, as indicated by TLC. The solution was then diluted with CH_2Cl_2 (80 mL) and washed successively with water (60 mL), 0.1M HCl (60 mL) and saturated aqueous $NaHCO_3$ (60 mL). The organic layer was dried over anhydrous Na_2SO_4 to afford the product as a brown oil (73% yield).[52] ^{1}H NMR ($CDCl_3$) δ 0.12 (m, 6H, $SiCH_3$), 0.94 (m, 9H, CH_3), 3.92 (m, 2H, NH_2), 6.81 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$) δ -4.91 ($SiCH_3$), -4.04 ($SiCH_3$), 17.73 (SiC), 116.79, 120.18, 120.29, 137.86, 148.64. HRMS (ESI): *m/z* calcd for $C_{12}H_{21}NOSi + H^+$ $(M + H)^+$: 224.1465; found: 224.1180.

General method I: imine preparation. The appropriate amine (10 mmol) was heated at reflux with the appropriate aldehyde (10 mmol) in ethanol (50 mL) for 3 h. The solvent was removed *in vacuo* and the resulting solid product was purified by recrystallisation from ethanol.

(4-Benzyloxybenzylidene)-[4-(*tert*butyldimethylsilanyloxy)phenyl]amine (10) was prepared from **9** and 4-benzyloxybenzaldehyde as white crystals according to general method I (82% yield), mp 146 °C. IR (KBr): $\tilde{\nu}$ = 1626 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$) δ 0.24 (m, 6H, $-SiCH_3$), 0.99 (m, 9H, CH_3), 5.18 (s, 2H, OCH_2), 6.93 (m, 4H, ArH), 7.12 (m, 2H, ArH), 7.42 (m, 5H, ArH), 7.86 (m, 1H, ArH), 8.43 (s, 1H, CH=N).

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^{13}C NMR ($CDCl_3$) δ -4.93 ($SiCH_3$), -4.85 ($SiCH_3$), 17.79 (SiC), 25.18 (CH_3), 25.25 (SiC), 69.82 (OCH_2), 114.69, 115.58, 119.94, 120.52, 120.62, 120.81, 121.97, 122.84, 125.14, 127.06, 127.13, 127.91, 128.03, 128.30, 128.34, 131.57, 135.47 (ArC), 190.39 (CH=N). HRMS (ESI): *m/z* calcd for $C_{26}H_{31}NO_2Si + H^+$ $(M + H)^+$: 418.2197; found: 418.2216.

4-(*tert*-Butyldimethylsilyloxy)-N-(3,4,5-trimethoxybenzylidene)aniline (11) was obtained from **9** and 3,4,5-trimethoxybenzenamine according to general method I. The product **11** was obtained as a brown oil in quantitative yield which was used without further purification. IR (NaCl): $\tilde{\nu}$ = 1623 cm^{-1} (s; ν(C=N)). ^1H NMR ($CDCl_3$) δ 0.04 (s, 6H, 2xCH_3), 0.84 (s, 3H, CH_3), 0.96 (s, 6H, 2xCH_3), 3.73(s, 3H, OCH_3), 3.86 (6H, s, 2xOCH_3), 7.797.25 (6H, m, ArH), 8.53(s, 1H, CH=N). ^{13}C NMR ($CDCl_3$) δ -4.52 (-$SiCH_3$), -3.21 (-$SiCH_3$), 17.94, 25.54 (CH_3), 55.86 (OCH_3), 60.13 (OCH_3), 105.55, 115.70, 120.37, 122.32, 132.00, 144.94, 153.10, 153.56 (ArC) 158.49 (CH=N). HRMS (ESI): *m/z* calcd for $C_{22}H_{31}NO_4Si + H^+$ $(M + H)^+$: 402.2095; found: 402.2083.

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***N*,1-bis(4-(benzyloxy)phenyl)methanimine (13)** was prepared from 4-benzyloxybenzaldehyde and 4-(benzyloxy)aniline according to general method I. The product **13** was obtained as a pale green solid (74% yield), mp 210-212 °C. IR (KBr): $\tilde{\nu}$ = 1615 cm^{-1} (s; ν(C=N)). ^1H NMR ($CDCl_3$) δ 5.11 (s, 2H, OCH_2Ph), 5.16 (s, 2H, OCH_2Ph), 7.02 (d, J = 8.5 Hz, 2H, ArH), 7.08 (d, J = 8.5 Hz, 2H, ArH), 7.32 (d, J = 8.5 Hz, 2H, ArH), 7.36-7.49 (m, 10H, ArH), 7.87 (d, J = 8.0 Hz, ArH), 8.42 (s, 1H, HC=N). ^{13}C NMR ($CDCl_3$) δ 69.6 (OCH_2), 69.8 (OCH_2), 114.6, 114.9, 121.6, 127.1, 127.6, 127.7,

128.2, 128.2, 129.9, 123.0, 136.5, 153.6, 156.8 (ArC) 157.5 (HC=N). HRMS (ESI): *m/z* calcd for $C_{27}H_{23}NO_2 + H^+$ $(M + H)^+$: 394.1802; found: 394.1812.

***N*-(4-Methoxybenzylidene)-4-anisidine (14)** was prepared from 4-methoxyaniline and 4-methoxybenzaldehyde according to general method I. The product **14** was obtained from ethanol as mint green coloured crystals (79% yield), mp 148 °C - 150 °C.[53] IR (KBr): $\tilde{\nu}$ = 1623 cm^{-1} (s; ν(C=N)). ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.93-7.00 (m, 4H, ArH), 7.22 (d, 2H, J = 9.0 Hz, ArH, 7.84-7.86 (m, 2H, ArH), 8.42 (s, 1H, -CH=N). ^{13}C NMR ($CDCl_3$): δ 55.4 (OCH_3), 55.5 (OCH_3), 114.2, 114.4, 122.0, 129.6, 130.2, 145.3, 157.8, 157.9 (ArC), 162.1 (CH=N). HRMS (ESI): *m/z* calcd for $C_{15}H_{15}NO_2 + H^+$ $(M + H)^+$: 242.1176; found: 242.1192.

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General method II: β-lactam preparation (Staudinger-type reaction). The appropriate imine (5 mmol) and triethylamine (15 mmol) were added to anhydrous CH_2Cl_2 (50 mL) in an inert atmosphere and the mixture was refluxed at 60 °C. The appropriately substituted acid chloride (7.5 mmol) was injected dropwise and the mixture was refluxed for 3 hours. Upon cooling to room temperature, the mixture was twice washed with distilled water (50 mL) and once with saturated aqueous $NaHCO_3$ solution (50 mL). The organic layer was dried by filtration through anhydrous Na_2SO_4. The organic layer containing the product was reduced *in vacuo*. The crude product was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient).

General method III: β-lactam preparation (Reformatsky-type reaction). Zinc powder (15 mmol) was activated using trimethylchlorosilane (5 mmol) in anhydrous benzene (5 mL) by heating for 15 min at 40 °C and subsequently for 2 min at 100 °C with microwave irradiation. After cooling, the appropriate imine (10 mmol) and ethyl 2-bromoacetate (12 mmol) were added to the reaction vessel and the mixture was placed in the microwave reactor for 30 min at 100 °C. The reaction mixture was filtered through Celite to remove the zinc catalyst and then diluted with CH_2Cl_2 (50 mL). This solution was washed with saturated ammonium chloride solution (20 mL) and 25% ammonium hydroxide (20 mL), and then with dilute HCl (40 mL), followed by water (40 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient).

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General method IV: desilylation of TBDMS-protected β-lactams. To a solution of the appropriately protected phenol (10 mmol) in THF (50 mL) was added 1.5 equivalents of 1M tetrabutylammonium fluoride. The solution was stirred in an ice-bath for 15 min and the reaction was monitored by TLC and IR. The reaction mixture was diluted with EtOAc (100 mL) and quenched with 10% HCl (100 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The organic layer was washed with water (100 mL) and brine (100 mL) and was dried with anhydrous Na_2SO_4. The crude product was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient) and was used immediately in the following reaction without any further purification.

General method V: debenzylation of Bn-protected β-lactams. The benzyloxy protected β-lactam (2 mmol) was dissolved in ethanol: ethyl acetate (50 mL; 1:1 mixture) and hydrogenated over 10%% Pd/C (1.2 g) at room temperature until complete, as indicated by TLC. The catalyst was filtered, the solvent was removed under vacuum and the product was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient).

