Bioorganic & Medicinal Chemistry 16 (2008) 9554–9573

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, biological evaluation, structural–activity relationship, and docking study for a series of benzoxepin-derived estrogen receptor modulators

Irene Barrett [a], Mary J. Meegan [a,*], Rosario B. Hughes [a], Miriam Carr [a], Andrew J. S. Knox [b], Natalia Artemenko [b], Georgia Golfis [b], Daniela M. Zisterer [c], David G. Lloyd [b]

[a] School of Pharmacy and Pharmaceutical Sciences, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin 2, Ireland
[b] Molecular Design Group, School of Biochemistry & Immunology, Trinity College Dublin, Dublin 2, Ireland
[c] School of Biochemistry & Immunology, Trinity College Dublin, Dublin 2, Ireland

ARTICLE INFO

Article history:
Received 12 May 2008
Revised 2 September 2008
Accepted 10 September 2008
Available online 16 September 2008

Keywords:
Benzoxepin
Estrogen receptor antagonist
Antiproliferative activity
Docking
QSAR

ABSTRACT

The estrogen receptors ERα and ERβ are recognized as important pharmaceutical targets for a variety of diseases including osteoporosis and breast cancer. A series of novel benzoxepin-derived compounds are described as potent selective modulators of the human estrogen receptor modulators (SERMs). We report the antiproliferative effects of these compounds on human MCF-7 breast tumor cells. These heterocyclic compounds contain the triarylethylene arrangement as exemplified by tamoxifen, conformationally restrained through the incorporation of the benzoxepin ring system. The compounds demonstrate potency at nanomolar concentrations in antiproliferative assays against an MCF-7 human breast cancer cell line with low cytotoxicity together with low nanomolar binding affinity for the estrogen receptor. The compounds also demonstrate potent antiestrogenic properties in the human uterine Ishikawa cell line. The effect of a number of functional group substitutions on the ER binding properties of the benzoxepin molecular scaffold is examined through a detailed docking and 2D-QSAR computational investigation. The best QSAR model developed for ERαβ selectivity yielded R^2 of 0.84 with an RMSE for the training set of 0.30. The predictive quality of the model was Q^2 of 0.72 and RMSE of 0.18 for the test set. One particular compound (**26b**) bearing a 4-fluoro substituent, exhibits 15-fold selectivity for ERβ and both our docking and QSAR studies converge on the correlation between enhanced lipophilicity and enhanced ERβ binding for this benzoxepin ring scaffold.

1. Introduction

The estrogen receptor (comprising of two subtypes ERα and ERβ) is a ligand inducible nuclear receptor which play a critical physiological role as mediator of the actions of the estrogen hormones. The estrogen receptors are widely distributed in the body tissues and are regarded as attractive therapeutic agents for diseases such as osteoporosis and breast cancer.[1–3] ERα is predominantly found in the uterus, bone, cardiovascular tissue, and liver and is the predominant ER expressed in breast cancer. ERβ is expressed in many tissues including prostate, breast, vascular endothelium, and ovary. The precise function of ERβ and its role in breast pathobiology are not clear. However, recent studies indicate that ERβ expression may have a potential protective effect on normal cells against ERα induced hyperproliferation.[4] The DNA binding domains of the two ER subtypes is well conserved, however, the amino acid sequence conservation in the ligand binding domain is only 59%. The LBD volume for ERβ is smaller than for ERα and differences are found in the amino acids of the LBD including replacement of Met421 and Leu384 in ERα with Ile and Met in ERβ. Design of ligands which can modulate the activity of the ER in a tissue selective manner continues to attract interest due to the regulating effects of the ER on many diseased states. Tamoxifen (**1a**) is a well-established antagonist for the estrogen receptor and has been a useful endocrine drug in the treatment of ER positive breast cancer.

X-ray crystallographic studies have determined the binding modes of several ligands (e.g., estradiol, 4-hydroxytamoxifen (**1b**) and raloxifene (**2**)) in the ligand binding domain of the ERα and ERβ.[5–9] It is evident that the binding of estrogen agonists, pure antiestrogens and SERMs (selective estrogen receptor modulators) to the ER-LBD results in induction of key conformational changes to Helix 12. Many alternative novel scaffolds for estrogen receptor modulators have been discovered through 'scaffold hopping' protocols which have facilitated the design of several novel agonist and

* Corresponding author. Tel.: +353 1 8962798; fax: +353 1 8962793.
E-mail address: mmeegan@tcd.ie (M.J. Meegan).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.09.035

antagonist-type ligands for the ER.[10] Examples of ER modulator scaffolds based on oxygen containing core heterocyclic systems have been reported, for example, benzopyrans such as EM-652 (**3**) which is the active metabolite of EM-800,[11] 7-oxabicyclo[2.2.1]heptene[12] (**4**), chromans,[13] bisbenzopyrans,[14] dihydrobenzoxathin (**5**),[15] bicyclo[3.3.1]nonene,[16] and oxachrysenol[17] while mono[18] and bis-benzo[*b*]oxepines[19] have been designed as agonists of the estrogen receptor. The carbocyclic benzocycloheptene (**6**) has been investigated as a nonisomerisable analogue of *Z*-4-hydroxytamoxifen[20] while benxocycloheptnenes have been reported as tissue selective estrogens.[21] The structure of estradiol together with selected SERMs (**2–6**) is illustrated in Figure 1. We have identified a novel estrogen receptor modulator core containing a central benzoxepin scaffold which demonstrated some potential as antiproliferative and antagonist ER binding ligands[18] and now report the further investigation of this novel core scaffold structure as tissue and subtype selective estrogen receptor modulators. We now report the syntheses of 22 benzoxepin derivatives which are substituted at C-4, C-7, and C-8 positions together with an evaluation of their antiproliferative activity and the relative binding affinities for ERα and ERβ. These novel ligands can be used to probe the size, shape, and flexibility of the ERα and ERβ ligand binding domains and could be potentially developed as pharmaceutical agents.

The compounds chosen for synthesis are arranged in four different structural classes as we wished to examine the response of the ER ligand binding domain to specific structural variations. Type I (compounds **15a–d, 26a–e, and 28**) representing the novel benzoxepin scaffold (with variations in the aromatic substituents in the Rings B and C, and also in the basic side chain component of the Ring A substituent) are considered to be ring fused analogues of (*Z*)-tamoxifen. We were interested in examining the effect on activity of the inclusion of a fluorine substituent at C-8 which would increase lipophilicity and also block an expected metabolic oxidation of these compounds, thus ensuring a longer plasma half life. Fluorotamoxifen has been investigated as a possible estrogen receptor imaging agent for positron emission tomography in breast cancer.[22] The inclusion of fluorine has been recently reported to contribute to the SERM activity in the oxachrysenol series of ER ligands.[23] Type II compound **32** contains the basic side chain substituent located on the alternative Ring C site and represents a ring fused analogue of (*E*)-tamoxifen. Compounds of Type III, **36a–e**, contain a novel heterocyclic or naphthyl Ring C structure while in Type IV compounds **41a–e**, Ring C is included as a benzylic type substituent.

To rationalize the observed ER binding selectivity of the benzoxepin products, a detailed 2D-QSAR study was undertaken involving calculation of MOE 2006.08 2D descriptors[24] and also a novel set of descriptors developed within our group known as Quasi-Fragmental descriptors. Finally, a thorough flexible docking study of the benzoxepin ligands was carried out to facilitate both visualization and corroboration of the biochemical biochemical results.

2. Chemistry

The synthesis of the Type I series of benzoxepin analogues containing the *p*-phenolic substituent in Ring C is illustrated in

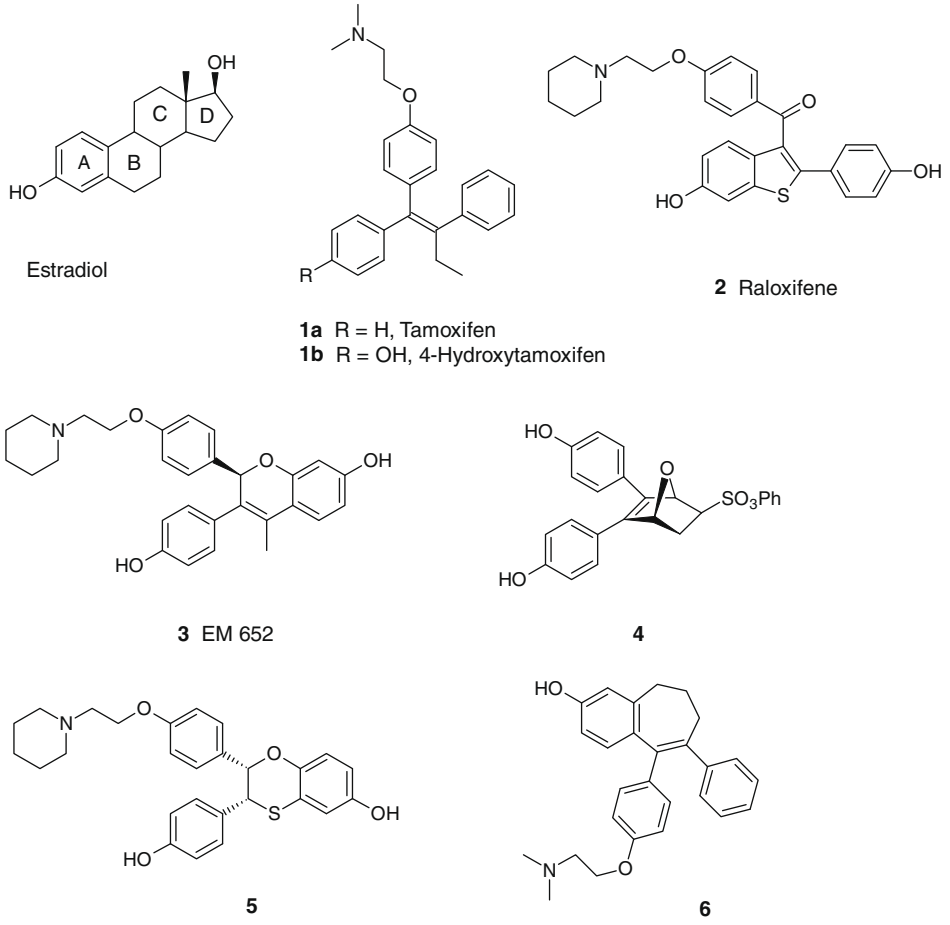
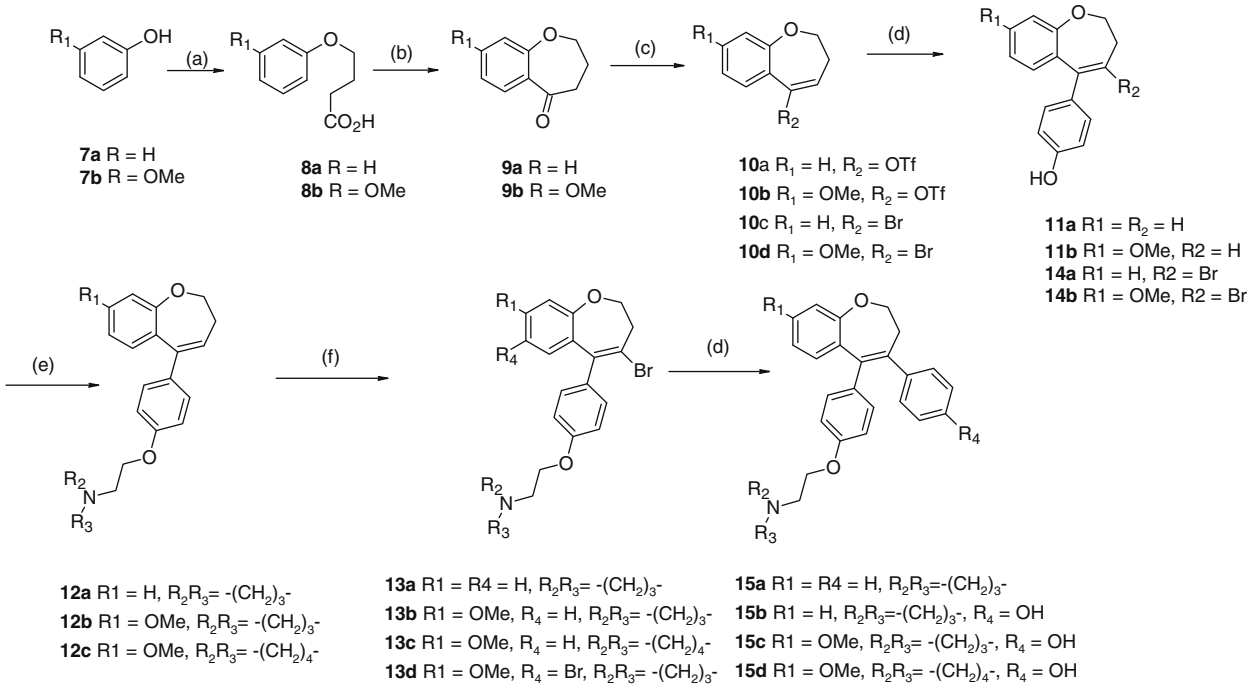


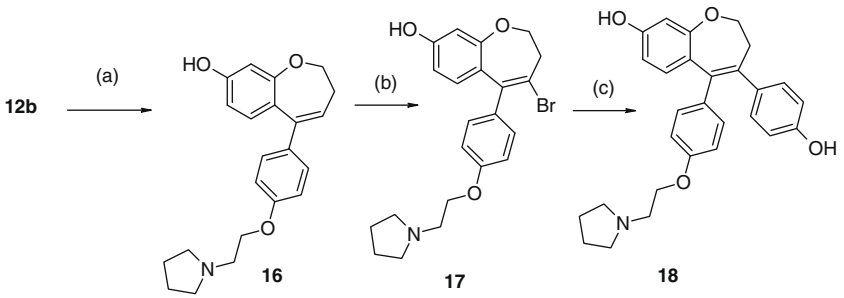
Figure 1. Structure of estradiol and SERMs.

Scheme 1. The 2,3,4,5-tetrahydro-1-benzoxepin-2-ones **9a–b** were obtained by acid promoted cyclization of the 4-phenoxy butyric acids **8a–b** (obtained by alkylation of the corresponding phenols **7a–b** with ethyl bromobutyrate). Many reagents, for example, polyphosphoric acid, methane sulfonic acid, trifluoro methane sulfonic acid, and the use of hafnium triflate as catalyst[25] were explored to optimize this cyclization reaction: PPA was found to be the most efficient reagent for the preparation of the unsubstitued benzoxepin **9a**, R = H, while Eatons reagent was found to be superior for **9b**, R = OMe with yields of up to 30% achieved. **9a,b** were converted to the triflates **10a,b** by treatment with triflic anhydride and then subsequently coupled with 4-hydroxyphenylboronic acid in a Suzuki reaction promoted by $Pd(PPh_3)_4$ to afford the 5-arylbenzoxepins **11a,b** in good yield (67–88%). The vinyl bromides **10c,d** was also obtained by reaction of **9a,b** with phosphorus tribromide; subsequent arylation of the bromides afforded the products **11a,b**, respectively. However, the bromides **10c,d** proved to be unstable and the triflates **10a,b** were preferred for subsequent arylation reactions. The basic pyrrolidine and piperidine ethers were then readily introduced under standard alkylating conditions to afford products **12a–c** which were converted to the vinylic bromides **13a–c** on treatment with pyridine hydrobromide perbromide. This reaction was completed rapidly and required careful monitoring to prevent the formation of the 4,7-dibromide product **13d**. An alternative route to **13a,b** involves the initial vinylic bromination of **11a,b** followed by alkylation of the bromide products **14a,b** to afford the products **13a,b**, respectively. A second Suzuki reaction was then completed on compounds **13a–c** to afford the required products **15a–d** in good yield. For synthesis of the diphenolic product **18**, it was necessary to first demethylate **12b** with pyridinium hydrochloride to produce the phenol **16** as the more usual demethylating reagent boron tribromide etherate caused extensive degradation of both **13b** and **15b**. Subsequent arylation of the bromide **17** afforded the required diphenolic product **18** (Scheme 2).