General method VI. A solution of β-lactam (1 mmol) in anhydrous THF (25 mL) was stirred at -78 °C under an inert atmosphere. Lithium diisopropylamide (LDA) (2M, 5 mmol) was added quickly and the solution was stirred at -78 °C for 5 min. A solution of 4-hydroxybenzaldehyde (3 mmol) in anhydrous THF (12.5 mL) was added to the reaction mixture and it was stirred at -78 °C for 30 min and then poured slowly into a saturated sodium chloride solution (50 mL). Ethyl acetate (25 mL) was added, the organic layer was separated and dried with anhydrous Na_2SO_4. Evaporation of the solvent yielded a yellow solid residue which was purified by column chromatography (eluent: *n*-hexane:ethyl acetate; 4:1).

1,4-bis(4-Hydroxyphenyl)azetidin-2-one (15) was prepared from imine **10** and ethyl 2-bromoacetate according to general method III. The crude product 4-(4-(benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)azetidin-2-one (**15a**) was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient) as a brown oil. This product was immediately desilylated according to general method IV

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to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)azetidin-2-one (**15b**), which was debenzylated according to general method V to afford the product **15** as a white solid (0.9% overall yield), mp 180 ˚C. IR (KBr): $\tilde{\nu}$ = 1715 (s; *v*(C=O)), 3306 cm^{-1} (s; *v*(O−H)). ^{1}H NMR (CD_3OD) *δ* 2.80-2.84 (m, 1H, H_3), 3.48 (dd, 1H, *J* = 5.0 Hz, 14.6 Hz, H_3), 4.98-4.99 (m, 1H, H_4), 6.67-6.72 (m, 2H, ArH), 6.79-6.81 (m, 2H, ArH), 7.13 (d, 2H, *J* = 8.5 Hz, ArH), 7.21 (d, 2H, *J* = 8.0 Hz, ArH). ^{13}C NMR (CD_3OD) δ 45.3 (C_3), 53.4 (C_4), 114.3, 114.3, 114.6, 114.9, 117.9, 121.7, 126.7, 128.3, 128.5, 129.6, 153.3, 156.9 (ArC), 164.8 (C=O). HRMS (ESI): *m/z* calcd for $C_{15}H_{13}NO_3 + H^+$ $(M + H)^+$: 256.0968; found: 256.0967.

1,4-bis(4-Benzyloxyphenyl)azetidin-2-one (17) was prepared from imine **13** and ethyl 2-bromoacetate according to general method III. The product was obtained as a yellow powder (32% yield), mp 135-136 ˚C. IR (KBr): $\tilde{\nu}$ = 1745 cm^{-1} (s; *v*(C=O)). ^{1}H NMR ($CDCl_3$) *δ* 2.83-2.86 (m, 1H, H_3), 3.52 (dd, 1H, *J* = 14.8 Hz, 5.3 Hz, H_3), 5.02 (s, 2H, OCH_2), 5.08 (s, 2H, OCH_2), 5.10-5.11 (m, 1H, H_4), 6.94 (d, *J* = 9.0 Hz, 2H, ArH), 7.02 (d, *J* = 8.5 Hz, 2H, ArH), 7.15-7.39 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) *δ* 46.3 (C_3), 52.6 (C_4), 69.2 (OCH_2), 69.3 (OCH_2), 115.1, 115.3, 117.8, 127.6, 127.7, 127.7, 127.8, 127.9, 128.4, 128.4, 130.5, 131.2, 136.9, 136.9, 154.4, 158.3 (ArC), 163.9 (C=O). HRMS (ESI): *m/z* calcd for $C_{29}H_{25}NO_3 + H^+$ $(M + H)^+$: 436.1907; found: 436.1920.

1,4-bis(4-Methoxyphenyl)azetidin-2-one (18) was prepared from imine **13** according to general method III. Evaporation of the solvent yielded a brown solid residue which was purified by column chromatography (eluent: CH_2Cl_2) to afford the β-lactam as brown

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crystals (36% yield), mp 134 °C.[54] IR (KBr): $\tilde{\nu}$ = 1751 cm^{-1} (s; ν(C=O)). ^{1}H NMR ($CDCl_3$) δ 2.92 (dd, 1H, J = 12.6 Hz, J = 2.5 Hz, H_3), 3.53 (dd, 1H, J = 9.5 Hz, J = 5.5 Hz, H_3), 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.94, (dd, 1H, J = 2.7 Hz, J = 5.5 Hz, H_4), 6.80 (d, 2H, J = 9.0 Hz, ArH), 6.92 (d, 2H, J = 8.5 Hz, ArH), δ 7.24-7.30 (2xd, overlapping, 4H, J = 8.5 Hz, ArH). ^{13}C NMR ($CDCl_3$) δ 46.6 (C_3, CH_2), 53.3 (C_4, CH), 54.9 (OCH_3), 55.3 (OCH_3), 113.8, 115.9, 117.7, 126.8, 129.7, 131.0, 155.4, 159.2, 163.8 (C=O). HRMS (ESI): *m/z* calcd for $C_{17}H_{17}NO_3$ + Na^+ $(M + Na)^+$: 306.1101; found: 306.1115.

1,4-bis(4-Hydroxyphenyl)-3-phenylazetidin-2-one (19). 4-(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-phenylazetidin-2-one (**19a**) was prepared from imine **10** and 2-phenylacetyl chloride according to general method II. This product was immediately desilylated according to general method IV to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-3-phenylazetidin-2-one (**19b**), which was debenzylated according to general method V to afford the product **19**. It was obtained by column chromatography (hexane: ethyl acetate gradient) as a yellow gel (29% yield). IR (KBr): $\tilde{\nu}$ = 1717 (s; ν(C=O)), 3327 cm^{-1} (s; ν(O−H)). ^{1}H NMR (CD_3OD) δ 4.24 (d, 1H, J = 2.5 Hz, H_3), 4.96 (d, 1H, J = 2.0 Hz, H_4), 6.72 (d, 2H, J = 9.0 Hz, ArH), 6.83 (d, 2H, J = 8.5 Hz, ArH), 7.21 -7.27 (m, 4H, ArH), 7.33-7.34 (m, 2H, ArH), 7.41-7.43 (m, 2H, ArH). ^{13}C NMR (CD_3OD) δ 65.7 (C_3), 66.6 (C_4), 117.1, 117.4, 120.8, 129.1, 129.2, 129.4, 130.6 (ArC). HRMS (ESI): *m/z* calcd for $C_{21}H_{17}NO_3$ + Na^+ $(M + Na)^+$: 354.1101; found: 354.1102.

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1,3,4-tris(4-Hydroxyphenyl)azetidin-2-one (20). 3,4-bis(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)azetidin-2-one (**20a**) was prepared from imine **10** and 4-benzyloxyphenylacetyl chloride according to general method II. This product was immediately desilylated according to general method IV to form 3,4-bis(4-benzyloxy)phenyl)-1-(4-hydroxyphenyl)azetidin-2-one (**20b**), which was debenzylated according to general method V to afford the product **20** as an off-white powder (13% yield), mp 124 °C. IR (KBr): $\tilde{\nu}$ = 1741 (s; ν(C=O)), 3427 cm^{-1} (s; ν(O−H)). ^1H NMR ($CDCl_3$) δ 4.14 (d, 1H, J = 2.5 Hz, H_3), 4.95 (d, 1H, J = 2 Hz, H_4), 6.67 (m, 1H, ArH), 6.69 (m, 2H, ArH), 6.78 (m, 4H, ArH), 6.95 (s, 1H, ArH), 7.10 (m, 4H, ArH), 7.20 (m, 1H, ArH), 9.35 (m, 3H, OH). ^{13}C NMR ($CDCl_3$) δ 62.5 (C_3), 63.6 (C_4), 114.9, 114.9, 115.5, 115.6, 115.7, 118.6, 120.7, 125.4, 127.7, 127.8, 128.6, 129.3, 129.7, 130.1, 130.9, 153.7, 155.4, 156.8, 157.5 (ArC), 165.3 (C=O). HRMS (ESI): m/z calcd for $C_{21}H_{17}NO_2$ + H^+ $(M + H)^+$: 348.1230; found: 348.1233.

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1,4-bis(4-Hydroxyphenyl)-3-phenoxyazetidin-2-one (21). 4-(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-phenoxyazetidin-2-one (**21a**) was obtained from imine **10** and 2-phenoxyacetyl chloride according to general method II. This product was immediately desilylated according to general method IV to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-3-phenoxyazetidin-2-one (**21b**), which was debenzylated according to general method V to afford the product **21** as a yellow powder (14% yield), mp 244 ˚C. IR (KBr): $\tilde{\nu}$ = 1727 (s; ν(C=O)), 3349 cm^{-1} (s; ν(O−H)). ^1H NMR (CD_3OD) δ 5.48 (dd, 1H, J = 4.5 Hz, H_4), 5.64 (d, 1H, J = 4.5 Hz, H_3), 6.67-6.73 (m, 4H, ArH), 6.80 (m, 2H, ArH), 6.88-6.91 (m, 1H, ArH), 7.15-7.24 (m, 6H, ArH). ^{13}C

NMR (CD_3OD) δ 61.4 (C_4), 79.9 (C_3), 108.6, 114.2, 114.5, 114.7, 117.4, 118.5, 121.0, 122.9, 128.4, 128.9, 153.9, 156.5, 157.1 (ArC), 162.8 (C=O). HRMS (ESI): *m/z* calcd for $C_{21}H_{17}NO_4 + Na^+$ $(M + Na)^+$: 370.1050; found: 370.1062.

1,4-bis(4-Hydroxyphenyl)-3-(naphthalen-2-yl)azetidin-2-one (22). 4-(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-(naphthalen-2-yl)azetidin-2-one (**22a**) was obtained from imine **10** and 2-(naphthalen-2-yl)acetyl chloride according to general method II. This product was immediately desilylated according to general method IV to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-3-(naphthalen-2-yl)azetidin-2-one (**22b**), which was debenzylated according to general method V to afford the product **22** as a cream powder (83% yield), mp 229 ˚C. IR (KBr): $\tilde{\nu}$ = 1707 (s; ν(C=O)), 3417 cm^{-1} (s; ν(O−H)). ^1H NMR (DMSO-d_6) δ 4.48 (s(br), 1H, H_3), 5.18 (s(br), 1H, H_4), 6.72 (d, 2H, *J* = 8.5 Hz, ArH), 6.79 (d, 2H, *J* = 8.0 Hz, ArH), 7.17 (d, 2H, *J* = 8.5 Hz, ArH), 7.30 (d, 2H, *J* = 8.0 Hz, ArH), 7.45 (d, 1H, *J* = 8.5 Hz), 7.52-7.54 (m, 2H, ArH), 7.90-7.97 (m, 4H, ArH), 9.36 (s, 1H, OH), 9.59 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ 61.9 (C_3), 64.2 (C_4), 115.5, 115.7, 118.7, 125.5, 126.1, 126.3, 126.5, 127.6, 127.6, 127.7, 127.9, 128.5, 129.2, 132.3, 132.7, 133.0, 153.9, 157.6 (ArC), 164.7 (C=O); HRMS (ESI): *m/z* calcd for $C_{25}H_{19}NO_3 + Na^+$ $(M + Na)^+$: 404.1257; found: 404.1249.

1,4-bis(4-Hydroxyphenyl)-3,3-diphenylazetidin-2-one (23). 4-(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3,3-diphenyl azetidin-2-one (**23a**) was obtained from imine **10** and 2,2-diphenylacetyl chloride according to general method II.

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This product was immediately desilylated according to general method IV to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-3,3-diphenyl azetidin-2-one (**23b**), which was debenzylated according to general method V to afford the product **23** as a white powder (75% yield), mp 115˚C. IR (KBr): $\tilde{\nu}$ = 1775 (s; ν(C=O)), 3359 cm^{-1} (s; ν(O−H)). ^{1}H NMR (DMSO-d_6) δ 5.95 (s, 1H, H_4), 6.53 (d, 2H, J = 8.0 Hz, ArH), 6.72 (d, 2H, J = 9.0 Hz, ArH), 6.96-7.09 (m, 7H, ArH), 7.21 (d, 2H, J = 9.0 Hz, ArH), 7.28-7.32 (m, 1H, ArH), 7.39-7.43 (m, 2H, ArH), 7.66 (d, 2H, J = 8.0 Hz, ArH), 9.37 (s, 1H, OH), 9.39 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ 65.3 (C_3), 71.1 (C_4), 115.0, 115.5, 118.9, 124.9, 126.54, 127.2, 127.9, 127.9, 128.7, 128.9, 128.9, 138.1, 141.1, 153.9, 156.9 (ArC), 165.7 (C=O). HRMS (ESI): m/z calcd for $C_{27}H_{21}NO_3 + H^+$ $(M + H)^+$: 408.1594; found: 408.1594.

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1,4-bis(4-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)azetidin-2-one (24). 4-(4-(Benzyloxy)phenyl)-1-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-(3,4,5-trimethoxyphenyl)azetidin-2-one (**24a**) was obtained from imine **10** and 2-(3,4,5-trimethoxyphenyl)acetyl chloride according to general method II. This product was immediately desilylated according to general method IV to form 4-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)azetidin-2-one (**24b**), which was debenzylated according to general method V to afford the product **24** as a white solid (46% yield), mp 122-124 ˚C. IR (KBr): $\tilde{\nu}$ = 1615 (s; ν(C=O)), 3414 cm^{-1} (s; ν(O−H)). ^{1}H NMR ($CDCl_3$) δ 3.30 (s, 2H, OH), 3.66 (s, 3H, OCH_3), 3.77 (s, 6H, 2xOCH_3), 4.23 (s, 1H, H_3), 5.13 (s, 1H, H_4), 6.63 (s, 2H, ArH), 6.69 (m, 2H, ArH), 6.78 (m, 2H, ArH), 7.13 (m, 2H, ArH), 7.26 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ 56.4 (OCH_3), 60.5 (OCH_3), 62.2 (C_3), 64.8 (C_4), 105.4, 115.9, 116.2, 119.2, 128.4, 128.3, 129.7, 131.1,

137.3, 153.6, 154.3, 158.1 (ArC), 165.1 (C=O). HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_6$ + Na^+ $(M + Na)^+$: 444.1418; found: 444.1440.

1,3-bis(4-Hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (25). 3-(4-(Benzyloxy)phenyl)-4-(3,4,5-trimethoxyphenyl)-1-(4-((trimethylsilyl)oxy)phenyl)azetidin-2-one (**25a**) was obtained from imine **11** and 4-benzyoxyphenylacetyl chloride, according to general method II. This product was immediately desilylated according to general method IV to form 3-(4-(benzyloxy)phenyl)-1-(4-hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**25b**), which was debenzylated according to general method V to afford the product **25** as a brown powder (54% yield), mp 221 ˚C. IR (NaCl): $\tilde{\nu}$ = 1727 (s; ν(C=O)), 3427 cm^{-1} (s; ν(O−H)). ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 5H, OCH_3), 3.87 (s, 3H, OCH_3), 4.20 (d, 1H, J = 2.5 Hz, H_3), 4.78 (d, 1H, J = 2.5 Hz, H_4), 6.56 (s, 2H, ArH), 6.77-6.83 (m, 4H, ArH), 7.17 (d, J = 8.6 Hz, 2H, ArH), 7.26-7.28 (m, 2H, ArH). ^{13}C NMR (DMSO-d_6) δ 55.9 (OCH_3), 59.9 (OCH_3), 62.7 (C_3), 63.3 (C_4), 103.7, 115.6, 115.6, 118.7, 125.2, 128.7, 129.3, 133.5, 137.4, 153.3, 153.9, 156.9 (ArC), 165.4 (C=O). HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_6^+$ $(M)^+$: 421.1525; found: 421.1531.

3-[(Hydroxy-4-hydroxyphenyl)methyl]-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (26) was prepared from β-lactam **16** according to general method VI. The product **26** was obtained as orange crystals (31% yield), mp 130-133 °C. IR (NaCl): $\tilde{\nu}$ = 1749 (s; ν(C=O)), 3210 cm^{-1} (s; ν(O−H)). ^{1}H NMR ($CDCl_3$) δ 3.43-3.44 (0.25H, m, H_3), 3.46 (dd, 0.75H, J = 2.0 Hz, 6.3 Hz, H_3), 4.78 (d, 0.75H, J =

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2.0 Hz, H_4), 5.08-5.11 (m, 1H, H_5), 5.28-5-29 (m, 0.25H, H_4), 6.97-7.42 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 55.3, 57.2 (CH, C_4), 65.4, 66.2 (CH, C_3) 70.2, 71.4 (CH, C_5), 116.7, 116.9, 123.7, 123.9, 125.3, 128.7, 132.9, 139.5 (ArC), 165.2, 165.8 (C_2, C=O). HRMS (ESI): *m/z* calcd for $C_{26}H_{27}NO_7 + Na^+$ $(M + Na)^+$: 488.1680; found: 488.1661.