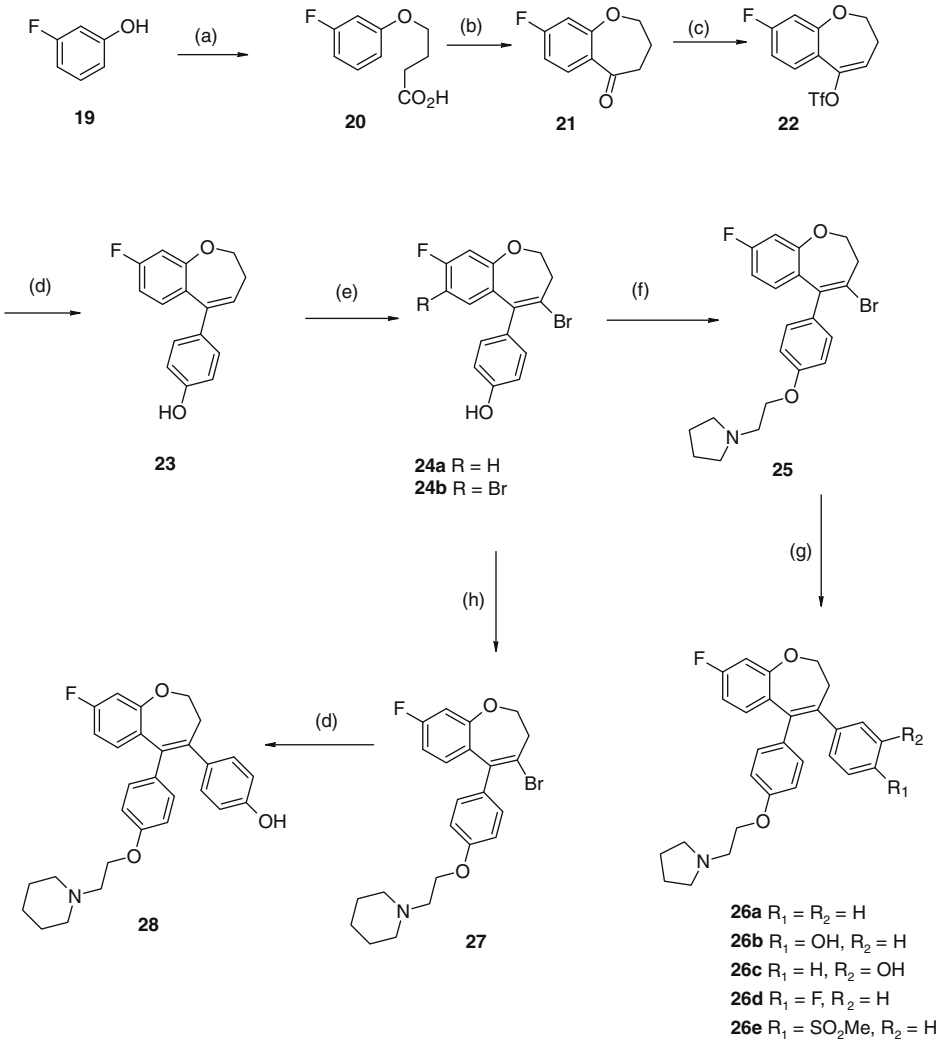
The fluorinated benzoxepin series was prepared in a similar procedure as illustrated in Scheme 3. Reaction of 3-fluorophenol **19** with ethyl 4-bromobutyrate followed by base catalyzed hydrolysis of the ester afforded the acid **20** which was cyclized with polyphosphoric acid to give the 8-fluorobenzoxepin-4-one **21**.[26]



Scheme 1. Synthesis of Type I benzoxepin SERMs **15a–d**. Reagents and conditions: (a) i—$BrCH_2CH_2CH_2CO_2Et$, K_2CO_3, DMF; ii—NaOH, EtOH; (b) R = H, F; PPA, 110 °C, 4 h; R = OMe, Eaton's reagent, 80 °C, 2 h; (c) $(TfO)_2O$, Na_2CO_3, 18 h, rt; (d) $Pd(PPh_3)_3$, 4-$HOC_6H_5B(OH)_2$, Na_2CO_3(aq), THF; (e) K_2CO_3, 1-(2-chloroethyl)pyrrolidine hydrochloride or 1-(2-chloroethyl)piperidine hydrochloride, acetone, 24 h, 85 °C; (f) $PyHBr_3$, CH_2Cl_2, 20 °C, 18 h; (g) Py·HCl, 190 °C, 4 h.



Scheme 2. Synthesis of Type I benzoxepin SERM **18**. Reagents and conditions: (a) Py·HCl, 190 °C, 4 h; (b) $PyHBr_3$, CH_2Cl_2, 20 °C, 18 h; (c) $Pd(PPh_3)_3$, 4-$HOC_6H_5B(OH)_2$, Na_2CO_3(aq), THF.



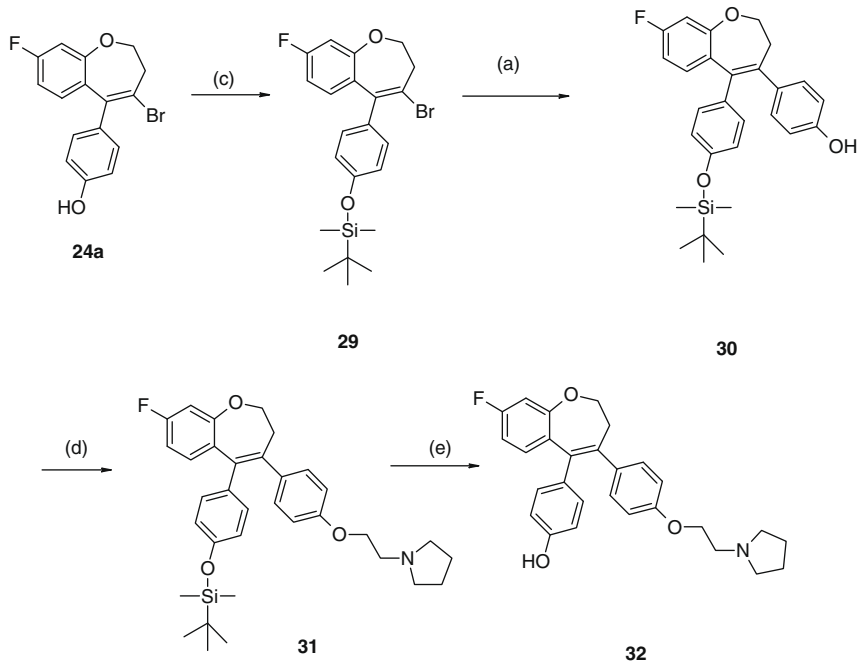
Scheme 3. Synthesis of Type 1 benzoxepin SERMs **26a–e** and **28**. Reagents and conditions: (a) i—$BrCH_2CH_2CH_2CO_2Et$, K_2CO_3, DMF; ii—NaOH, EtOH; $BrCH_2CH_2CH_2COOH$; (b) PPA, 110 °CC, 4 h; (c) $(TfO)_2O$, Na_2CO_3, 18 h, rt (d) $Pd(PPh_3)_3$, 4-$HOC_6H_4B(OH)_2$, Na_2CO_3(aq), THF; (e) PyHBr$_3$, CH_2Cl_2, rt, 18 h; (f) K_2CO_3, 1-(2-chloroethyl)pyrrolidine hydrochloride, acetone, 24 h, 85 °C; (g) $Pd(PPh_3)_3$, $R_1R_2C_6H_3B(OH)_2$, Na_2CO_3(aq), THF; (h) K_2CO_3, 1-(2-chloroethyl)piperidine hydrochloride, acetone, 24 h, 85 °C.

The phenol **23** was obtained in yield of >90% by Suzuki arylation of the triflate **22** catalyzed by $Pd(PPh_3)_4$ without the need for isolation of the intermediate triflate **22**. Careful bromination of **23** was required to obtain the product **24a** free from contamination with the dibrominated compound **24b**. The bromide **24a** was then alkylated with 1-(2-chloroethyl)pyrrolidine to give **25** which was subsequently arylated in a second Suzuki reaction with a selection of boronic acids to afford the products **26a–e**. Introduction of the piperidine type basic substituent required the initial alkylation of **24a** to give **27** which was subsequently arylated to afford the phenolic product **28**.

The synthesis of the isomeric compound **32**, which could be compared to the (*E*)-tamoxifen stereochemistry was also accomplished (Scheme 4). The phenol **24a** was converted to the protected TBDMS ether **29** by treatment with *tert*-butyltrimethylsilyl chloride, in DMF in the presence of imidazole. Subsequently arylation in a second Suzuki reaction with the phenolic boronic acid provided the phenol **30**. Introduction of the basic substituent onto Ring C was accompanied by in situ deprotection of **31** to afford the required product **32** in 60% yield.

The introduction of a number of alternative Ring C types, for example, pyridine, naphthalene, furan, thiophene, and benzofuran at C-4 of the benzoxepin system (Type III compounds) was achieved as outlined in Scheme 5. The products were obtained by first bromination of the benzoxepin **11b** and then demethylation of **33** with boron tribromide to afford the phenol **34**.[18] Subsequent Suzuki arylation of the bromide **34** with the appropriate boronic acids afforded the products **35a–e**. The basic ethers **36a–e** were then produced by standard alkylation of the phenols **35a–e** with *N*,*N*-dimethylaminoethyl chloride.

A flexible methylene linking group was introduced between C-4 of the benzoxepin system and the aromatic ring C (Type IV compounds) as illustrated in Scheme 6. Aldol condensation of the benzoxepin **9a** with the appropriate aryl aldehyde in the presence of HCl afforded the 4-benzylidenebenzoxepin-5-one **37**[27] which was then hydrogenated to form **38**. Direct reaction of **38** with *N*,*N*-dimethylaminoethoxyphenyl bromide and *n*-butyllithium was not successful. However, treatment of **38** with *n*-butyllithium and 4-methoxybromobenzene followed by acid promoted dehydration of the intermediate alcohol resulted in isolation of the alkene products **39**. The target products were obtained following demethylation of **39** with boron tribromide and subsequent alkylation of the phenolic products **40** to afford the desired basic ethers **41a–e.**



Scheme 4. Synthesis of Type II SERM, **32**. Reagents and conditions: (a) TBDMSiCl, imidazole, DMF; (b) $Pd(PPh_3)_3$, 4-$HOC_6H_5B(OH)_2$, Na_2CO_3(aq), THF; (c) K_2CO_3, 1-(2-chloroethyl)pyrrolidine hydrochloride, acetone, 24 h, 80 °C; (d) $(n\text{-}Bu)_4NF$, THF.

3. Biochemistry

The compounds were evaluated in a series of estrogen dependent in vitro assays which measured their affinity for the estrogen receptors and also their ability to act as functional antagonists or agonists of estrogen.

3.1. Antiproliferative activity in MCF-7 breast cancer cells

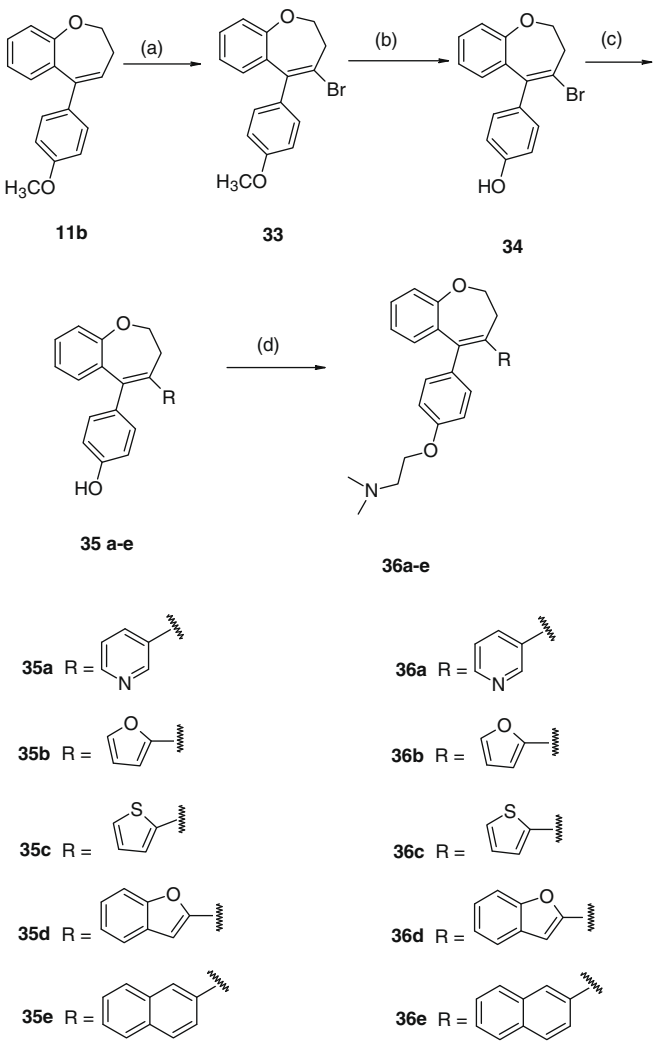
The products were initially assessed for their antiproliferative action using the ER expressing (ER dependent) human MCF-7 breast cancer cell line and the results are displayed in Table 1. Examination of the antiproliferative data of compounds of type I show that many of the products are active at submicromolar concentrations; the most active compounds are those containing the OH group in Ring C and either the F or OH substituent at the C-8 position. Compounds **18** and **26b**, with IC_{50} values of 11 nM and 21 nM, respectively, were the most potent in the series. The replacement of the OH group on compound **18** (C log P = 5.53) with lipophilic fluorine in **26b** (C log P = 6.30) results in a slight decrease in the IC_{50} value from 11.9 to 21.4 nM. The nature of the substituent in the Ring B is therefore critical in determining the antiproliferative activity and ER binding ability of these compound series. The monohydroxy compound **15b** and difluoro compound **26d** were found to have moderate antiproliferative activity (IC_{50} 446 and 450 nM), although considerably greater than tamoxifen, see Table 1.[28] Introduction of the phenolic group on Ring C at the *meta*-position as in compound **26c**, reduces the potency of the compound (IC_{50} 304 nM) while the presence of a methyl sulfone grouping compound **26e**, resulted in a dramatic loss of activity for the compound, (IC_{50} 4.56 μM). The methyl sulfone group has been exploited as a Ring C phenol replacement in naphthalene based SERMs to improve pharmacokinetic properties as it cannot undergo conjugation metabolism to the glucuronide or sulfate ester.[23]

The antiproliferative results obtained in this study compare favorably with the activity reported for products based on the benzocycloheptene and dibenzothiepin scaffold structures.[3,20,29–32] The structurally related benzocycloheptene compound **6**, a ring fused analogue of 4-hydroxytamoxifen was reported McCague et al. to have a close correlation of stereochemistry between the triarylbutene and the seven membred ring structure, particularly in the orientation of the aryl rings as determined by X-ray crystallography.[20] Compound **6** was shown to have similar antiproliferative effect as 4-hydroxytamoxifen in MCF-7 breast cancer cells at 10^{-8} M and blocks the growth stimulation caused by 10^{-8} M estradiol.

Introduction of the pyrrolidine basic ether on the alternative Ring C site, (compound **32**) resulted in significant reduction of antiproliferative activity (IC_{50} = 6.503 μM) which is consistent with the previous reports of the poorer antiproliferative activity of the E-type tamoxifen and the less favorable stereochemical arrangement for antagonist binding in the ER-LBD, possibly contributing to agonist effects observed for E-tamoxifen.[33]

From our previous investigations,[18] the nature of ring C was found to also be crucial in determining the ER affinity of the benzoxepin ligand. Structural type III compounds (**36a–e**) containing a heterocyclic (3-pyridyl, 2-furyl, 2-thienyl, and 2-benzofuryl) or 1-naphthyl Ring C substituent displayed poor antiproliferative activity with IC_{50} in the range 16–20 μM for MCF-7 cells (e.g., for compound **36c**, IC_{50} MCF-7 = 16.7 μM) and poor ERα binding value; IC_{50} = 828 nM) and were not further investigated. Introduction of a flexible hinge to link Ring B to the central ethylene core has been achieved in many SERMs, for example, the carbonyl group in raloxifene,[34] the oxygen ether in arzoxifene,[35] the oxygen ether and methylene linker groups in naphthalene[23] and tamoxifen based SERM examples.[36] However, introduction of a lipophilic benzylic Ring C type substituent as in structural type IV (compounds **41a–e**) resulted in low antiproliferative activity for the series (e.g., compound **41d**, IC_{50} = 10.4 μM) and poor ERα binding (IC_{50} 14.2 μM).