3-[Hydroxy-(4-hydroxyphenyl)methyl]-1,4-bis(4-hydroxyphenyl)azetidin-2-one (28). 1,4-bis(4-Benzyloxyphenyl)-3-[hydroxy-(4-hydroxyphenyl)]methylazetidin-2-one (**27**) was prepared from β-lactam **17** according to general method VI to afford **27** as an oil (12% yield). IR (NaCl): $\tilde{\nu}$ = 1731 (s; ν(C=O)), 3429 cm^{-1} (s; ν(O−H)). ^{1}H NMR ($CDCl_3$) δ 3.41-3.42 (m, 1H, H_3), 4.73 (d, *J* = 2.0 Hz, 1H, H_4), 5.01 (s, 2H, OCH_2), 5.03 (s, 2H, OCH_2), 5.08 (d, *J* = 7.5 Hz, 1H, H_5), 6.83-7.42 (m, 22H, ArH). HRMS (ESI): *m/z* calcd for $C_{36}H_{31}NO_5 - H^+$ $(M - H)^+$: 556.2129; found: 556.2173. β-Lactam **28** was prepared from **27** according to general method V. The crude product was isolated by flash column chromatography over silica gel (hexane: ethyl acetate gradient) to afford the product **28** as an amber oil (65% yield). IR: NaCl film υ_{max}: 3325 (OH), 1721 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.32-3.40 (m, 1H, H_3), 4.70-4.72 (m, 0.5H, H_4), 5.00 (d, 0.5H, *J* = 6.5 Hz H_5), 5.13 (s, 0.5H, H_4), 5.14 (d, *J* = 4.0 Hz, 0.5H, H_5), 6.65-7.13 (m, 12H, ArH). ^{13}C NMR ($CDCl_3$) δ 59.6 (C_3), 60.4 (C_4), 74.5 (C_5), 114.2, 114.4, 114.5, 114.6, 114.8, 118.1, 126.6, 126.6, 127.4, 129.2, 132.2, 132.6, 153.4, 155.3, 157.7 (ArC), 167.3 (C_2). HRMS (ESI): *m/z* calcd for $C_{22}H_{19}NO_5 + Na^+$ $(M + Na)^+$: 400.1155; found: 400.1149.

3-[Hydroxy-(4-hydroxyphenyl)-methyl]-1,4-bis-(4-methoxyphenyl)-azetidin-2-one (29) was prepared from β-lactam **18** according to general method VI. Evaporation of

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solvent yielded a brown solid residue which was purified using column chromatography (DCM: EtOAc 4:1) to obtain the product **29** as an orange gel (20% yield). IR (NaCl): $\tilde{\nu}$ = 1731 (s; ν(C=O)), 3384 cm^{-1} (s; ν(O−H)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.34-3.36 (q, 0.38H, J = 2.0 Hz, H_3), 3.40-3.42 (q, 0.62H, J = 3.4 Hz, H_3), 3.69 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 4.72-4.73 (d, 0.6H, J = 2.0 Hz, H_4), 4.97-4.99 (d, 0.6H, J = 6.2 Hz, H5), 5.11 (s, 0.4H, H_5), 5.16 (d, 0.4H, J = 4.1 Hz, H_4), 6.72-6.79 (m, 6H, ArH), 6.96-7.00 (m, 2H, ArH), 7.11-7.23 (m, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.0, 55.9, 57.2 (C_4), 65.4, 66.1 (C_3), 69.5, 71.7 (C_5), 113.9, 115.1, 115.2, 118.2, 118.3, 126.6, 126.8, 127.7, 128.4, 128.9, 130.3, 130.4, 131.7, 132.4, 155.5, 155.9, 158.8, 159.1, 165.4 (C=O), 165.5 (C=O). HRMS (ESI): *m/z* calcd for $C_{24}H_{23}NO_5$ + Na^+ $(M + Na)^+$: 428.1468; found: 428.1494.

3-(Hydroxy-[4-(2-pyrrolidin-1-ylethoxy)phenyl]methyl-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30). β-Lactam **26** (10 mmol) was dissolved in anhydrous acetone (100 mL) and anhydrous potassium carbonate (160 mmol) was added. The mixture was stirred gently for 10 min under a nitrogen atmosphere and 1-(2-chloroethyl)pyrrolidine hydrochloride (40 mmol) was added. The mixture was refluxed until the reaction was complete, as indicated by TLC. The solution was filtered, the solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent: CH_2Cl_2). The product **30** was obtained as a yellow gel (30% yield). ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 4H, $C(CH_2)_2C$), 2.81 (s, 4H, CH_2NCH_2), 3.04 (s, 2H, CH_2N), 3.41-3.48 (m, 1H, H_3), 3.71 (s, 6H, OCH_3), 3.78 (s, 6H, OCH_3), 4.21 (s, 2H, CH_2-O), 4.83 (d, 0.5H, J = 2.0 Hz, H_4), 5.08 (d, 0.5H, J = 5.5 Hz, H_5), 5.16 (d, 0.5H, J =

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2.5 Hz, H_4), 5.34 (d, 0.5H, *J* = 4.0 Hz, H_5), 6.97-7.42 (m, 14H, ArH). HRMS (ESI): *m/z* calcd for $C_{32}H_{38}N_2O_7 + H^+$ $(M + H)^+$: 563.2752; found: 563.2769.

Biochemical Evaluation. All biochemical assays were performed in triplicate on at least three independent occasions for the determination of mean values reported.

ER Fluorescent Polarisation Assay. Competitive binding affinity experiments were carried out using purified baculovirus-expressed human ERα and ERβ and fluoromone, a fluorescein-labeled estrogen ligand. The ERα and ERβ fluorescence polarization based-competitor assay kits were obtained from Invitrogen (P2698 [α] and P2700 [β]). The assay was performed using a protocol described by the manufacturer.[37-38] The recombinant ER and the fluorescent estrogen ligand were removed from the -80 °C freezer and thawed on ice (4 °C) for one-hour prior to use. The fluorescent estrogen (2 nM) was added to the ER (40 nM for ERα and 20 nM for ERβ) and screening buffer (provided; 100 nM potassium phosphate (pH 7.4), 100 μg/ml BGG, 0.02 M NaN_3) was added to make up a final volume that was dependent on the number of tubes used. Test compound, 1 μL, in the desired range of concentrations, was added to the wells of a 96-well black plate (Greiner, 6 mm diameter) to which 49 μL screening buffer was then added. 50 μL of the fluorescent estrogen/ER complex was added to make up a total volume of 100 μL. A vehicle control contained 1% ethanol (v/v). A negative control contained 50 μL of screening buffer and 50 μL of fluorescent estrogen/ER complex. This control was used to determine the polarization value when no competitor was present (theoretical maximum polarization). 1 μL of 1 mM β-estradiol (endogenous ligand) (final

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concentration in well of 10 μM) was used as a positive control (minimum polarization value). The plates were incubated in the dark at room temperature for 2 hours and were mixed by shaking on a plate shaker. The fluorescence polarization values were read using 485 nM excitation and 530 nM emission interference filters. IC_{50} values were calculated using GraphPad Prism software.[55]

ER FRET Assay for Determination of Agonist or Antagonist Binding. The Lanthascreen® ER FRET assay (Invitrogen) was used to determine the binding mode of test compound **20** as described in the manufacturers protocol.[56] Briefly, nuclear receptor buffer K was prepared by adding DTT to give a final concentration of 5 mM DTT. This buffer was used for all other dilutions. A 'no ligand' control of DMSO (2% v/v final concentration) was used to show minimum binding. β-Estradiol (2 μM final concentration) was used as a positive control. Compound serial dilutions in DMSO were prepared in a 96-well plate before transfer to a 384-well plate. 1 μL of a 10 mM solution of compound **20** in DMSO was added to 49 μL buffer. 10 μL of this dilution was added into each well. To make a 4X (12 nM) dilution of Fluoromone ES2 using a stock concentration of 1800 nM, 6.7 μL of fluoromone was added to 993 μL of buffer. 5 μL of this mixture was added to each well. ERα was thawed for one hour on ice prior to use and diluted with cold buffer. The TR-FRET buffer is prepared immediately prior to use. Receptor and fluorescein solution are added to the 384-well assay plate and the plate is read at wavelengths of 520nm and 495nm. IC_{50} values were calculated using GraphPad Prism Software.[55]