The most potent examples were also tested in hormone independent MDA-MB-231 breast cancer cells. The growth was not inhibited by the benzoxepin compounds up to 100 nM. At higher concentrations all compounds showed a cytotoxic effect but the IC_{50} value determined in the MDA-MB 231 cell line for the most potent compounds were at least 10-fold greater than that obtained for the same compounds in the MCF-7 cell line (Compounds **15b,**



Scheme 5. Synthesis of Type III SERMs **36a–e**. Reagents and conditions: (a) $PyHBr_3$, CH_2Cl_2, 20 °C, 18 h; (b) BBr_3, DCM; (c) $Pd(PPh_3)_4$, $RB(OH)_2$, Na_2CO_3(aq), THF; (d) $ClCH_2CH_2N(CH_3)_2$, K_2CO_3, acetone, 24 h, 85 °C.

15c, 26b, and **18** gave IC_{50} values of 5.10 μM, 5.24 μM, 6.75 μM, 2.60 μM, respectively, when evaluated in the MDA-MB-231 cell line).

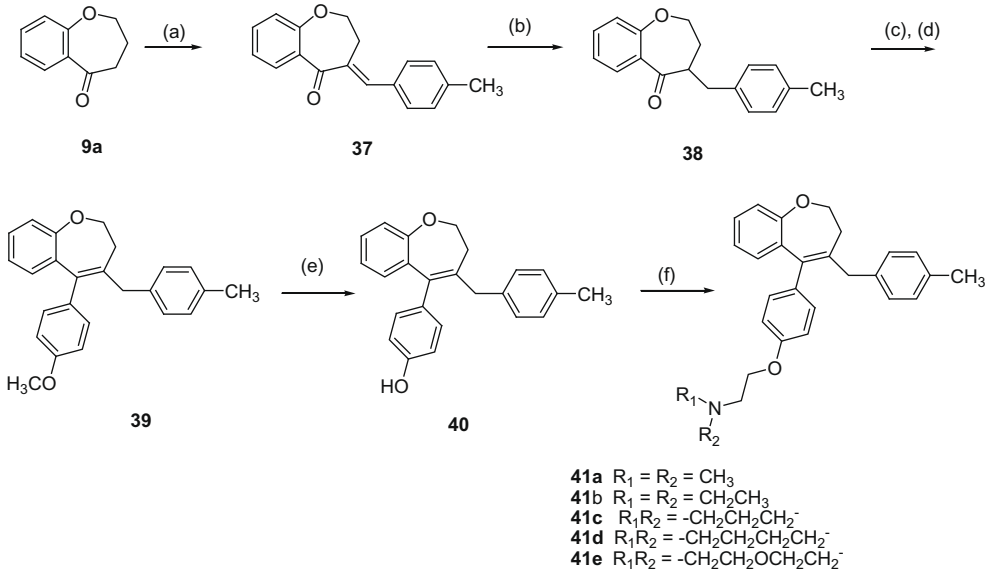
3.2. Cytotoxicity

The cytotoxicity of the compounds in the MCF-7 cell line was also determined in the standard LDH assay as previously reported as we wished to confirm that the antiproliferative effects of the compounds were due to cytostasis rather than cellular necrosis. The compounds all demonstrated low cytotoxicity profiles suggesting that their action is cytostatic rather than cytotoxic. Typical values for cytotoxic induced antiproliferative effects for compound **15a** at 10 μM is 8%, compound **18** is 11%, and compound **11** is 17% whereas value for tamoxifen is observed to be 24% at testing concentration of 10 μM. The cytotoxicity values obtained were less than that for tamoxifen for all compounds evaluated.

3.3. Estrogen receptor binding studies

Estrogen receptor binding studies were carried out for the most potent compounds of the series with ERα and ERβ using a fluorescence polarization procedure.[36] The displacement of fluorescein labeled estradiol (fluoromone) in a competitive binding assay from the human recombinant full length receptor proteins ERα and ERβ expressed from baculovirus-infected insect cells by the synthesized ligands was observed as a decrease in polarization values. The IC_{50} values are calculated from resulting sigmoidal inhibition curves as illustrated in Table 1.

For the Type I benzoxepin scaffold structures, most compounds showed potent ERα binding affinity with $IC_{50} < 20$ nM. The presence of a hydroxyl or fluoro substituent at position 8 in Ring B together with a hydroxyl group at C-4 in Ring C proved to be very effective as demonstrated in compounds **18** and **26b** and with IC_{50} values of 8.4 nM and 10.4 nM, respectively. The piperidine basic group present in compound **28** proved to equally effective (IC_{50} = 6.5 nM) as the pyrrolidine in its role as antiestrogenic basic binding group for ERα LBD Aspartic acid 351. The values compare very favorably with the values obtained for tamoxifen and hydroxytamoxifen, (Table 1). These benzoxepin compounds also displayed considerably more potent binding to ERα and ERβ than



Scheme 6. Synthesis of Type IV SERMs **41a–e**. Reagent and conditions: (a) 4-CH_3-C_6H_4CHO, HCl, EtOH; (b) H_2/Pd, EtOH; (c) 4-MeO-C_6H_4Br, nBuLi, THF; (d) H_3PO_4, EtOH; (e) BBr_3, CH_2Cl_2; (f) K_2CO_3, $R_1R_2NCH_2CH_2Cl$, acetone, 24 h, 85 °C.

Table 1
Antiproliferative effects and estrogen receptor binding affinities for benzoxepin type SERMs

Compound	Structure type	Yield (%)	MCF-7 IC_{50}[a](μM)	ERα IC_{50}[b] (nM)	ERβ IC_{50}[b] (nM)	ER αβ
15a	I	89	1.584 ± 0.262	8.77	46.1	5.3
15b	I	37	0.446 ± 0.111	1.9	5.9	3.1
15c	I	39	1.280 ± 0.147	6.7	4.61	0.69
15d	I	30	1.540 ± 0.274	10.3	3.63	0.35
18	I	12	0.0119 ± 0.005	8.4	2.9	0.35
26a	I	40	1.869 ± 1.140	16.2	31.0	1.9
26b	I	68	0.0214 ± 0.0063	10.4	0.70	0.07
26c	I	50	0.359 ± 0.163	14.0	6.0	0.43
26d	I	31	0.450 ± 0.321	126	262	2.07
26e	I	22	4.560 ± 1.00	>15 μM	>15 μM	—
28	I	84	0.156 ± 0.050	6.5	8.78	1.35
32	II	60	6.503 ± 0.290	5.57	17.33	3.11
36c	III	71	16.70 ± 2.10	828[c]	828 [c]	—
41d	IV	31	10.40 ± 2.70	14.2 μM*c*	14.2 μM [c]	—
Tamoxifen[d](1)	—	—	4.12 ± 0.038	70	170	2.42
4-Hydroxy tamoxifen (2)	—	—	0.107 ± 0.081	40	20	0.5

[a] Experimental values represent the average for two experiments performed in triplicate along with the standard deviation (SD) between the assay values. Values without SD values were run once. IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 cells.
[b] Values are an average of at least nine replicate experiments for ERα with typical standard errors below 15%; and six replicate experiments for ERβ with typical standard errors below 15%.
[c] The ER binding values for compounds **36c** and **41d** were obtained in a competitive radiometric binding assay with [^{3}H]estradiol rat uteri cytosol and are expressed as K_i values.
[d] The value for tamoxifen IC_{50} 4.12 ± 0.38 μM is in good agreement with the reported IC_{50} value for Tamoxifen using the MTT assay on human MCF-7 cells.

that reported for the binding of related benzocycloheptene compound **6** to estrogen receptors in rat cytosol,[20] indicating that the benzoxepin ring is more favorably bound to the receptor than the more lipophilic benzocycloheptene structure.

It is evident that the presence of the hydroxy group in Ring B is not essential for the potent binding of these compounds. These binding studies are also broadly consistent with the antiproliferative activity of these compounds as observed in the MCF-7 study in which the most potent compounds were identified as **18** and **26b**. Since MCF-7 cells predominantly express ERα, this antiproliferative activity may be mediated by the ERα receptor subtype, however the contribution of ERβ subtype to antiproliferative activity is also possible. Pure antiestrogens are known to degrade ERα and stabilize ERβ and this process can alter the ratio of these subtypes during treatment. A notable exception in the series was compound **26e**, containing a methyl sulfone substituent in Ring B. This substituent was introduced to prevent unfavorable metabolic hydroxylation on Ring B.[23] However it seems to prevent the usual antiestrogenic binding of the compound associated with the ERα LBD residues Argenine 394 and Glutamic acid 353. For the benzoxepin compounds, the increase in lipophilicity[37] on replacement of the OH (calculated C log *P* = 5.53) at C-8 of compound **18** with F in compound **26b** (C log *P* = 6.30) resulted in small increase in both antiproliferative and ER binding activity. For ERβ binding, the benzoxepin Type I compounds **15b**, **15c**, **26c**, **15d**, and **28** were potent as ERβ ligands with IC_{50} values of <10 nM. With compounds **18** and **26b** being the most potent, with IC_{50} binding values of 2.9 nM and 0.7 nM, respectively.

Many of the compounds displayed a low selectivity for ERα, for example, compounds **15b** and **28** with αβ ratio of 3.1 and 1.35, respectively. The selectivity of some of the compounds for ERβ could be related to the presence of the F substituent on Ring B as demonstrated for compounds **26b** which showed βα ratio of 15:1. The possible binding orientation of this ligand in the ER-LBD is explored in the modeling section below. The corresponding diphenolic compound **18** showed much lower selectivity with β/α ratio of 2.9 which is closer to the β/α ratio value of 1.67 obtained for 4-hydroxytamoxifen in this experiment.

In the Type II compounds, introduction of the pyrrolidine basic ether on the alternative Ring C site (compound **32**) resulted in unexpected potent ERα binding result IC_{50} 5.57 nM, and ERβ IC_{50} 17.33 nM. The Type III and Type IV compounds containing heterocyclic and benzylic Ring C structures were shown to be ineffective ligands for the ERα and ERβ with IC_{50} values >15 μM.

3.4. Estrogenic stimulation

The estrogen stimulation and antagonistic properties of the most active compounds **18** and **26b** and was determined in the estrogen bioassay which is based on the stimulation of alkaline phosphatase (AlkP) in the Ishikawa human endometrial adenocarcinoma cell line.[38] Compounds are tested as estrogen antagonists by their effect on the inhibition of estradiol stimulation in the Ishikawa cells in a dose-dependent manner (Table 2). Compound **26b** was approximately equivalent to tamoxifen in its ability as an estrogen antagonist with IC_{50} value of 194 nM. Compound **18** was much more potent than tamoxifen as an estrogen antagonist with IC_{50} values of 15.9 nM, respectively, and correlates with the potent ERα and ERβ binding observed for these compounds. The estrogenic stimulatory properties of these compounds could be determined in the Ishikawa cells by measuring the stimulation of alkaline phosphatase(AlkP) in these cells in the absence of estradiol. Compounds **18** and **26b**, the most potent benzoxepin-derived compound in the series, showed low stimulatory value (6% and 9.7%, respectively, at concentration of 1 μM). The reverse compound **32** displayed stronger stimulatory activity of 23% at 1 μM concentration in the absence of estradiol. This compound which is a ring fused analogue of *E*-tamoxifen, also displayed estrogen antagonist activity approximately equivalent to tamoxifen with IC_{50} value of 216 nM, which is confirmed in the MCF-7 antiproliferative assay result above. The results from this AlkP assay for estrogen antagonism and stimulation provide useful information in selection of the structural features for optimum antiestrogenic activity without associated adverse estrogenic effect on tissues such as the uterus.

3.5. Computational Investigation

To rationalize the observed ERαβ selectivity of the novel benzoxepin compounds (**15a–d, 18, 16a–e, 28, and 32**) shown in Table 1, a QSAR model for ERαβ selectivity in the benzoxepin scaffold structure was developed using the general core structure

for the benzoxepin antiestrogens **15a–d** as illustrated in Scheme 1. A data set of 27 estrogen receptor antagonist compounds was selected (see Supplementary information, Tables 5 and 6) utilizing ER active ligands extracted from literature including three ligands with similar structures to the benzoxepin scaffolds of this series (benzothiepin-derived SERMs) previously published by our group.[39] A number of different initial descriptor sets, including only substructural, 2D-MOE, normalized 2D-MOE descriptors, and different combinations of all, were chosen to analyze their impact on observed activity. The ratio of selectivity of ER β/α binding values (pIC_{50} values) was used as input for the prediction of selectivity of the benzoxepin series. This data was represented in the form of the natural logarithm for convenience. The original data set was divided several times into a training set of 20 ligands and an external test set of seven ligands, respectively. Cross-validation procedure was implemented by using leave-one-out (LOO) cross-validation procedure implying exclusion of each compound of the training set and the prediction of its activity by the model developed using the remaining compounds of the training set.

The variety of PLS (partial least squares) models (1–11) based on different descriptors sets and utilizing different selections of training and test sets is represented in Table 3, together with the predictive ability of each model.

Analysis of selected descriptor sets revealed that substructural descriptors play a dominant role in prediction of ERαβ selectivity. These consisted of mainly chains of 3, 7–15 atoms with different bond environmental (i.e., single, double, and aromatic) and few branched fragments of four atoms. Among the list of other 2D descriptors appeared some physicochemical ones, such as hydrophobicity (log*P*), topological polar surface area, total negative and positive van der Waals surface area, mass density, molar refractivity (MR) descriptors. Also some other indices such as the number of chiral centers, Petitjean graph shape coefficient, and total atom information content were deemed as important contributors. Our best model (Table 3, No. 8) could be described by the following equation

$$\text{Log } IC_{50}(\text{Er } \beta/\alpha) = 2.49844 + -0.891999 * \text{sqrt PEOE\_VSA-3\_norm} + -6.94527 * \text{* logP(o/w)\_norm} + -0.113787 * \text{p8.\_\_\_\_\_.1444114} + 0.0794729 * \text{pe.\_\_\_\_\_.1444111144444}$$

$n = 20$; $RMSE_{tr} = 0.303$; $R^2_{tr} = 0.836$; $RMSE_{val} = 0.402$; $Q^2 = 0.715$; $RMSE_{test} = 0.177$.

The propensity for a ligand to bind with higher affinity to ERβ is correlated with higher LogP (ERα binding affinity decreases with increase in lipophilicity),[40] lower van Der Waals surface area, but most importantly the presence of a seven-membered benzoxepin heterocycle within the nucleus of the antiestrogenic core.

Figure 2 illustrates correlation between experimental and predicted values of the activity for all subsets for the best model (Table 3, No. 8). The ER subtype selectivity effect of the 7-memebered fused-ring system is more apparent when one examines the docked solutions of **26b** in both estrogen receptor isoforms.

3.6. Docking

To further rationalize the observed ERβ selectivity (15-fold) of compound **26b**, a flexible ligand–receptor docking investigation was undertaken. At present there are no crystal structures of human ERα and ERβ with the same antagonist co-crystallized and thus it is difficult to compare docking of the same ligand in different isoforms with accuracy. To overcome this, Autodock4.0[41] was employed with movement of several key active site residues allowed. A summary of RMSD differences between active site residues in ERα obtained by superposition of data from 10 crystal structures is presented in Figure 3.

We examined the ability of our procedure in reproducing the binding position for raloxifene in PDB entry 1ERR, when docked in another X-ray structure (PDB: 3ERT) with a co-crystallized antagonist. The procedure was successful in reproducing the binding position for raloxifene in 3ERT (Glu353, 2.70 Å; Arg394, 2.80 Å; His524, 3.20 Å; Asp351, 4 Å) when compared with 1ERR (Glu353, 2.40 Å; Arg394, 3.00 Å; His524, 2.70 Å; Asp351, 2.70 Å) exhibiting the same patterns for hydrogen bonding.

Next, we examined the possible binding mode of the most ERβ selective benzoxepin, compound **26b**, when docked in ERα (3ERT). Firstly and importantly, two possible conformations (1 and 2) of the 7-membered benzoxepin ring system can be observed for compound **26b** as illustrated in Figure 4. Both conformations (1 and 2) were docked in each ER isoform to establish any differences in binding of the two ring conformations. The most stable solutions for **26b** complexed in ERα exhibited a propensity towards binding

Table 2
Antiestrogenic activity for selected Benzoxepin type SERMs

Compound	Ishikawa IC_{50} (nM)[a]	% Stimulation (1 μM)
18	20.8 ± 5.4	6.0
26b	194 ± 36.6	9.7
32	216 ± 141nM	23
Tamoxifen	170 ± 0.0	16.5%

[a] Ishikawa IC_{50} values are the compound concentrations required to inhibit by 50% the stimulation produced by 1 nM estradiol as determined by the alkaline phosphatase quantitation. Experimental values represent the average for triplicate determinations along with the standard deviation (SD) between the assay values.