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MTT Assay for Measurement of Antiproliferative Effects. The human breast tumour cell line MCF-7 was cultured in Eagles minimum essential medium in a 95% O_2/5% CO_2 atmosphere at 37ºC with 10% fetal bovine serum, 2 mM L-glutamine and 100 µg/mL penicillin/streptomycin. The medium was supplemented with 1% non-essential amino acids. Cells were trypsinised and seeded at a density of 5 x 10^3 cells/well in a 96-well plate and incubated at 37ºC for 24 hr. After this time they were treated with 2 µL volumes of test compound in ethanol (nine final concentrations between 1 nM-100 µM) Control wells contained the equivalent volume of the vehicle ethanol (1% v/v). After 72 hr, the culture medium was carefully removed, the cells were washed with 100 µL phosphate buffered saline (PBS) and 50 µL MTT (dissolved in PBS) was added, to give a final concentration of 1 mg/mL MTT. Cells were incubated for 3 hours in darkness at 37ºC. After this, 200 µL DMSO was added to each well, cells were kept in darkness for 20 min at room temperature and the absorbance at 595 nm was read using a Dynatech MR5000 plate reader. The absorbance value of control cells (no compound added) was set as 100% cell viability and, from this, graphs of absorbance versus cell density per well were prepared to assess cell viability using GraphPad Prism software.[55]

Lactate Dehydrogenase Assay for Measurement of Cytotoxicity. Cytotoxicity was determined using the CytoTox 96 non-radioactive cytotoxicity assay (Promega) following the manufacturer's protocol.[57] Briefly, MCF-7 cells were seeded in 96-well plates, incubated for 24 hr and then treated with test compounds **16**, **26** and **30** as described in the MTT assay above. After 72 hr, 20 µL of 'lysis solution (10X)' was added to control wells and the plate was incubated for a further 1 hr to ensure 100% death. 50

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μL of supernatant was carefully removed from each well and transferred to a new 96-well plate. 50 μL of reconstituted 'substrate mix' was added and the plate was placed in the dark at room temperature for 30 min. After this period, 50 μL of 'stop solution' was added to each well and the absorbance was read at a wavelength of 490 nm using a Dynatech MR5000 plate reader. The percentage cell death at 10 μM was calculated.

Cell Cycle Analysis using Flow Cytometry. MCF-7 cells were seeded at a density of 18 x 10^5 cells/mL in medium (5 mL)(900,000 cells per flask). After 24 hr the cells were treated with 50 μL of ethanol (1% v/v)(vehicle control) and range of concentrations of selected compounds **16**, **26** or **30** (10 nM-10 μM 1% v/v). They were incubated for 24 hr, 48 hr (compound **26**, 10 μM) or 72 hr (compound **26**, 10 μM). Following incubation, media was transferred to a vial and the cells were trypsinized. Cells were centrifuged for 10 min at 600*g*. The supernatant was decanted, the pellet resuspended in ice-cold PBS (200 μL) and added to the vial containing the media. Subsequently ice-cold 70% ethanol/PBS (2 mL) was slowly added to the tube as it was gently vortexed. Samples were kept at -20 °C for a minimum of one hour. After the fixation FBS (5 μL) was added to the samples. Ethanol was removed by centrifugation and pellets were incubated in FACSflow sheath fluid (400 mL) supplemente)d with RNase A (10 mg/mL)(Sigma Aldrich, St Louis, MO, USA) and propidium iodide (100 mg/mL (Sigma Aldrich, St Louis, MO, USA). The samples were incubated in the dark for a minimum of 30 min at 37 °C. The samples were read at 488 nM using FACSCalibur™ flow cytometer (Becton Dickinson). The FACS data for 10,000 cells was analysed using BD CellQuest™ and the data was stored as frequency histograms.

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Western Blots

Tubulin Polymerisation Assay. Tubulin depolymerization was quantified by using a modified version of a previously documented method.[58] MCF-7 cells were treated with vehicle [0.1% ethanol (v/v)] or indicated concentrations of **26** (1 μM), paclitaxel (1 μM) or nocodazole (1 μM) for 4 hours. Cells were harvested into MT-preserving buffer (0.1 M PIPES (pH 6.9), 2 M glycerol, 5 mM $MgCl_2$, 2 mM EGTA, 0.5% Triton X-100, and protease inhibitors (Roche Diagnostics Ltd, UK) supplemented with 4 μM paclitaxel to maintain stability of assembled microtubules during isolation. The supernatant containing unpolymerized tubulin was clarified by centrifugation (16,000*g* for 45 min) using a Sorvell and separated from the pellet containing polymerized tubulin. The pellet was washed once in MT-preserving buffer before being denatured in PARP buffer (300 μL)(62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% Bromophenol Blue, 6M Urea and 5% β-mercaptoethanol). Samples were stored at -80 °C. Before use 1M DTT (20 μL) was added to both the supernatant and pellet samples. 2X Laemmli buffer (180 μL)(62.5 mM Tris-Hcl, pH 6.8, 6 M urea, 2% SDS, 10% glycerol, and 0.00125% bromphenol blue) was added to the supernatants. All samples were boiled at 100 °C for 3 minutes and loaded equally (30 μg protein). Proteins were separated on 10% SDS-PAGE gels and transferred onto nitrocellulose (Millipore). Membranes were blocked in 5% non-dry fat milk/TBST for 1 hour, anti-α-tubulin primary antibody [1:1000] (Millipore) for 2 hours and anti-mouse HRP-conjugated secondary antibody [1:1000] (Promega) for 1 hour at RT. All blots were probed with anti-GAPDH antibody [1:1000] (Millipore) to confirm equal loading. Proteins were detected using Immobilon™ western

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electrochemiluminescence reagent (Milipore) on Kodak X-Omat LS film and developed using a Fuji X-rayprocessor.

Evaluation of expression levels of anti-apoptotic proteins Bcl-2 and Mcl-1

MCF-7 cells were seeded at a density of 500,000 cells/flask in T25 flasks. After 48 or 72 hr, whole cell lysates were prepared from untreated cells or cells treated with vehicle control (EtOH, 0.1% v/v) or compound **26** (10 μM). Cells were harvested in RIPA buffer supplemented with protease inhibitors (Roche Diagnostics), phosphatase inhibitor cocktail 2 (Sigma-Aldrich) and phosphatase inhibitor cocktail 3 (Sigma-Aldrich). Equal quantities of protein (as determined by a BCA assay) were resolved by SDS-PAGE (12%) followed by transfer to PVDF membranes. Membranes were blocked in 5% non-dry fat milk/TBST for 1 hr. Membranes were incubated in the relevant primary antibodies at 4 ºC overnight, washed and incubated in horseradish peroxidase conjugated secondary antibody for 1 hr at rt and washed again. Enhanced chemiluminescence was used for detection of protein expression. Western blot analysis was performed using antibodies directed against Mcl-1 [1:1000] (Millipore) and Bcl-2 [1:500] (Millipore) followed by incubation with a horseradish peroxidase-conjugated anti-mouse antibody [1:1000] (Promega, Madison, WI, USA). All blots were probed with anti-GAPDH antibody [1:1000] (Millipore) to confirm equal loading. Proteins were detected using chemiluminescent western blot detection (Clarity Western ECL substrate) (Bio Rad) on the ChemiDoc MP System (Bio Rad).