Table 3
Statistical parameters of PLS models for ERβ/ERα binding selectivity (IC_{50} values) of estrogen receptor active ligands

Descriptor type in the initial set	Model	$RMSE_{tr}$	R^2	$RMSE_{val}$	Q^2	$RMSE_{test}$
Substructural	1	0.305	0.846	0.432	0.705	0.542
Substructural	2	0.314	0.821	0.436	0.665	0.231
Substructural + MOE 2D	3/1	0.300	0.841	0.402	0.718	0.260
Substructural + MOE 2D	4/2	0.312	0.839	0.462	0.656	0.457
Substructural + MOE 2D	5/3	0.306	0.832	0.419	0.694	0.240
Substructural + MOE 2D	6/4	0.319	0.831	0.439	0.689	0.453
Substructural + MOE 2D	7/5	0.320	0.831	0.726	0.158	0.390
Substructural + MOE 2D + normalised MOE 2D	8/1	0.303	0.836	0.402	0.715	0.177
Substructural + MOE 2D + normalised 2D	9/2	0.326	0.824	0.476	0.649	0.459
Substructural + MOE 2D + normalised 2D	10/3	0.355	0.792	0.453	0.668	0.457
Substructural + MOE 2D + normalised 2D	11/4	0.334	0.789	0.431	0.655	0.164

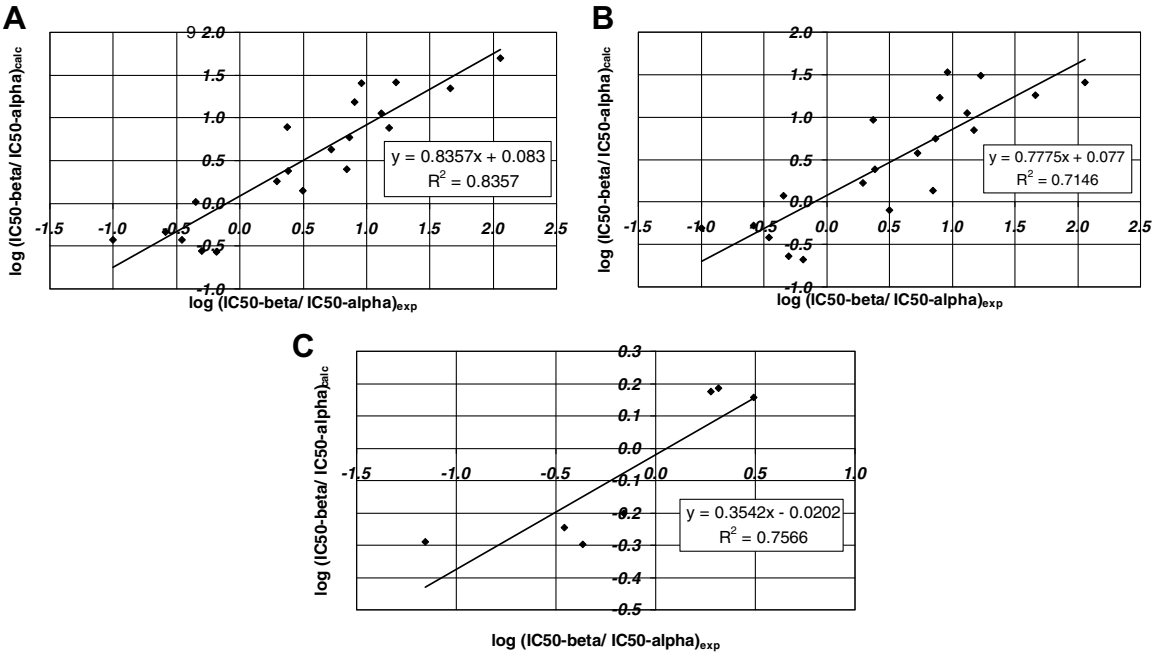


Figure 2. Correlation between experimental and predicted ERβ/ERα selectivity values ($\log IC_{50}$ERβ/$\log IC_{50}$ERα) for ensemble of estrogen receptor active ligands: (A) training; (B) validation; (C) test set.

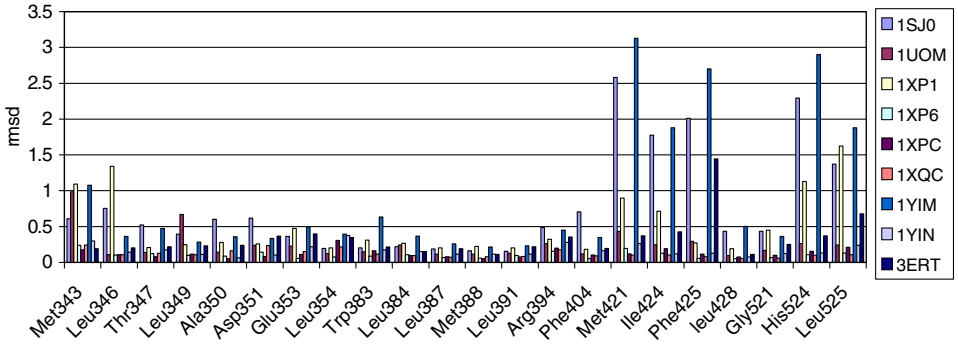


Figure 3. Summary of RMSD differences between active site residues in ERα obtained by superposition of data from 10 crystal structures.

as illustrated in Figure 3 irrespective of their ring conformations, (free energy of binding, ΔG – (conformation 1) = –17.72 kcal/mol; (conformation 2) = –17.50 kcal/mol). Compound **26b** clearly docks in an antiestrogenic manner in ERα making H-bonding contacts as outlined in Table 4.

In the case of docking both ring conformations of **26b** in ERβ (1QKN), conformation 1 is preferred binding mode, with a binding energy of –16.73 kcal/mol. Unlike in ERα, two distinct binding orientations of **26b** (for conformation 1) are observed in ERβ as illustrated in Figure 5. Met421 and Ile424 adjust their positions accordingly to allow both ring conformations of **26b** to bind in the same manner in ERα. In the case of ERβ, only Ile328 and Ile321 move position slightly to accommodate the alternative binding modes.

Virtually all the same binding mode contacts are made in both orientations of **26b** in ERβ as detailed in Table 4. However, what is immediately apparent and different to those binding modes observed in ERα, is that the ligand is devoid of H-bonding interaction with His524 in ERβ as illustrated in Figure 6. The substitution of Met421 (ERα) with Ile328 (ERβ) causes steric interaction with the ligand, supplementary to that imposed by Phe332, preventing the ligand from docking in the same orientation as observed with ERα. Ile328 aids this process moving out of position to accommodate the ligand in its alternative binding mode, and consequently His430 can make a hydrogen bonding contact with Gly327 instead of to the ligand. As mentioned earlier the lowest energy binding mode of **26b** is actually the alternative 'ring flipped' binding mode

Table 4
Hydrogen bonding contacts for **26b** in both ring conformations (1 and 2) in both isoforms ERα and ERβ8

Conformation	1	1	2	2
Isoform	3ERT (α)	1QKN (β)	3ERT (α)	1QKN (β)
Binding energy (kcal/mol)	–17.72	–16.73	–17.50	–16.24
Distance (Å) Asp 351 (258)	2.6	2.7	2.7	2.7
Glu 353 (260)	2.99	2.8	3.67	2.71
Arg 394 (301)	2.58	2.7	2.46	2.7
Gly 521 (427)	2.7	2.9	2.8	2.9
His 524 (430)	3.2	—	3.0	—

in which the *para*-fluoro of the ligand is directed towards Histidine 524. The strength of the bond between this fluorine and His430 would be less than if it hydrogen bonds to Gly327 so this relocation of His430 would be preferable.

Celik et al.[42] have recently carried out a 158 ns unrestrained all-atom MD simulation of ERα LBD sampling the conformational changes upon binding of estradiol and to establish the importance of His524 in the ligand binding process. Surface exposure of His524 was seen to translate to movement in H12 via disruption of the conserved hydrogen bonding network between Helix 3 and Helix 11. We suggest from this and our analysis that this rotation of His430 in ERβ may also result in this plasticity in Helix12 and consequently, ease of hydrogen bonding contact between Asp258 and the amine of the basic side chain of the ligand.

It has been suggested previously by a number of groups[43,44] that enhanced ERβ selectivity is achieved through a ligand that can differentiate between ERα Met421 and ERβ Ile328. Compound **26b** exhibits these preferences through ligand ring conformations and ability to bind in two alternative ring 'flipped' orientations because of the presence of this mutation from Met421 to Ile328 in ERβ. Both our QSAR and docking analysis converge on the importance of the position of the fluorine substituent (enhanced lipophilicity) and the fused benzoxepin ring system in providing binding selectivity towards ERβ.

4. Conclusions

We have identified a number of fluoro substituted benzoxepin compounds which demonstrate antiproliferative activity against the MCF-7 human breast cancer cell line, and in addition display up to 15-fold selectivity for ERβ together with subnanomolar binding affinity for the ERβ. Initial molecular modeling studies indicate that these compounds may dock in an unexpected mode in the binding site of the ER. Metabolic oxidation of these compounds would be blocked by the presence of the F atom in Ring B thus ensuring a longer plasma half life. These compounds which are ring

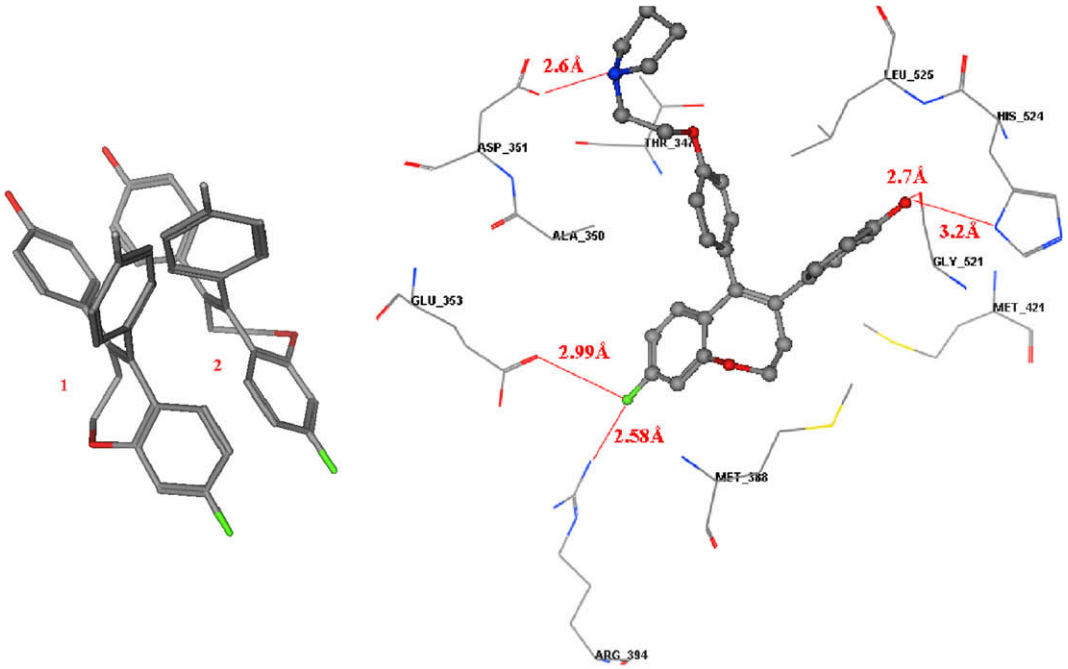


Figure 4. *Left*: Two possible conformations (1 and 2) of benzoxepin **26b** ring system. *Right*: Binding mode of **26b** in ERα active site.

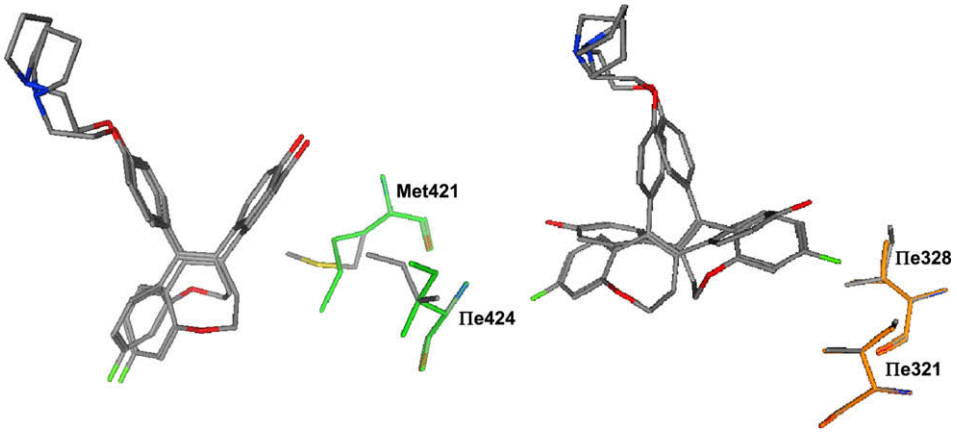


Figure 5. *Left*: Two possible conformations of benzoxepin ring system **26b** docked in ERα. *Right*: Alternate 'ring flipped'orientations of benzoxepin ring system **26b** docked in ERβ.

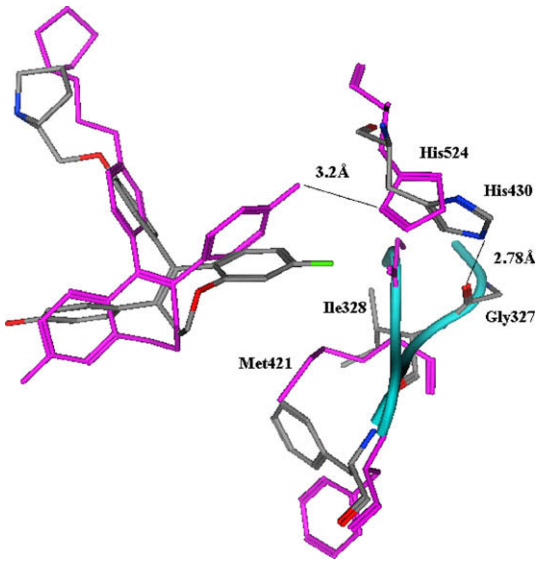


Figure 6. Lowest energy docked structures of **26b** in ERα and ERβ superposed by heavy atom. Hydrogen bond contact for **26b** with His524 in ERα clearly shown.

fused analogue of (*Z*)-tamoxifen, expand the structural diversity of ligands that can act as potent antagonists for the estrogen receptor and without metabolic complications introduced by the facile isomerisation of the tamoxifen type triarylethylene antiestrogen structures. Selectivity for ERβ for the most potent compound **26b** has been examined in both docking and QSAR studies and suggest a correlation between enhanced lipophilicity and enhanced ERβ binding for this benzoxepin ring scaffold.

5. Experimental

5.1. Materials and methods: chemistry

All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous THF was obtained by distillation from benzophenone-sodium under nitrogen immediately before use. All reactions were performed under a nitrogen atmosphere unless specifically noted. IR spectra were recorded as thin films on NaCl plates or as KBr disks 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 °C, 400.13 MHz for ^{1}H spectra, 100.61 MHz for ^{13}C spectra, in either $CDCl_3$, CD_3COCD_3, or CD_3OD (internal standard 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 in the Department of Chemistry, Trinity College Dublin. Thin-layer chromatography was performed using Merck Silica gel 60 TLC aluminum 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. All samples were analyzed using reversed phase high performance liquid chromatography (Waters Alliance system). The analysis was performed at 254 nm using a Phenomonex column (4 μ, 250 × 4.60 mm) using a mobile phase of acetonitrile/water (0.1% TFA) 70:30 deliverated at a flow rate of 1.0 mL/min.