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Computational Procedures. Compound **26** was drawn in Accelrys Draw v4.1 with alternate isomers represented and converted to a 3D structure using CORINA v3.4.[59] Subsequent enumeration of correct protonation states and conformers were carried out using pkatyper (QUACPAC v1.6.3.1[60-61]) and OMEGA v 2.5.1.4 respectively.[62-63] Compound **26** was docked using the ligand guided docking tool HYBRID (OEDocking v3.0.1[64]) into the binding site of ERα (PDB ID: 2OUZ[65]) and the colchicine site of tubulin (PDB ID: 4O2B[66]). All hybrid dockings were refined by energy minimisation under the Pfrosst force field in MOE v2011.10.[39]

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Non-Standard Abbreviations

ER	Estrogen receptor
FRET	Fluorescence resonance energy transfer
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TBDMS	*Tert*-butyldimethylsilane
TMCS	Trimethylchlorosilane

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Supporting Information Available: Top 5 ranked docking solutions for compound **26** in the ligand binding domain of ERα. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. ERα and ERβ binding affinity values and antiproliferative activity in MCF-7 cells for β-lactam compounds

Compound	R_6 R_1 R_4 R_5 R_6 O N R_3 R_2 R_3						IC_{50} MCF-7 (μM)[a]	IC_{50} ERα (μM)[b]	IC_{50} ERβ (μM)[b]
	R_1	R_2	R_3	R_4	R_5	R_6			
15	OH	OH	H	H	H	H	>50	13	56
16	OCH_3	OCH_3	OCH_3	H	H	H	0.04[25]	Inactive	Inactive
19	OH	OH	H	C_6H_5	H	H	>50	0.23	0.98
20	OH	OH	H	*p*-C_6H_4OH	H	H	>50	1.1	4.8
21	OH	OH	H	OC_6H_5	H	H	>50	0.14	1.2
22	OH	OH	H	2-naphthyl	H	H	>50	0.15	1.1
23	OH	OH	H	C_6H_5	C_6H_5	H	45	0.04	0.019
24	OH	OH	H	3,4,5-$OCH_3C_2H_6$	H	H	>50	1.47	23
25	OCH_3	OH	H	*p*-C_6H_4OH	H	OCH_3	>50	18	144
26	OCH_3	OCH_3	OCH_3	OH HO	H	H	0.21	0.008	0.015
28	OH	OH	H	OH HO	H	H	49	Nd[c]	Nd[c]

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29	OCH_3	OCH_3	H		H	H	32	Nd[c]	Nd[c]
30	OCH_3	OCH_3	OCH_3		H	H	3.8	Inactive	Inactive
1	β-Estradiol						-	0.0057	0.0056
2	Tamoxifen						4.1	0.061	0.19

[a]*In vitro* cytotoxicity data for compounds **15**, **16**, **19-26** and **28-30**. IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 cells. Values represent the mean for three independent experiments performed in triplicate. The IC_{50} value obtained for **2** is in good agreement with the reported IC_{50} value for tamoxifen in human MCF-7 cells.[67-68]

[b]Competition assay for ERα and ERβ for compounds **15**, **16**, **19-26** and **28-30** using a human recombinant ERα and ERβ proteins and a fluorescent estrogen. IC_{50} values: the concentration of competitor that results in a half maximum shift in polarisation is calculated as the IC_{50} of the competitor. The ER binding values obtained are in agreement with the reported IC_{50} binding data for **2** (ERα: 60.9 nM; ERβ: 188 nM) (Invitrogen). Values represent the mean for three independent experiments performed in duplicate.

[c]IC_{50} values for compounds **28** and **29** were not determined (nd). The % inhibition of ERα for these compounds at a concentration of 1 μM was 40% and 37% respectively.

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The % inhibition of ERβ for these compounds at a concentration of 1 μM was 43% and 75% respectively. For comparative purposes, compound **26** (1 μM) caused 93% inhibition of ERα and 94% inhibition of ERβ.

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Figure Legends

Figure 1. Estrogen Receptor Ligands

Figure 2A. Evaluation of G_2M arrest in MCF-7 cells exposed to compounds 16, 26, and 30. Cell cycle analysis of MCF-7 cells treated with vehicle control [1% (v/v) ethanol], or 10 nM, 100 nM, 1 μM and 10 μM (final concentrations) of compounds **16**, **26** and **30** at 24 hours. Cells were analysed by FACScan flow cytometry. Percentages of cells in different phases of the cell cycle are indicated. Values represent the mean ± S.E.M deviation for three separate experiments.

Figure 2B. Differential effects of compound 26 on the cell cycle and apoptosis in MCF-7 cells. Cells were treated with either vehicle [0.1% ethanol (v/v)] or 10 μM compound **26** for 24, 48 and 72 hours. Cells were then fixed, stained with PI, and analyzed by flow cytometry. Cell cycle analysis was performed on histograms of gated counts per DNA area (FL2-A). The number of cells with <2N (pre-G_1), 2N (G_0G_1), and 4N (G_2M) DNA content was determined with CellQuest software. The sub-G_1 peak is indicative of apoptosis. Statistical analysis was performed using GraphPad Prism software.[55] A two-way ANOVA was employed to determine significant differences between vehicle controls and treated samples. Values represent the mean ± S.E.M. for two separate experiments.

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Figure 3. β-Lactam 26 induces depolymerization of tubulin in MCF-7 cells. The effect of β-lactam **26** on the microtubule network of MCF-7 breast cancer cells was examined by a sedimentation assay and western blotting. Cells were treated with vehicle [0.1% ethanol (v/v)], β-lactam **26**, paclitaxel or nocodazole (1μM) for 4 hr before being lysed in MT preserving buffer. Unpolymerized and polymerized fractions were separated by centrifugation and collected as supernatant (S) and pellet (P) fractions respectively. Samples were separated by western blotting and probed with anti-α-tubulin antibody [1:1000] and anti-mouse secondary antibody [1:1000]. The soluble supernatant faction (S) contains unpolymerised tubulin and the insoluble pellet fraction (P) contains polymerized tubulin. GAPDH was used as a loading control [1:1000]. Results are representative of three separate experiments.

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Figure 4. β-Lactam 26 downregulates expression of the anti-apoptotic proteins Bcl-2 and Mcl-1. MCF-7 cells were untreated (UT), treated with ethanol (0.1% v/v) (EtOH) or treated with compound **26** (10 μM). After 72 hr, cells were harvested and separated by SDS PAGE. The membrane was probed with anti-Bcl-2 [1:500] or anti-Mcl-1 [1:1000] antibodies. GAPDH was used as a loading control [1:1000]. Results are representative of three separate experiments.

Figure 5. Predicted binding mode of β-lactam 26 in the ligand binding domain of ERα. Atoms are coloured as follows: carbon – grey; nitrogen – blue; oxygen – red. Dashed lines represent the non-covalent interaction between the ligand and receptor.

Figure 6. Predicted binding mode of β-lactam 26 in the tubulin-colchicine binding site. Colchicine is shown with a green scaffold and β-lactam **26** is shown with a grey scaffold. Atoms are coloured in both molecules as follows: nitrogen – blue; oxygen – red. Dashed lines represent the non-covalent interaction between the ligand and receptor.

Figure 1.

1 2 3

4 5 6

7 8

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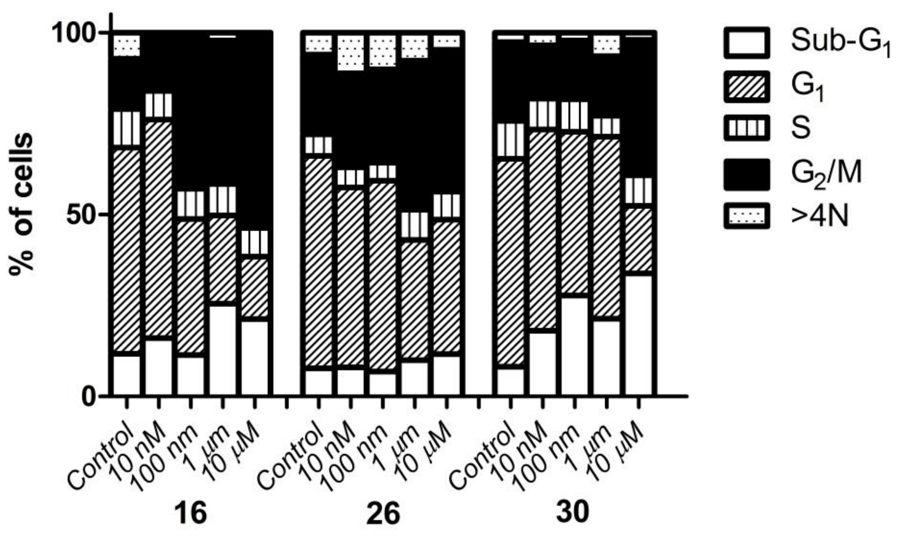
Figure 2.

A.



B.

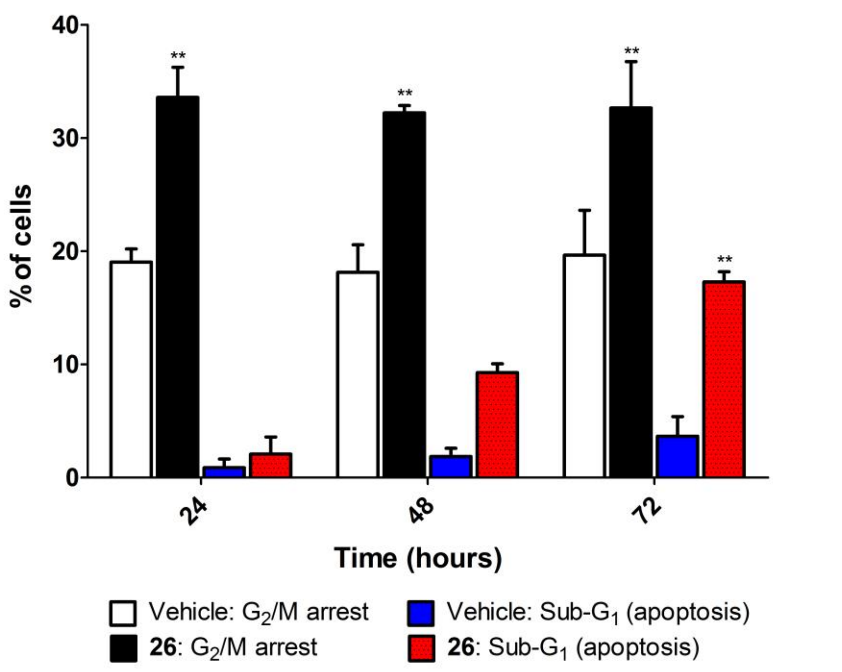


Figure 3.

Vehicle β-Lactam 26 Paclitaxel Nocodazole

S P S P S P S P

50 kDa α-Tubulin

38 kDa GAPDH

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Figure 4.

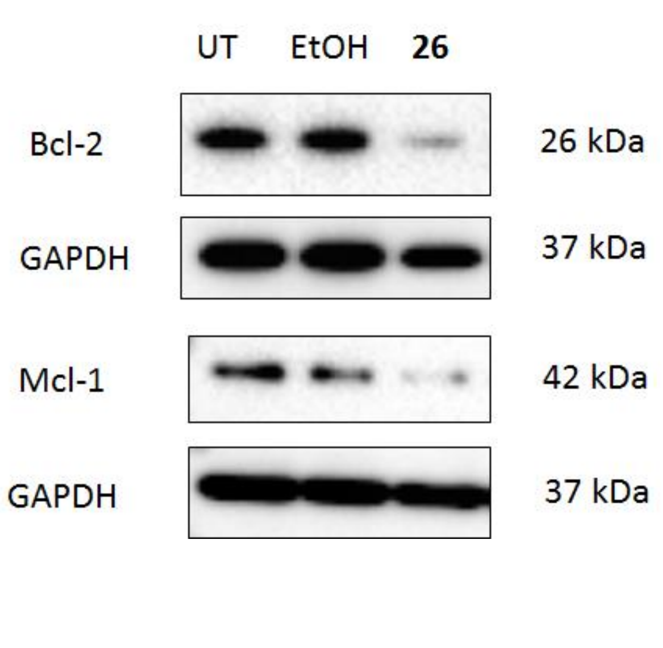

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Figure 5.