5.2. 5-Bromo-2,3-dihydrobenzo[*b*]oxepine (10c)

A mixture of 3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one (**9a**) (0.33 g, 2.04 mmol) and phosphorus tribromide (1.00 mL, 10.4 mmol) was heated at 90 °C for 24 h. The reaction was cooled to room temperature, added slowly dropwise to ice-water (20 mL), and the aqueous layer extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with water (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by chromatography (5% diethyl ether/hexanes) to give an unstable yellow oil (0.26 g, 56%), which was used immediately in the next reaction without further purification.

5.3. 4-(2,3-Dihydrobenzo[*b*]oxepin-5-yl)-phenol (11a)

Method A: $Pd(PPh_3)_4$ (40 mg) was added to a solution of **10c** (1.12 g, 5 mmol) in THF (20 mL) and stirred at room temperature of 30 min. 4-Hydroxyphenylboronic acid (0.76 g, 5.5 mmol) and 2 M Na_2CO_3 (12.8 mL, 25 mmol) was added and the solution heated to 80 °C for 24 h. The solution was cooled, water was added (20 mL), and the aqueous layer extracted with dichloromethane (4× 50 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (5% diethyl ether/hexanes) to give the prooduct as a white solid (0.62 g, 52%).IR ν_{max} (KBr): 3384, 1606 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.23 (1H, m, Ar*H*), 7.14 (3H, m, Ar*H*), 7.0 (2H, m, Ar*H*), 6.77 (2H, d, *J* = 6.0, Ar*H*), 6.27 (1H, t, *J* = 6.0, C*H*), 2.51 (2H, t, *J* = 6.0, OCH_2), 2.46 (2H, m, CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.6, 154.8, 140.9, 135.3, 133.0, 131.0, 129.8, 128.4, 126.9, 123.3, 121.9, 114.9, 78.0, 29.7.

Method B: Trifluoromethanesulfonic anhydride (5.0 mL, 30.9 mmol) in dichloromethane (10 mL) was added to a suspension of **9a** (1.66 g, 10.3 mmol) and sodium carbonate (3.28 g, 30.9 mmol) in dichloromethane (30 mL) at 0 °C. The suspension was stirred for 18 h at room temperature, filtered and the filtrate washed with water (50 mL), brine (50 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude triflate **10a** was dissolved in dry THF (50 mL) and $Pd(PPh_3)_4$ (100 mg) was added and the solution stirred under nitrogen for 15 min. 4-Hydroxyphenylboronic acid (1.79 g, 12.9 mmol) and 2 M sodium carbonate (25 mL) was added and the solution refluxed for 6 h. The solution was cooled to room temperature and acidified with 2 M hydrochloric acid. The aqueous layer was extracted with dichloromethane and the combined organic layers were washed with water (30 mL), brine (30 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by flash chromatography (5% diethyl ether/hexanes) to afford the product **11a** as a white solid (2.15 g, 88%).

5.4. 4-(4-Bromo-2,3-dihydro-1-benzo[*b*]oxepin-5-yl)-phenol (14a)

Pyridinium tribromide (0.99 g, 2.8 mmol) was added to a solution of **11a** (0.67 g. 2.8 mmol) in dry dichloromethane (30 mL) 0 °C and the solution was warmed to room temperature and stirred for 1 h. A solution of sodium hydrogen carbonate (10%, 20 mL) was added and the aqueous layer extracted with dichloromethane (3× 50 mL), washed with water, dried over sodium sulfate, and the solvent removed under reduced pressure. The crude productwas purified by flash chromatography (CH_2Cl_2/hexane 1:1) to give the product as a white solid (0.59 g, 67%). Mp 116 °C; IR ν_{max} (KBr): 3384, 1606 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.20 (1H, t, *J* = 6.0, m, Ar*H*), 7.14 (2H, d, *J* = 8.5, Ar*H*), 7.07 (1H, dd, *J* = 8.0, 1.0, Ar*H*),

6.95 (1H, t, J = 6.5, Ar*H*), 6.85–6.77 (3H, m, Ar*H*), 4.59 (2H, t, J = 6.0, O*CH*$_2$), 3.00 (2H, t, J = 6.0, *CH*$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 156.4, 154.8, 131.4, 128.9, 123.4, 122.0, 114.9, 77.4, 41.1; HRMS (ESI) calculated for $C_{16}H_{14}BrO_2$ (M^+) 317.1848; found: 317.1842.

5.5. 1-{2-[4-(2,3-Dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (12a)

Potassium carbonate (0.94 g, 6.82 mmol) was added to a solution of **14a** (0.46 g, 1.36 mmol) in acetone (50 mL). The suspension was stirred for 15 min and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.34 g, 2.04 mmol) was added and the mixture refluxed for 6 h. The solution was cooled, filtered, and the solvent removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL), washed with water (30 mL), brine (30 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2.5% methanol/ CH_2Cl_2) to give the product as a yellow oil (0.45 g, 95%). IR υ_{max} (film): 2962, 1606 cm^{-1}; ^{1}H NMR (400 Mz, CDCl$_3$) δ *7.21–7.14 (3H,* m, Ar*H*), 7.06 (1H, d, J = 8.0, Ar*H*), 6.95–6.89 (3H, m, Ar*H*), 6.78 (1H, d, J = 8.0, Ar*H*), 4.57 (2H, t, J = 6.0, O*CH*$_2$), 4.13 (2H, J = 6.0, O*CH*$_2$), 2.99 (2H, t, J = 6.0, *CH*$_2$), 2.92 (2H, t, J = 6.0, *CH*$_2$), 2.65–2.62 (4H, m, *CH*$_2$N*CH*$_2$), 1.83–1.79 (4H, m, *CH*$_2$*CH*$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 157.7, 156.4, 139.3, 134.9, 133.5, 131.0, 129.4, 128.8, 123.4, 121.9, 121.8, 114.5, 113.9, 66.1, 54.8, 54.6, 41.1, 23.4; HRMS (ESI) calculated for $C_{22}H_{24}BrNO_2$ (M^+) 414.3461; found:, 414.3395.

5.6. 1-{2-[4-(4-Phenyl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (15a)

This compound was prepared from 1-{2-[4-(4-bromo-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine[18] (**13a**) using a procedure similar to that described for **11a.** The crude product was purified by flash chromatography (hexane/dichloromethane/methanol 48:48:2) to give the product as a brown oil (76 mg, 89%). IR υ_{max} (film): 2962, 1606 cm^{-1}; HPLC (t_r = 2.422 min; 100%); ^{1}H NMR (400 Mz, CDCl$_3$) δ 6.64 (2H, d, J = 8.5, Ar*H*), 4.61 (2H, J = 6.0, O*CH*$_2$), 4.11 (2H, t, J = 5.5, *CH*$_2$), 2.99 (2H, t, J = 5.5, *CH*$_2$), 2.79 (4H, m, *CH*$_2$N*CH*$_2$), 2.70 (2H, t, J = 6.0, *CH*$_2$), 1.89–1.86 (4H, m, *CH*$_2$*CH*$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 157.0, 156.0, 142.5, 137.8, 137.2, 137.1, 134.0, 132.5, 130.9, 129.5, 128.4, 127.9, 126.3, 123.5, 122.0, 113.6, 80.5, 65.9, 54.8, 54.6, 35.6, 23.3; HRMS (ESI) calculated for $C_{28}H_{30}NO_2$ (M^+): 412.2277; found: 412.2277.

5.7. 4-{5-[4-(2-Pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (15b)

This compound was prepared from **13a**[18] using a procedure similar to that described for **11a**. The crude product was purified by flash chromatography (hexane/CH_2Cl_2/CH_3OH; 48:48:2) to give the product as a beige solid (0.17 g, 37%). IR υ_{max} (film): 2962, 1606 cm^{-1}; ^{1}H NMR (400 Mz, CDCl$_3$) δ *7.21–7.14 (3H,* m, Ar*H*), 7.06 (1H, d, J = 8.0, Ar*H*), 6.95–6.89 (3H, m, Ar*H*), 6.78 (1H, d, J = 8.0, Ar*H*), 4.57 (2H, t, J = 6.0, O*CH*$_2$), 4.13 (2H, J = 6.0, O*CH*$_2$), 2.99 (2H, t, J = 6.0, *CH*$_2$), 2.92 (2H, t, J = 6.0, *CH*$_2$), 2.65–2.62 (4H, m, *CH*$_2$N*CH*$_2$), 1.83–1.79 (4H, m, *CH*$_2$*CH*$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 157.7, 156.4, 139.3, 134.9, 133.5, 131.0, 129.4, 128.8, 123.4, 121.9, 121.8, 114.5, 113.9, 66.1, 54.8, 54.6, 41.1, 23.4; HRMS (ESI) calculated for $C_{28}H_{29}NO_3$ (M^+) 414.3461; found: 427.5479.

5.8. 4-(3-Methoxy-phenoxy)-butyric acid (8b)[19]

A mixture of 3-methoxyphenol (**7b**) (5.5 mL, 50 mmol) and potassium carbonate (6.9 g, 55 mmol) in acetone (100 mL) was stirred for 30 min. Ethyl 4-bromobutyrate (7.9 mL, 55 mmol) was added dropwise via syringe and catalytic KI was added. The reaction mixture was refluxed for 24 h. The reaction mixture was cooled to ambient and the solid was removed by filtration. The solid was washed with acetone (50 mL) and the combined filtrate and washings were concentrated under reduced pressure. The residue was taken up in diethyl ether (150 mL), washed with 5% sodium hydroxide (50 mL) and the solvent removed under reduced pressure. The residue was dissolved in ethanol (20 mL) and treated with 10% sodium hydroxide in water (100 mL) and heated at 110 °C for 3 h or until the solution went clear. The solution was cooled and acidified with concd hydrochloric acid and the product which precipitated was filtered and dried (8.05 g, 76%). mp 89–91 °C[19]; IR ν_{max} (film): 3220, 1725, 1559 cm^{-1}; ^{1}H NMR (400 Mz, CDCl$_3$) δ 7.16 (1H, m, Ar*H*), 6.51–6.45 (3H, m, Ar*H*), 3.99 (2H, t, J = 6.02 Hz, O*CH*$_2$CH$_2$CH$_2$), 3.77 (3H, s, O*CH*$_3$), 2.58 (2H, t, J = ?, OCH$_2$*CH*$_2$CH$_2$), 2.11 (2H, m, OCH$_2$*CH*$_2$CH$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 179.6, 160.8, 159.9, 129.8, 106.6, 106.4, 100.8, 66.4, 55.2, 30.5, 24.3; HRMS (ESI) calculated for $C_{11}H_{14}O_4Na$ (M^++Na) 233.0790; found: 233.0787.

5.9. 8-Methoxy-3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one (9b)

A mixture of **8b** (3.50 g, 16.7 mmol) and Eaton's reagent (27 mL) was stirred for 2 h at 100 °C under nitrogen. The mixture was poured into water (100 mL) and extracted into ethyl acetate (4× 200 mL). The organic layer was washed with water (100 mL), brine (100 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude oil was purified by flash chromatography (5% diethyl ether in hexanes) to give the product as a yellow oil (0.94 g, 29%).[19,26,45] A similar procedure using polyphosphoric acid gave a 14% of the benzoxepinone product. IR υ_{max} (film): 2934, 1607 cm^{-1}; ^{1}H NMR (400 Mz, CDCl$_3$) δ *(2H,* d,), 7.76 (2H, d, J = 6.0 HZ, Ar*H*), 6.66 (1H, d, J = 9.0 HZ, Ar*H*), 6.56 (1H, s, Ar*H*), 4.24 (2H, t, J = 6.0 HZ, O*CH*$_2$), 3.83 (3H, s, *CH*$_3$), 2.87 (2H, t, J = 7.0 Hz, *CH*$_2$), 2.0 (2H, m, *CH*$_2$); ^{13}C NMR (101 Mz, CDC1$_3$) δ 157.7, 156.4, 139.3, 134.9, 133.5, 131.0, 129.4, 128.8, 123.4, 121.9, 121.8, 114.5, 113.9, 66.1, 54.8, 54.6, 41.1, 23.4; HRMS (ESI) calculated for $C_{11}H_{13}O_3$ 193.0865 (M^+); found: 193.0858.

5.10. 5-Bromo-8-methoxy-2,3-dihydro-benzo[*b*]oxepine (10d)

A mixture of **9b** (0.48 g, 2.5 mmol) and phosphorus tribromide (1.20 mL, 12.5 mmol) was heated at 90 °C under nitrogen for 22 h. The solution was cooled and was added carefully dropwise to ice-water (25 mL). The aqueous layer was extracted with ethyl acetate (3× 50 mL), washed with brine (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The yellow oil, which decolorises rapidly was purified by flash chromatography (5% diethyl ether in hexanes) to give the product **10d** (0.31 g, 50%), which was used immediately in the next reaction.

5.11. 4-(8-Methoxy-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (11b)

Method A: This compound was prepared from **10d** using a procedure similar to that described for **11a.** The residue was purified by flash chromatography (5% diethyl ether in hexanes) to give the product as a colorless gel (0.62 g, 52%) which was used in the following reaction without further purification. IR υ_{max} (film): 3423, 2939, 1609 cm^{-1}; ^{1}H NMR (400 Mz, CDCl$_3$) δ 7.11 (2H, d, J = 8.5, Ar*H*), 6.90 (1H, dd, J = 6.5, 8.5 Hz, Ar*H*), 6.80 (1H, m, Ar*H*), 6.7 (2H, m, Ar*H*), 6.77 (2H, d, J = 8.5 Hz, Ar*H*), 6.72 (1H, m, Ar*H*), 6.20 (1H, t, J = 6.3 Hz, = C*H*), 5.47 (1H, br s, O*H*), 4.50 (2H, t, J = 6.0 Hz, O*CH*$_2$CH$_2$), 2.42 (2H, m, OCH$_2$*CH*$_2$); ^{13}C NMR (101 Mz,

$CDC1_3$) δ 162.2, 159.7, 157.5, 153.8, 139.0, 133.9, 130.8, 128.6, 127.8, 125.4, 114.1, 109.2, 108.1, 107.9, 107.6, 107.6, 77.8, 28.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.65 ppm.

Method B: This compound was prepared from **9b** using a procedure similar to that described for **11a.** The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to afford the product (0.35 g, 44%).

5.12. 4-(4-Bromo-8-methoxy-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (14b)

This compound was prepared from **11b** using a procedure similar to that described for **14a**. The residue was purified using flash chromatography (10% diethyl ether in hexanes) to give the product as a yellow oil (0.63 g, 63%) which was used in the following reaction without further purification. IR υ_{max} (film): 3310, 1234 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.09 (2H, d, J = 8.52 Hz, Ar*H*), 6.82 (2H, d, J = 8.04 Hz, Ar*H*), 6.67 (2H, d, J = 8.56 Hz, Ar*H*), 6.62 (2H, d, J = 2.52 Hz, Ar*H*), 6.48–6.51 (1H, q (dd), J = 2.52 Hz, 7.04 Hz, Ar*H*), 4.56 (2H, t, J = 5.76 Hz, CH_2), 3.77 (3H, s, OCH_3), 3.02 (2H, t, J = 5.76 Hz, CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 159.8, 157.5, 154.8, 138.9, 135.0, 132.4, 131.2, 125.4, 120.3, 114.9, 109.7, 106.8, 77.3, 55.3, 41.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.65.

5.13. 1-{2-[4-(4-Bromo-8-methoxy-2,3-dihydrobenzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (13b)

This compound was prepared from **12b** using a procedure similar to that described for **12a**. Flash chromatography (1% methanol in dichloromethane) afforded the product as a yellow oil, (0.77 g). IR υ_{max} (film): 2934, 1607 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 6.91 (2H, d, J = 6.0, Ar*H*), 6.89 (2H, d, J = 6.0 Hz, Ar*H*), 6.61 (1H, s, Ar*H*), 6.50 (1H, d, J = 6.0 Hz, Ar*H*), 6.47 (1H, d, J = 6.0 Hz, Ar*H*), 4.55 (2H, t, J = 6.0 Hz, OCH_2), 4.17 (2H, t, J = 6.0 Hz, CH_2), 3.77 (3H, s, CH_3), 3.04 (2H, t, J = 6.0 Hz, CH_2), 3.01 (2H, t, J = 6.0, 6.0 Hz, CH_2), 2.97 (4H, m, CH_2NCH_2), 1.84 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.8, 157.8, 135.2, 132.4, 130.9, 125.2, 120.4, 113.9, 109.5, 106.7, 76.5, 66.5, 56.2, 55.3, 56.4, 41.5, 23.4; HRMS (ESI) calculated for $C_{23}H_{26}BrNO_3$ 444.3726 (M^+); found: 444.3723.

5.14. 4-{8-Methoxy-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (15c)

This compound was prepared from **13b** using a procedure similar to that described for **11a**. The residue was purified by flash chromatography (10% methanol in dichloromethane) to give the product as a brown solid (0.31 g, 39%). HPLC (t_r = 2.354 min; 93.2%); IR υ_{max} (film): 2934, 1607 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 6.95 (2H, d, J = 6.0 Hz, Ar*H*), 6.85 (2H, d, Ar*H*), 7.01 (4H, m, Ar*H*), 6.57 (1H, d, J = 6.0 Hz, Ar*H*), 6.49 (2H, d, J = 6.0 Hz, Ar*H*), 4.59 (2H, t, J = 6.0 Hz, OCH_2), 4.08 (2H, t, J = 6.0 Hz, CH_2), 3.80 (3H, s, CH_3), 3.26 (2H, br t, CH_2), 3.14 (4H, m, CH_2–CH_2), 2.67 (2H, br t, CH_2), 1.97 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.8, 157.3, 139.2, 133.5, 132.4, 130.9, 125.2, 120.4, 113.9, 109.5, 106.7, 66.1, 55.3, 54.8, 54.6, 41.1, 23.4; HRMS (ESI) calculated for $C_{29}H_{32}NO_4$ 458.2331 (M^+); found: 458.2338.

5.15. 1-{2-[4-(8-Methoxy-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (12b)

This compound was prepared from **11b** using a procedure similar to that described for **12a**. The crude product was purified by flash chromatography (2.5% methanol/CH_2Cl_2) to give the product as an oil which was used in the subsequent reaction without further purification (0.45 g, 95%). IR υ_{max} (KBr) cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.06 (2H, d, J = 8.56 Hz, Ar*H*), 6.86–6.83 (3H, q, J = 6.52 Hz, Ar*H*), 6.62 (1H, d, J = 3Hz, Ar*H*), 6.54–6.51 (1H, q, J = 3.84 Hz, Ar*H*), 6.08 (1H, t, J = 6.28 Hz, =C*H*), 4.43 (2H, t, J = 6.02 Hz, CH_2), 4.09 (2H, t, J = 6.02 Hz, OCH_2), 3.76 (3H, s, O–CH_3), 2.87 (2H, t, J = 6.02 Hz, CH_2); 2.79 (2H, t, J = 7.04 HZ, CH_2), 2.53 (s, 4H, $(CH_2)_2$), 1.78 (s, 4H, $(CH_2)_2$).

5.16. 1-{2-[4-(8-Methoxy-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-piperidine (12c)

This compound was prepared from **11b** using a procedure similar to that described for **12a**. The residue was purified by flash chromatography (55:35:10 dichloromethane/hexane/methanol) to give a yellow oil (292 mg, 65%). IR υ_{max} (film): 2934, 1607 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.17 (2H, d, J = 8.5 Hz, Ar*H*), 6.89–6.84 (3H, m, Ar*H*), 6.65 (1H, d, J = 2.5 Hz, Ar*H*), 6.56 (1H, dd, J = 8.5, 2.5 Hz, Ar*H*), 6.11 (2H, t, J = 6.0 Hz, C*H*), 4.46 (2H, t, J = 6.0 Hz, OCH_2), 4.11 (2H, t, J = 6.0 Hz, CH_2), 3.79 (3H, s, CH_3), 2.78 (2H, t, J = 6.0 Hz, CH_2), 2.51–2.47 (6H, m, CH_2NCH_2), 1.64–1.59 (4H, m, CH_2CH_2), 1.45 (2H, m, CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 159.6, 159.0, 157.9, 140.4, 135.6, 131.9, 129.6, 125.6, 124.9, 114.1, 109.2, 106.7, 77.2, 65.8, 57.9, 55.3, 54.9, 30.2, 25.9, 24.1; HRMS (ESI) calculated for $C_{24}H_{30}NO_3$ 380.2226 (M^+); found: 380.2233.

5.17. 1-{2-[4-(4-Bromo-8-methoxy-2,3-dihydro-benzo[*b*]xepin-5-yl)-phenoxy]-ethyl}-piperidine (13c)

Method A: This compound was prepared from **12c** using a procedure similar to that described for **14a**. The residue was purified using flash chromatography (10% diethyl ether in hexanes) to give the product as a yellow oil (0.81 g, 89%).

Method B: This compound was prepared from **14b** using a procedure similar to that described for **12a**. The residue was purified by flash chromatography (silica, 55:35:10 dichloromethane/hexane/methanol) to give a yellow oil (187 mg, 52%). IR υ_{max} (film): 2933, 1606 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.13 (2H, d, J = 8.5 Hz, Ar*H*), 6.89 (3H, m, Ar*H*), 6.67–6.60 (2H, m, Ar*H*), 6.48 (1H, dd, J = 8.0, 2.5 Hz, Ar*H*), 4.54 (2H, t, J = 6.0, OCH_2), 4.14 (2H, t, J = 6.0 Hz, OCH_2), 3.76 (3H, s, CH_3), 3.04 (2H, t, J = 6.0 Hz, CH_2), 2.81 (2H, t, J = 6.0 Hz, CH_2), 2.54 (4H, m, CH_2NCH_2), 1.66–1.60 (4H, m, CH_2CH_2), 1.46 (2H, m, CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 159.8, 157.9, 156.8, 155.7, 138.9, 135.1, 132.3, 130.9, 129.1, 125.2, 120.3, 113.9, 109.5, 106.7, 65.6, 57.8, 56.2, 55.2, 54.9, 41.4, 25.7, 24.0; HRMS (ESI) calculated for $C_{24}H_{28}BrNO_3$ (M^+) 458.3997; found: 458.4001.

5.18. 4-{8-Methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (15d)

This compound was prepared from **13c** using a procedure similar to that described for **11a**. The crude product was purified by flash chromatography (hexane/diethyl ether 1:1) to give the product as a yellow solid (0.25 g, 30%). HPLC (t_r = 2.667 min; 88.1%); IR υ_{max} (KBr) 2970, 1609 cm^{-1}; ^{1}H NMR (400 Mz, DMSO-*d*) δ 6.83 (10H, m, Ar*H*), 6.53 (2H, d, J = 6.0, Ar*H*), 4.60 (2H, t, J = 6.0, CH_2), 4.33 (2H, t, J = 6.0 Hz, CH_2), 3.55 (2H, t, J = 6.0 Hz, CH_2), 3.30 (4H, m, CH_2–CH_2), 2.72 (2H, t, J = 6.0 Hz, CH_2), 1.67 (4H, m, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 156.8, 155.8, 155.4, 138.1, 134.9, 132.9, 131.1, 129.9, 114.9, 113.9, 66.1, 55.3, 55.4, 45.5, 25.8, 23.2; HRMS (ESI) calculated for $C_{30}H_{34}NO_4$ (M^+) 472.2480; found: 472.2490.

5.19. 5-[4-(2-Pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-8-ol (16)

A mixture of pyridinium hydrochloride (1.41 g, 12.2 mmol) and **12b** (0.89 g, 2.44 mmol) was heated at 180–190 °C for 3 h. The hot

mixture was poured into a 5% aqueous solution of sodium carbonate (40 mL) and extracted into ethyl acetate (5× 100 mL) and methanol (20 mL). The organic extracts were combined, washed with water, and the solvent removed under reduced pressure. The residue was purified by flash chromatography (2.5% methanol/dichlorormethane) to give a brown oil (111 mg, 13%). ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.10 (2H, d, J = 8.5 Hz, Ar*H*), 6.74–6.67 (4H, m, Ar*H*), 6.44 (2H, dd, J = 8.5, 2.5 Hz, Ar*H*), 6.02 (1H, t, J = 6.0 Hz, Ar*H*), 4.40 (2H, t, J = 6.0 Hz, CH_2), 4.12 (2H, t, J = 5.5 Hz, CH_2), 3.03 (2H, t, J = 5.5 Hz, CH_2), 2.82 (4H, m, CH_2NCH_2), 2.46 (2H, t, J = 6.0 Hz, CH_2), 1.89 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 159.0, 157.4, 157.3, 140.5, 135.9, 131.9, 129.5, 125.1, 123.8, 113.9, 111.0, 108.9, 65.6, 54.8, 54.4, 30.2, 23.2; HRMS (ESI) calculated for $C_{22}H_{26}NO_3$ (M^+) 352.1913; found: 352.1914.

5.20. 4-Bromo-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-8-ol (17)

This compound was prepared from **16** using a procedure similar to that described for **14a**. The residue was purified using flash chromatography (10% diethyl ether in hexanes) to give the product as a yellow oil (84 mg, 64%). ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.01 (2H, d, J = 8.0 Hz, Ar*H*), 6.86 (2H, d, J = 8.0 Hz, Ar*H*), 6.52–6.42 (2H, m, Ar*H*), 6.30 (1H, d, J = 6.5 Hz, Ar*H*), 4.41 (2H, t, J = 6.0 Hz, CH_2), 4.11 (2H, br t, CH_2), 3.05 (2H, t, CH_2), 2.93 (2H, br t, CH_2), 2.92–2.79 (4H, m, CH_2NCH_2), 1.84 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.7, 156.4, 139.3, 134.9, 133.5, 131.0, 129.4, 128.8, 123.4, 121.9, 121.8, 114.5, 113.9, 66.1, 54.8, 54.6, 41.1, 23.4; HRMS (ESI) calculated for $C_{22}H_{24}BrNO_3$ (M^+) 458.2331; found: 430.3450.

5.21. 4-(4-Hydroxyphenyl)-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]*oxepin-8-ol* (18)

This compound was prepared from **17** using a procedure similar to that described for **11a**. The residue was purified by flash chromatography (5% diethyl ether in hexanes) to give the product as a beige solid (11 mg, 12%), Mp 148 °C. HPLC (t_r = 2.404 min; 87%); ^{1}H (400 MHz, DMSO-*d*, Me_4Si): 6.95 (2H, d, J = 6.5, Ar*H*), 6.89 (2H, d, J = 6.5 Hz, Ar*H*), 6.76 (2H, d, J = 8.5 Hz, Ar*H*), 6.67–6.54 (4H, m, Ar*H*), 6.47 (1H, dd, J = 8.5, 2.5 Hz, C*H*), 4.53 (2H, t, J = 6.0, CH_2), 4.22 (2H, t, J = 5.0 Hz, CH_2), 3.45 (2H, t, J = 5 Hz, CH_2), 3.36–3.27 (6H, m, CH_2NCH_2), 2.68 (2H, t, J = 6.0 Hz, CH_2), 2.05 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.7, 156.4, 139.3, 134.9, 133.5, 131.0, 129.4, 128.8, 123.4, 121.9, 121.8, 114.5, 113.9, 66.1, 54.8, 54.6, 34.9, 21.9; HRMS (ESI) calculated for $C_{28}H_{30}NO_4$ (M^+) 444.2175; found: 444.2194.

5.22. 4-(3-Fluoro-phenoxy)-butyric acid (20)

To a suspension of potassium carbonate (7.59 g, 55 mmol) in acetone (100 mL) was added 3-fluorophenol (4.5 mL, 50 mmol) and the mixture stirred under nitrogen for 15 min. Ethyl-4-bromobutyrate (7.2 mL, 50 mmol) was added and the mixture refluxed for 6 h, cooled to ambient then filtered. The filtrate was concentrated under reduced pressure and the residue taken up in diethyl ether (100 mL). The organic layer was washed with 10% sodium hydroxide solution (50 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The oily residue refluxed in 10% sodium hydroxide (100 mL) and ethanol (20 mL) until the solution became clear. The solution was cooled, acidified with concd hydrochloric acid, and beige crystals were collected by filtration (4.75 g, 79%), mp 42–45 °C.[26] IR υ_{max} (KBr): 3436, 1713 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.87 (1H, br s, O*H*), 7.22 (1H, m, Ar*H*), 6.61 (3H, m, Ar*H*), 4.00 (2H, t, J = 6.0 Hz, OCH_2CH_2CH_2), 2.58 (2H, t, avg. J = 7.3, OCH_2CH_2CH_2), 2.10 (2H, m, OCH_2CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 179.2, 164.8, 162..4, 160.4, 159.9, 130.2, 110.2, 107.5, 102.2, 66.7, 30.5, 24.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −112.1; HRMS (ESI) calculated for $C_{10}H_{11}O_3NaF$ (M+Na^+) 221.2042; found: 221.0484.

5.23. 8-Fluoro-3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one (21)

A mixture of **20** (4.75 g, 27.8 mmol) and polyphosphoric acid (51 g) was heated at 80 °C for 3 h and then poured into ice-water. The product was extracted with ethyl acetate (4× 100 mL) and the combined organic layers were washed with 10% sodium hydroxide (100 mL), brine (100 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow oil (1.56 g, 31%).[26] IR υ_{max} (film): 1685; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.85 (1H, m, Ar*H*), 6.82 (2H, m, Ar*H*), 4.26 (2H, t, avg. J = 6.8 Hz, OCH_2CH$_2$CH$_2$), 2.90 (2H, t, avg. J = 7.0 Hz, OCH$_2$CH$_2$CH_2), 2.22 (2H, m, OCH$_2$CH_2CH$_2$); ^{13}C NMR (101 Mz, $CDC1_3$) δ 198.9, 166.9, 164.3, 163.8, 131.5, 125.6, 110.3, 107.6, 73.2, 40.4, 26.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −105.3 ppm; HRMS (ESI) calculated for $C_{10}H_9O_2NaF$ (M+Na^+) 203.18; found: 203.0479.

5.24. 4-(8-Fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (23)

This compound was prepared from **21** and **22** using a procedure similar to that described for **11a**, *Method B:* The product was purified by flash chromatography (5% diethyl ether/hexanes) to give the product was a yellow oil (1.50 g, 67%, 96%) which was used in the following reaction without further purification. IR υ_{max} (film): 3310 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.11 (2H, d, J = 8.5 Hz, Ar*H*), 6.90 (1H, dd, J = 6.5, 8.5 Hz, Ar*H*), 6.80 (1H, m, Ar*H*), 6.70 (2H, m, Ar*H*), 6.77 (2H, d, J = 8.5 Hz, Ar*H*), 6.72 (1H, m, Ar*H*), 6.20 (1H, t, J = 6.3 Hz, =C*H*), 5.47 (1H, br s, O*H*), 4.50 (2H, t, J = 6.0 Hz, OCH_2CH$_2$), 2.42 (2H, m, OCH$_2$CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 162.2, 159.7, 157.5, 153.8, 139.0, 133.9, 130.8, 128.6, 127.8, 125.4, 114.1, 109.2, 108.1, 107.9, 107.6, 107.6, 77.8, 28.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ: −113.65.

5.25. 4-(4-Bromo-8-fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (24a)

This compound was prepared from **23** using a procedure similar to that described for **14a** The crude product purified by flash chromatography (5% diethyl ether/hexanes) to give the pure product as a yellow oil (1.79 g, 91%). IR υ_{max} (film) 3402 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.09 (2H, d, J = 9.0 Hz, Ar*H*), 6.84–6.73 (4H, m, Ar*H*), 6.68–6.63 (1H, m, Ar*H*), 4.59 (2H, t, J = 6.0 Hz, OCH_2), 3.00 (2H, t, J = 6.0 Hz, CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.5, 161.0, 157.4, 151.6, 138.6, 134.7, 132.5, 130.9, 129.3, 121.5, 115.0, 110.8, 110.5, 109.4, 109.2, 77.4, 41.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −112.47; HRMS (ESI) calculated for $C_{16}H_{12}FO_2BrNa$ (M^+) 359.1650; found: 359.0339.