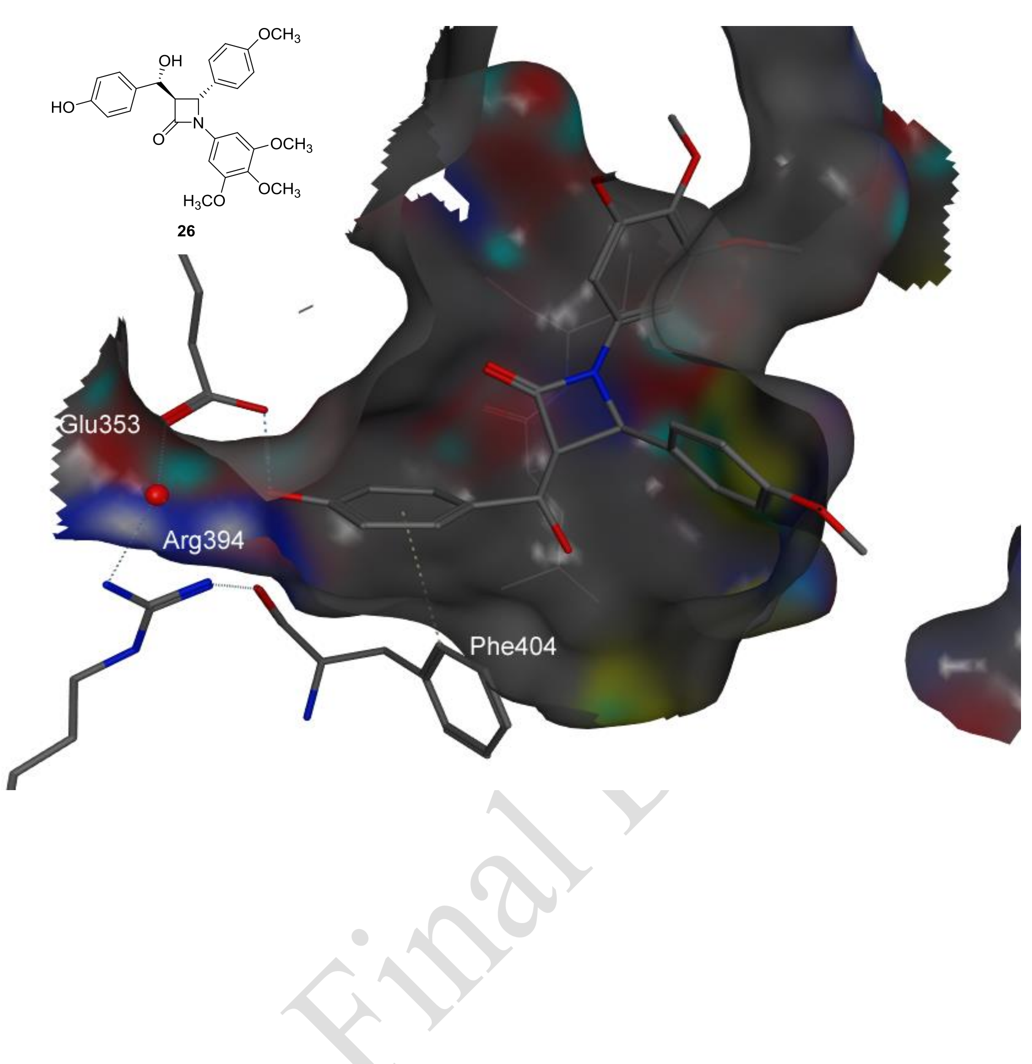

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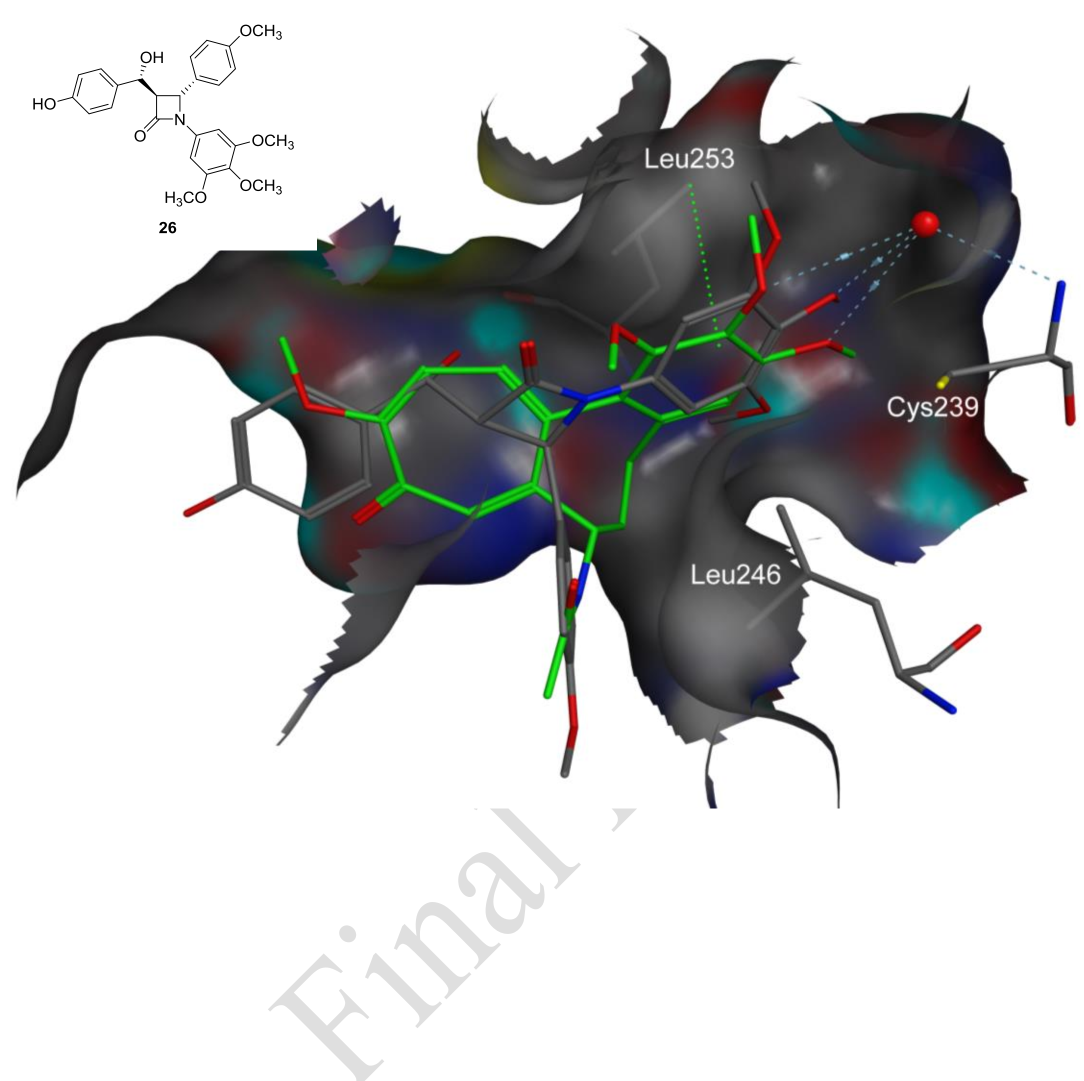
Figure 6.



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Scheme Legends

Scheme 1. Synthesis of compound 9 and imines 10-14 [a]

[a]Reagents and conditions: (a) *t*-$BuMe_2SiCl$, DBU, CH_2Cl_2, rt, until complete as indicated by TLC, 73%; (b) EtOH, reflux, 3 h, 74−100%.

Scheme 2. Synthesis of β-lactams 15-25 [a]

[a](a) Zn dust, $(CH_3)_3SiCl$, 40 °C, 15 min then 100°C, 2 min, microwave; $BrCH_2CO_2Et$, C_6H_6, 100 °C, 30 min, microwave, 32−43%; (b) TBAF, THF, 0 ˚C, 15 min; (c) H_2, Pd/C, EtOH:EtOAc (1:1), rt, until complete as indicated by TLC; (d) $R_1R_2CHCOCl$, Et_3N, CH_2Cl_2, reflux, 3 h.

Scheme 3. Synthesis of β-lactams 26-30 [a]

[a](a) HOC_6H_4CHO, LDA, THF, -78 ºC, 30 min, 12−31%; (b) H_2, Pd/C, EtOH:EtOAc (1:1), rt, until complete as indicated by TLC, 65%; (c) 1-(2-Chloroethyl)pyrrolidine hydrochloride, CH_3COCH_3, reflux, 2 h, 30%.

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Scheme 1.

HO NH$_2$ —*a*→ TBDMSO NH$_2$

9

R_1 NH$_2$ + H(C=O) R_2 R_3 R_3 —*b*→ R_1 N= R_2 R_3 R_3

10: R_1=OTBDMS; R_2=OBn; R_3=H
11: R_1=OTBDMS; R_2=R_3=OCH_3
12: $R_1 = R_2 = R_3 = OCH_3$
13: $R_1 = R_2$ = OBn, R_3 = H
14: $R_1 = R_2 = OCH_3$, R_3 = H

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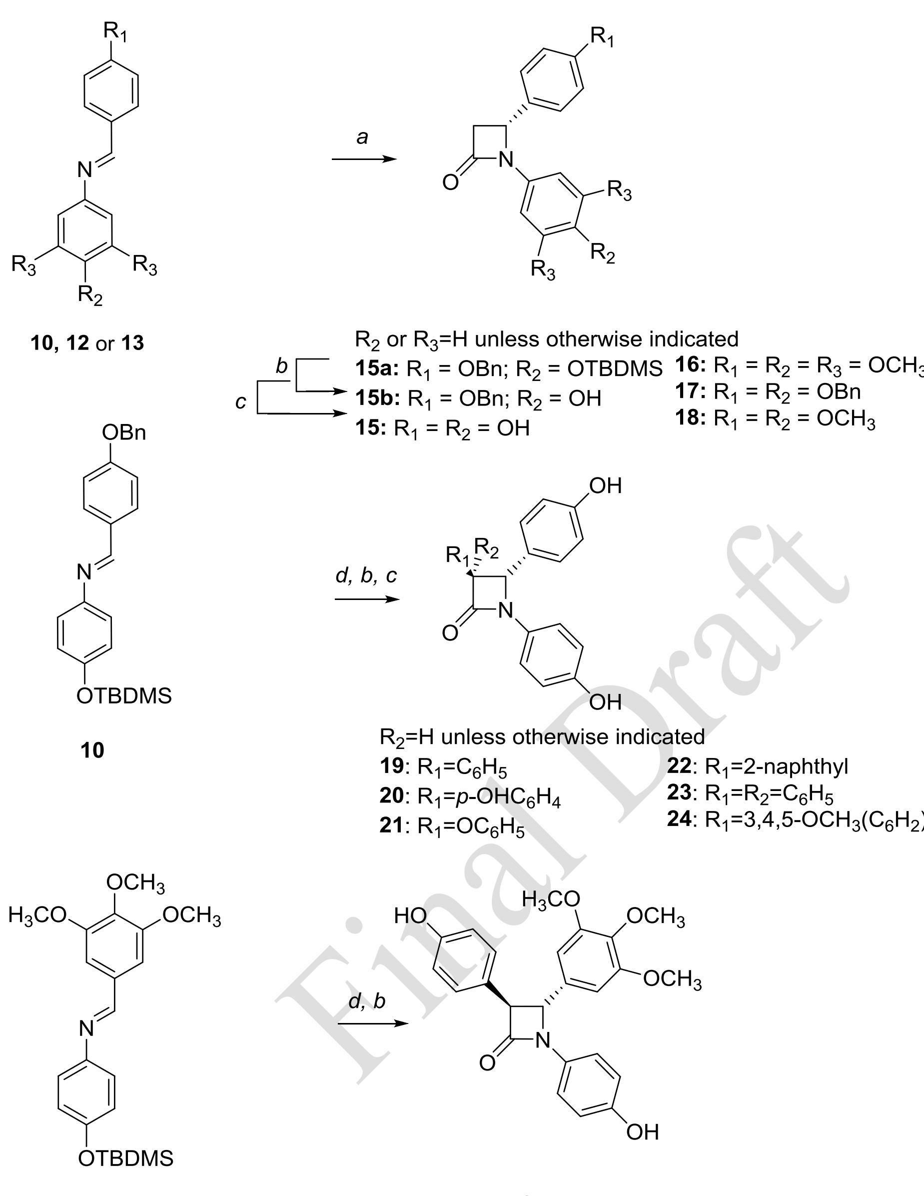
Scheme 2.



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Scheme 3.

a

16: $R_1 = R_2 = R_3 = OCH_3$
17: $R_1 = R_2 = OBn$, $R_3 = H$
18: $R_1 = R_2 = OCH_3$, $R_3 = H$

b

26: $R_1 = R_2 = R_3 = OCH_3$
27: $R_1 = R_2 = OBn$, $R_3 = H$
28: $R_1 = R_2 = OH$, $R_3 = H$
29: $R_1 = R_2 = OCH_3$, $R_3 = H$

26 → c → **30**

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