5.26. 1-{2-[4-(4-Bromo-8-fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (25)

This compound was prepared from **24a** using a procedure similar to that described for **12a**. The crude product was purified by flash chromatography (2.5% methanol/dichloromethane) to give the product as a yellow oil (0.48 g, 81%). ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.13 (2H, d, J = 8.5 Hz, Ar*H*), 6.79 (2H, d, J = 8.5 Hz, Ar*H*), 6.68 (1H, m, Ar*H*), 4.58 (2H, t, avg. J = 5.8 Hz, OCH_2CH$_2$N–), 4.13 (2H, t, avg. J = 5.8 Hz, OCH_2CH$_2$), 3.01 (2H, t, J = 6.0 Hz, OCH$_2$CH_2N–), 2.90 (2H, J = 6.0 Hz, OCH$_2$CH_2), 2.63 (4H, m, CH_2NCH_2), 1.80, (4H, m, CH_2–CH_2), ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.4, 160.9, 158.1,

157.6, 138.6, 134.6, 132.6, 130.9, 121.4, 114.0, 110.6, 110.4, 109.3, 109.0, 77.3, 66.8, 54.9, 54.6, 41.0, 23.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ *−112.66;* HRMS (ESI) calculated for $C_{22}H_{24}NO_3FBr$ (M^+) 432.0974; found: 432.0958.

5.27. 4-{8-Fluoro-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (26b)

This compound was prepared from **25** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (2.5% methanol/dichloromethane) to give the product as a foamy yellow solid (152 mg, 68%), mp 112–120 °C. HPLC (t_r = 2.499 min; 97.6%); IR υ_{max} (KBr) 3431 (OH) cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 6.96 (2H, d, J = 8.5 Hz, Ar*H*), 6.86–6.67 (7H, m, Ar*H*), 6.56 (2H, d, J = 9.0 Hz, Ar*H*), 4.60 (2H, t, avg. J = 6.2 Hz, OCH_2CH_2N–), 4.20 (2H, t, J = avg. 5.0 Hz, OCH_2CH_2), 3.22 (6H, m, 4× CH_2NCH_2, 2× CH_2NCH_2), 2.68 (4H, m, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.1, 156.9, 156.0, 155.4, 138.2, 135.1, 133.6, 132.5, 131.8, 130.6, 115.2, 113.7, 110.8, 110.5, 109.5, 109.3, 80.9, 63.9, 54.4, 54.1, 35.6, 23.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ *−113.7;* HRMS (ESI) calculated for $C_{28}H_{29}NO_3F$ (M^+) 446.2131; found: 446.2114.

5.28. 3-{8-Fluoro-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (26c)

This compound was prepared from **25** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (2.5% methanol/dichloromethane) to give the product as brown crystals, mp 105 °C. HPLC (t_r = 2.489 min; 93.7%); IR υ_{max} (KBr) 3435, 2922, 1607 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.02 (1H, t, J = 7.78 Hz, ArH), 6.83 (4H, m,Ar*H*), 6.72 (1H, m, Ar*H*), 6.66 (1H, d, J = 7.52 Hz, Ar*H*), 6.60 (1H, d, J = 8.52 Hz, Ar*H*), 6.55–6.53 (3H, m, Ar*H*), 4.76 (1H, br s, OH), 4.59 (2H, t, J = 5.78 Hz, OCH_2), 4.06 (2H, t, J = 4.78 Hz, OCH_2), 3.04 (2H, t, J = 5.04 Hz, OCH_2), 2.90 (4H, s, CH_2–N–CH_2), 2.67 (2H, t, J = 5.78 Hz, CH_2); 1.90 (4H, s, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.4, 160.9, 157.2, 157.0, 156.3, 143.8, 137.9, 136.2, 134.2, 132.9, 132.1, 131.9, 129.21, 120.8, 116.8, 113.9, 113.7, 110.5, 109.3, 80.8, 64.9, 54.5, 54.4, 35.6, 23.2,; ^{19}F NMR (376 MHz, $CDCl_3$) δ −112.9; HRMS (ESI) calculated for $C_{28}H_{28}NO_3F$ (M^+) 446.2131; found: 446.2130.

5.29. 1-(2-{4-[8-Fluoro-4-(4-fluorophenyl)-2,3-dihydro-benzo[*b*]-oxepin-5-yl]-phenoxy}-ethyl)-pyrrolidine (26d)

This compound was prepared from **25** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (2.5% methanol/dichloromethane) to give the product as a yellow solid (67 mg, 31%), mp 130–135 °C. IR υ_{max} (KBr) 3431 (OH) cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.12 (2H, m, Ar*H*) 6.85 (6H, m, Ar*H*), 6.73 (1H, m, Ar*H*), 6.65 (2H, d, J = 8.0 Hz, Ar*H*), 4.60 (2H, t, J = 6.0, OCH_2CH_2N), 4.20 (2H, t, J = 6.0 Hz, CH_2), 3.19 (2H, t, J = 6.0 Hz, CH_2), 2.94 (4H, m, CH_2NCH_2) 2.68 (2H, t, J = 6.0 Hz, CH_2), 1.93 (4H, m, CH_2CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 157.5, 138.2, 136.8, 136.5, 133.4, 132.4, 132.0, 131.9, 131.0, 130.9, 115.0, 114.8, 113.8, 110.8, 110.6, 109.5, 109.3, 80.7, 66.7, 55.0, 54.7, 35.6, 23.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.3, 116.2; HRMS (ESI) calculated for $C_{28}H_{28}NO_2F_2$ (M^+) 448.2088; found: 448.2072.

5.30. 1-{2-[4-(8-Fluoro-4-phenyl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-pyrrolidine (26a)

This compound was prepared from **25** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (1:10:30 methanol/dichloromethane/hexanes) to give the product as a beige solid (82 mg, 40%). HPLC (t_r = 3.037min; 93.9%); IR υ_{max} (KBr) 2970, 1609 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 6.96 (5H, m, Ar*H*), 6.86–6.67 (4H, m, Ar*H*), 6.56 (1H, m, Ar*H*), 4.60 (2H, d, J = 6.2 Hz, CH_2), 4.20 (2H, t, J = 6 Hz avg., OCH_2CH_2), (2H, t, J = 6 Hz avg., OCH_2CH_2), 3.22 (2H, t, 4× CH_2NCH_2, 2× CH_2NCH_2), 2.68 (4H, m, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 165, 157.5, 157.1, 142.3, 137.5, 136.5, 133.5, 132.9, 132.3, 132.0, 129.3, 127.9, 127.5, 126.3, 113.7, 110.7, 110.5, 109.4, 109.2 (dd, C–F), 80.7, 66.8, 55.0, 54.7, 35.5, 23.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ 113.45; HRMS (ESI) calculated for $C_{28}H_{29}NO_2F$ (M^+) 430.2181; found: 430.2173.

5.31. 1-(2-{4-[8-Fluoro-4-(4-methanesulfonyl-phenyl)-2,3-dihydro-benzo[*b*]oxepin-5-yl]-phenoxy}-ethyl)-pyrrolidine (26e)

This compound was prepared from **25** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (5% diethyl ether/hexanes) to give the product as a white solid (66 mg, 22%). ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.74 (2H, d, J = 8.5 Hz, Ar*H*), 6.88–6.78 (5H, m, Ar*H*), 6.67 (2H, d, J = 8.9 Hz, Ar*H*), 4.60 (2H, t, J = 6.0, OCH_2), 4.06 (2H, t, J = 6.0 Hz, OCH_2CH_2), 3.04 (3H, s, CH_3), 2.91 (2H, t, J = 6.0 Hz, OCH_2CH_2), 2.73 (2H, t, J = 6.0 Hz, OCH_2CH_2), 2.71–2.65 (4H, m, CH_2NCH_2), 1.82 (4H, m, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.9, 161.3, 157.8, 157.1, 149.5, 139.2, 135.9, 132.5, 132.2, 130.3, 127.1, 114.0, 111.0, 110.8, 109.6, 109.4 (dd, C–F), 80.5, 66.3, 54.8, 54.6, 44.4, 35.3, 23.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −112.27; HRMS (ESI) calculated for $C_{29}H_{30}NO_4FS$ (M^+) 507.6292; found: 507.6100.

5.32. 1-{2-[4-(4-Bromo-8-fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-piperidine (27)

This compound was prepared from **24a** using a procedure similar to that described for **12a**. The product was purified by flash chromatography (5% diethyl ether/hexanes) to give the product as an orange gel. IR υ_{max} (film) cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.66 (2H, d, J = 8.56 Hz, Ar*H*), 6.91 (2H, d, J = 8.52 Hz, Ar*H*), 6.77–6.66 (2H, m, Ar*H*), 6.64–6.62 (1H, m, Ar*H*), 4.59 (2H, t, J = 5.78 Hz, OCH_2), 4.12 (2H, t, J = 6.02 Hz, OCH_2), 3.01 (2H, t, J = 5.76 Hz, OCH_2), 2.79 (2H, t, J = 6.02 Hz, CH_2); 2.53 (4H, s, CH_2–N–CH_2), 1.62 (6H, s, CH_2–CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.1, 160.6, 157.7, 138.3, 134.2, 132.1, 132.0, 131.8, 129.6, 128.9, 120.9, 113.7, 110.3, 110.0, 108.9, 108.7, 76.9, 65.4, 57.5, 57.2, 54.6, 40.6, 25.6, 23.8; HRMS (ESI) calculated for $C_{23}H_{25}BrFNO_2$ (M^+) 446.1131; found: 446.1129.

5.33. 4-{8-Fluoro-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-4-yl}-phenol (28)

This compound was prepared from **27** using a procedure similar to that described for **11a**. The residue was purified by flash chromatography (5% diethyl ether in hexanes) to give the product as a beige solid (0.84 g, 84%), mp 188 °C. HPLC (t_r = 2.614 min; 96.2%); IR υ_{max} (KBr) 2970, 1609 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ ^{1}H NMR (400 MHz, acetone-*d*) δ 6.83 (10H, m, Ar*H*), 6.53 (2H, d, J = 6.0 Hz, Ar*H*), 4.60 (2H, t, J = 6.0 Hz, CH_2), 4.33 (2H, t, J = 6.0 Hz, CH_2), 3.55 (2H, t, J = 6.0 Hz, CH_2), 3.30 (4H, m, CH_2–CH_2), 2.72 (2H, t, J = 6.0 Hz, CH_2), 1.91 (4H, m, CH_2 CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 156.8, 155.8, 155.4, 138.1, 134.9, 132.9, 131.1, 129.9, 114.9, 113.9, 109.7, 109.7, 108.5, 108.2 (dd, C–F), 80.1, 66.1, 55.3, 52.9, 34.9, 22.2, 20.7; ^{19}F (376 MHz, $CDCl_3$) δ −115.8; HRMS (ESI) calculated for $C_{28}H_{29}NO_2F$ (M^+) 430.2181; found: 430.2173.

5.34. 4-(4-Bromo-8-fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-*tert*-butyldimethyl- silane (29)

To a mixture of **24a** (8.05 mmol) and imidazole (10.5 mmol) were dissolved in DMF (10 mL). *tert*-Butyldimethylsilyl chloride (9.66 mmol) was added in six portions over 4 h. Stirring was continued at room temperature for a further 10 h. The reaction mixture was diluted with ethyl acetate (150 mL) and quenched with 10% HCl (20 mL). The organic layer was separated, washed with water (40 mL) and brine (40 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane/diethyl ether = 20:1) to yield the product 8 (1.54 g, 69%) as a brown solid, (69%) which was used in the following reaction without further purification. ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.06 (2H, d, J = 8.52 Hz, Ar*H*), 6.84 (2H, d, J = 10.52 Hz, Ar*H*), 6.80–6.73 (4H, m, Ar*H*), 6.71–6.62 (1H, m, Ar*H*), 4.58 (2H, t, J = 6.02 Hz, OCH_2), 3.01 (2H, t, J = 6.02 Hz, CH_2); 0.95 (9H, s, C-(CH_3)$_3$), 0.23 (6H, s, Si-(CH_3)$_2$), ^{13}C NMR (101 Mz, $CDC1_3$) δ 163.1, 160.6, 157.1, 138.4, 134.7, 132.3, 130.6, 129.0, 120.9, 119.2, 110.3, 108.9, 76.9, 40.7, 25.3, 20.3, 17.8, −4.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −112.7;

5.35. [4-{5-(4-*tert*-Butyldimethylsilanyloxy)-phenyl]-8-fluoro-2,3-dihydro-benzo[*b*]oxepin-5-yl}-phenol (30)

This compound was prepared from **29** using a procedure similar to that described for **11a**. The crude product was purified by column chromatography (5% diethyl ether/hexanes) to give 4-(2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol as a yellow solid (52%) which was used in the following reaction without further purification. ^{1}H NMR (400 Mz, $CDCl_3$) δ 6.99 (2H, d, J = 8.52 Hz, Ar*H*), 6.85–6.78 (4H, m, Ar*H*), 6.76–6.71 (1H, m, Ar*H*), 6.62–6.58 (4H, m, Ar*H*), 4.61 (2H, t, J = 5.78 Hz, OCH_2), 2.68 (2H, t, J = 6.02 Hz, CH_2); 0.95 (9H, s, C-(CH_3)$_3$), 0.15 (6H, s, Si-(CH_3)$_2$), ^{13}C NMR (101 Mz, $CDC1_3$) δ 162.8, 160.4, 156.4, 153.8, 136.8, 134.1, 134.0, 132.8, 131.9, 131.5, 131.4, 130.4, 130.3, 119.0, 114.4, 110.4, 109.1, 108.8, 80.5, 35.0, 25.2, 17.8, −4.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.7;

5.36. [4-{8-Fluoro-4-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2,3-dihydro-benzo[*b*]oxepin-5-yl}-phenol (32)

This compound was prepared from **30** using a procedure similar to that described for **12a**. The crude product was purified by flash chromatography (2.5% methanol/dichloromethane) to give the deprotected product **32** as yellow crystals (81%). HPLC (t_r = 2.481 min; 91.9%); IR υ_{max} (KBr) 3445, 2927, 1608 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.01 (1H, d, J = 8.56 Hz, Ar*H*), 6.94 (1H, d, J = 8.52 Hz, Ar*H*), 6.85–6.80 (3H, m, Ar*H*), 6.76–6.71 (2H, m, Ar*H*), 6.61–6.47 (4H, m, Ar*H*), 4.61 (2H, t, J = 5.26 Hz, OCH_2), 4.08-4.03 (2H, m, OCH_2), 3.01–2.98 (2H, m, OCH_2), 2.79 (4H, s, CH_2–N–CH_2), 2.67 (2H, t, J = 5.02 Hz, CH_2); 1.87 (4H, s, CH_2–CH_2); ^{13}C NMR (101 Mz, $CDC1_3$) δ 162.6, 160.1, 156.4, 153.1, 135.6, 134.6, 133.9, 132.1, 131.9, 131.6, 131.5, 130.3, 130.1, 114.9, 113.4, 110.3, 108.8, 80.6, 65.0, 54.4, 53.9, 35.1, 22.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.9; HRMS (ESI) calculated for $C_{28}H_{28}NO_3F$ (M^+) 446.2131; found: 446.2137.

5.37. 4-(4-Pyridin-3-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (35a)

This compound was prepared from 4-(4-bromo-2,3-dihydro-benzo[b]oxepin-5-yl)-phenol (**34**)[18] using a procedure similar to that described for **11a**. The product was purified by flash chromatography (C_6H_{12}/CH_2Cl_2/EtOAc; 40:50:10) to afford a yellow solid (83%) and used without further purification. IR ν_{max} (film) 3430, 3068–2970, 2926, 1658, 1590 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 2.71 (2H, t, J = 6.04 Hz, CH_2), 4.61 (2H, t, J = 6.02 Hz, CH_2), 6.66–7.71 (12H, m, Ar*H*); ^{13}C NMR (101 Mz, $CDC1_3$) δ 34.7, 79.8, 114.7, 121.6, 127.1, 128.0, 130.7, 130.9, 131.51, 132.92, 136.39, 139.67, 146.45, 149.65; MS (EI) m/z 315 (M^+).

5.38. 4-(4-Furan-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (35b)

This compound was prepared from **34** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (C_6H_{12}/CH_2Cl_2/EtOAc; 55.45:5) to afford an oil (81%). This compound was used in subsequent reactions without further purification. IRν_{max} (film) 3429, 2953–2929, 2853, 1601 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 2.86 (2H, t, J = 6.04 Hz, CH_2), 4.70 (2H, t, J = 6.02 Hz, CH_2), 5.74, (1H, m, Ar*H*), 6.25 (1H, m, Ar*H*), 6.70–7.24 (9H, m, Ar*H*); ^{13}C NMR (101 Mz, $CDC1_3$) δ 35.7, 80.5, 109.2, 110.5, 113.0, 114.9, 120.0, 121.7, 123.6, 127.9, 128.0, 129.9, 131.1, 133.9, 136.6, 140.3, 141.4; MS (EI) m/z 304 (M^+).

5.39. 4-(4-Thiophen-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (35c)

This compound was prepared from **34** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (C_6H_{12}/CH_2Cl_2/EtOAc; 30:60:10) to afford an oil (64%). This compound was used in subsequent reactions without further purification. IR ν_{max} (film) 3397, 2926, 2882–2854, 2227, 1617 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 2.73 (2H, t, J = 6.02 Hz, CH_2), 4.65 (2H, t, J = 6.26 Hz, CH_2), 6.66–7.24 (11H, m, Ar*H*); ^{13}C NMR (101 Mz, $CDC1_3$) δ 34.8, 80.2, 114.4, 121.3, 121.6, 123.8, 125.4, 127.9, 128.2, 128.6, 129.7, 130.4, 131.3, 132.0, 136.8, 142.1; MS (EI) m/z 320 (M^+).

5.40. 4-(4-Benzofuran-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (35d)

This compound was prepared from **34** using a procedure similar to that described for **11a**. Purification by flash chromatography (C_6H_{12}/CH_2Cl_2/EtOAc; 55:45:5) afforded the product as an oil (46%) which was used without further purification. IR ν_{max} (film) 3408, 2926, 2853, 1603 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 2.93 (2H, t, J = 6.02 Hz, CH_2), 4.72 (2H, t, J = 6.26 Hz, CH_2), 6.16 (1H, s, C*H*), 6.79–7.50 (12H, m, Ar*H*); ^{13}C NMR (101 Mz, $CDC1_3$) δ 41.0, 81.1, 110.7, 114.9, 115.6, 120.7, 121.6, 123.14, 124.0, 125.6, 128.5, 129.2, 131.0, 132.0, 155.14, 155.7; MS (EI) m/z 354 (M^+).

5.41. 4-(4-Naphthalen-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol (35e)

This compound was prepared from **34** using a procedure similar to that described for **11a**. The product was purified by flash chromatography (C_6H_{12}/CH_2Cl_2/EtOAc; 60:40:2) to afford an oil (50%) which was used without further purification. IR ν_{max} (film) 3397, 3080–2924, 2852, 1601 cm^{-1}; ^{1}H NMR (400 Mz, $CDCl_3$) δ 2.64 (2H, m, CH_2), 4.64 (2H, m, CH_2), 6.40–6.42 (2H, d, J = 8.56 Hz, Ar*H*), 6.80–7.71 (13H, m, Ar*H*); ^{13}C NMR (101 Mz, $CDC1_3$) δ 35.7, 79.7, 113.9, 114.6, 121.6, 123.1, 124.9, 125.5, 126.1, 127.9128.1, 131.0, 132.2133.3; MS (EI) m/z 364 (M^+).

5.42. Dimethyl-{2-[4-(4-pyridin-3-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-amine (36a)

This compound was prepared from **35a** using a procedure similar to that described for **12a.** The product was purified by flash

chromatography (CH_2Cl_2/EtOAc/MeOH; 70.25:5) to afford an oil (49%). IRν_{max} (film) 3047–2919, 2850, 1662, 1570 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.36 (6H, s, N(CH_3)$_2$), 2.72–2.75 (4H, m, NCH_2, CH_2), 4.02 (2H, t, J = 5.76 Hz, CH_2O), 4.63 (2H, t, J = 6.02 Hz, CH_2), 6.68–7.61 (12H, m, ArH; ^{13}C NMR (101 Mz, $CDC1_3$) δ 34.8, 45.2, 57.6, 65.2, 79.7, 113.6, 121.6, 123.2, 128.4, 129.3, 130.6, 132.0, 136.3, 146.7, 139.5, 149.8; HRMS (ESI) calculated for $C_{25}H_{26}N_2O_2$ 386.1998 (M^+); found: 386.1994.

5.43. {2-[4-(4-Furan-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-dimethyl-amine (36b)

This compound was prepared from **35b** using a procedure similar to that described for **12a**. Flash chromatography (CH_2Cl_2/EtOAc/MeOH; 60:40:2) afforded the product as an oil (38%). IR ν_{max} (film) 3050–2916, 2848–2775, 1606 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.40 (6H, s, N(CH_3)$_2$), 2.76 (4H, m, NCH_2, H-3), 4.11 (2H, m, CH_2O), 4.68 (2H, m, CH_2), 5.71-5.75 (1H, m, ArH), 6.23–6.26 (1H, m, ArH), 6.77–7.25 (9H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 32.3, 45.7, 58.1, 65.6, 80.0, 109.7, 111.5, 113.4114.3, 120.4, 121.1, 123.5, 128.3, 128.5, 129.2, 131.6, 132.3, 140.5; HRMS (ESI) calculated for $C_{24}H_{25}NO_3$ (M^+) 375.1845; found: 375.1834.

5.44. Dimethyl-{2-[4-(4-thiophen-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-amine (36c)

This compound was prepared from **35c** using a procedure similar to that described for **12a**. The product was purified by flash chromatography (CH_2Cl_2/EtOAc/MeOH; 50:40:10) to afford an oil (71%). IR ν_{max} 2927–2869, 2821–2772, 1606 cm^{-1}. ^1H NMR (400 Mz, $CDCl_3$) δ 2.41 (6H, s, (CH_3)$_2$), 2.73 (2H, t, J = 6.04 Hz, NCH_2), 2.81 (2H, t, J = 5.78 Hz, CH_2), 4.09 (2H, t, J = 5.78 Hz, CH_2O), 4.65 (2H, t, J = 6.02 Hz, CH_2), 6.73-7.28 (11H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 34.8, 45.1, 57.6, 65.1, 80.2, 113.5, 121.6, 122.5, 123.0, 124.24, 127.8, 128.6, 130.4, 131.4, 132.0; HRMS (ESI) calculated for $C_{24}H_{25}NO_2S$, 391.1609 (M^+); found: 391.1606.

5.45. {2-[4-(4-Benzofuran-2-yl-2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenoxy]-ethyl}-dimethylamine (36d)

This compound was prepared from **35d** using a procedure similar to that described for **12a.** Flash chromatography (CH_2Cl_2/EtOAc/MeOH; 60:40:2) afforded the product as an oil (68%). IR ν_{max} (film) 2924, 2853–2772, 1606 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.45 (6H, s, N(CH_3)$_2$), 2.80 (2H, m, NCH_2), 2.94 (2H, t, J = 5.76 Hz, CH_2), 4.40 (2H, t, J = 5.52 Hz, CH_2O), 4.61 (2H, t, J = 5.78 Hz, CH_2), 6.82–7.89 (12H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 41.0 (C-3), 45.6, 58.0, 65.9, 78.3, 111.7, 114.0, 116.2, 121.3, 121.9, 123.3, 124.0, 125.9, 128.2, 131.0, 132.0, 155.22 156.3; HRMS (ESI) calculated for $C_{28}H_{27}NO_3$, 425.1990 (M^+); found: 425.1991.

5.46. Dimethyl-{2-[4-(4-naphthalen-2-yl-2,3-dihydro-benzo[*b*]-oxepin-5-yl)-phenoxy]-ethyl}-amine (36e)

This compound was prepared from **35e** using a procedure similar to that described for **12a**. The product was purified by flash chromatography (CH_2Cl_2/EtOAc/MeOH; 70:20:5) to afford an oil (48%). IR ν_{max} (film) 3056–2928, 2864–2771, 1606 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.90 (6H, s, N(CH_3)$_2$), 2.64 (2H, t, J = 5.76 Hz, NCH_2), 2.89–2.96 (2H, m, CH_2), 3.91 (2H, t, J = 5.78 Hz, CH_2O), 4.61–4.68 (2H, m, CH_2), 6.49–8.03 (15H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 35.7, 45.3, 57.7, 65.2, 79.6, 113.0, 113.3, 121.6, 123.0, 124.0–125.5, 126.4, 127.9, 128.0, 131.9, 133.2, 133.3; HRMS (ESI) calculated for $C_{30}H_{29}NO_2$ 435.2208 (M^+); found: 435.2198.

5.47. 4-(4-Methylbenzyl)-3,4-dihydro-2H-benzo[*b*]oxepin-5-one (38)

4-(4-Methylbenzylidene)-3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one (**37**)[27] (18 mmol) was dissolved in ethanol (40 mL). Ten percent of Pd (17mmol) on activated charcoal was added and the reaction mixture was stirred at room temperature under an atmosphere of H_2. Stirring was maintained until TLC analysis verified that hydrogenation of the starting material was complete. The catalyst was then removed via filtration, washed with ethanol, and the solvent was evaporated under reduced pressure. The product was isolated using column chromatography (eluant C_6H_{12}/EtOAc; 90:10) as yellow oil in (83%) yield. IR ν_{max} (film) 3080–2996, 2977–2827 (CH), 1687 (C=O), 1600 (C=C) cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 1.79 (1H, m, CH), 2.35 (3H, s, CH_3), 2.37–2.49 (1H, m, CH), 2.79 (1H, dd, J = 13.89, 8.06 Hz, CH), 3.29 (1H, dd, J = 13.98, 6.31 Hz, CH), 3.40 (1H, m, CH), 3.92–3.99 (1H, dt, J = 12.10, 4.99 Hz, CH), 4.49 (1H, ddd, J = 12.42, 6.95, 2.59 Hz, CH), 6.94–7.12 (6H, m, ArH), 7.42 (1H, dt, J = 7.88, 2.00 Hz, ArH), 7.74 (1H, dd, J = 7.79, 1.67 Hz, ArH). ^{13}C NMR (101 Mz, $CDC1_3$) δ 20.9 (CH_3), 34.4, 34.8, 51.3, 72.3, 119.9, 122.2, 128.9, 128.9, 129.0, 132.7, 135.2, 136.1, 161.9, 201.5; HRMS (ESI) calculated for $C_{19}H_{20}O$ (M^+) 264.1574; found: 375.1514.

5.48. 5-(4-Methoxy-phenyl)-4-(4-methylbenzyl)-2,3-dihydro-benzo[*b*]oxepine (39)

n-BuLi (11 mmol, 2.5 M in hexane) was added dropwise to a stirred solution of 4-bromoanisole (4.1 mmol) in dry THF (25 mL) at −78 °C under an atmosphere of nitrogen. This solution was stirred for 1 h and then a solution of **38** (2.1 mmol) in dry THF (20 mL) was added slowly at −78 °C. This mixture was stirred for 1 h at −78 °C and then stirred at ambient temperature for 12 h. The reaction mixture was extracted with diethyl ether (3× 20 mL), washed with water, saturated NaC1 (20 mL), dried over $MgSO_4$, and concentrated. The intermediate alcohol product was isolated using flash chromatography (CH_2Cl_2/EtOAc; 98:2) as an oil (74%) then dissolved in EtOH (30 mL). Eighty five percent of polyphosphoric acid (120 mmol) was added and the mixture refluxed for 2–3 h. The resulting solution was neutralized with NaOH (20% w/v, 20 mL), washed with water, extracted with ethyl acetate (3× 25 mL), dried over Na_2SO_4, and concentrated. Flash chromatography (C_6H_{12}/CH_2Cl_2; 60:40) afforded the product an oil (79%). IR ν_{max} (film) 2984–2881, 2872–2809, 1605 cm^{-1}. ^1H NMR (400 Mz, $CDCl_3$) δ 2.30 (2H, t, J = 6.28 Hz, CH_2), 2.35 (3H, s, CH_3), 3.64 (2H, s, CH_2), 3.83 (3H, s, OCH_3), 4.34 (1H, t, J = 6.26 Hz, CH), 6.83–6.87 (2H, dd, J = 9.04, 1.70 Hz, ArH), 6.98-7.28 (11H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 20.5, 31.9, 40.0, 54.7, 79.9, 113.0, 121.4, 122.7, 127.3, 128.3, 128.6, 130.5, 130.4, 133.3, 136.0, 136.1, 136.3, 136.4, 155.3; MS (EI) m/z 305 (M^+). The product was used without further purification.

5.49. 4-[4-(4-Methylbenzyl)-2,3-dihydro-benzo[*b*]oxepin-5-yl]-phenol (40)

To a solution of **39** (0.4 mmol) in dry DCM (15 mL) was added dropwise BBr_3 (8.2 mmol, 1 M solution in DCM) which was diluted with dry DCM (3 mL). The mixture was stirred for 1 h at −78 °C and then quenched with 10% (w/w) $NaHCO_3$ (20 mL). The dark precipitate was dissolved in EtOAc/MeOH 10:1 (v/v) (20 mL) with vigorous stirring. The aqueous phase was separated and extracted twice with EtOAc/MeOH. The combined organic fractions were dried with $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (CH_2Cl_2/EtOAc; 99:1) afforded the product as oil (98%). IR ν_{max} (film) 3678–3202, 3078–2924, 2914–2839,

1608 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.28 (2H, t, J = 6.28 Hz, CH_2), 2.45 (3H, s, CH_3), 3.63 (2H, s, CH_2), 4.33 (2H, t, J = 6.28 Hz, CH_2), 6.79-7.12 (11H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 21.0, 32.4, 40.48, 80.45, 114.9, 121.9, 123.2, 127.8, 128.7, 129.1, 130.9, 154.4, 155.7; HRMS (ESI) calculated for $C_{24}H_{22}O_2$ (M^+) 342.1619; found: 342.1620.

5.50. Dimethyl-(2-{4-[4-(4-methylbenzyl)-2,3-dihydro-benzo[*b*]-oxepin-5-yl]-phenoxy}-ethyl)-amine (41a)

This compound was prepared from **40** using a procedure similar to that described for **12a.** Preparative thin layer chromatography (CH_2Cl_2/EtOAc/ MeOH; 50:50:10) afforded an oil (20%). IR ν_{max} (film) 3002–2889, 2873–2791, 1605 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 2.28 (2H, t, J = 8.48 Hz, CH_2), 2.32 (6H, s, N(CH_3)$_2$), 2.34 (3H, s, CH_3), 2.75 (2H, m, NCH_2), 3.60 (2H, s, CH_2), 4.05 (2H, m, (CH_2O), 4.30 (2H, t, J = 8.34 Hz, CH_2), 6.78–6.83 (2H, d, J = 9.04 Hz, ArH), 6.86–7.15 (10H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 20.3, 29.6, 40.3, 45.6, 58.1, 65.8, 80.4, 114.0, 120.4, 120.9, 127.7, 127.9–128.3, 130.2, 130.3, 130.7, 134.5, 138.1, 158.3, 159.6; HRMS (ESI) calculated for $C_{28}H_{31}NO_2$ (M^+) 413.2355; found: 413.2355.

5.51. Diethyl-(2-{4-[4-(4-methylbenzyl)-2,3-dihydro-benzo[*b*]-oxepin-5-yl]-phenoxy}-ethyl)-amine (41b)

This compound was prepared from **40** using a procedure similar to that described for **12a.** The product was isolated by preparative thin layer chromatography (CH_2Cl_2/EtOAc/MeOH; 50:50:10) as an oil (27%). IR ν_{max} (film) 3063–2985, 2881–2807, 1608 cm^{-1}. ^1H NMR (400 Mz, $CDCl_3$) δ 2.19 (5H, s, N(CH_3–CH_2)), 2.29 (2H, t, J = 6.28 Hz, CH_2), 2.34 (8H, s, N(CH_3CH_2), CH_3), 2.73 (2H, m, NCH_2), 3.62 (2H, s, H–CH_2), 4.11 (2H, m, CH_2O), 4.32 (2H, t, 6.82 Hz, CH_2), 6.86–6.89 (2H, dd, J = 9.04, 2.48 Hz, ArH), 6.86–7.15 (10H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 22.6, 29.6, 32.3, 40.4, 47.5, 53.4, 70.7, 80.7, 113.3, 114.0, 121.9, 123.2, 127.8, 128.7, 129.2, 130.7, 130.9, 135.6, 136.4, 136.8, 155.7, 157.6; HRMS (ESI) calculated for $C_{30}H_{35}NO_2$ (M^+) 441.2669; found: 441.2668.

5.52. 1-(2-{4-[4-(4-Methylbenzyl)-2,3-dihydro-benzo[*b*]oxepin-5-yl]-phenoxy}-ethyl)-pyrrolidine (41c)

This compound was prepared from **40** using a procedure similar to that described for **12a**. The product was obtained as an oil by preparative thin layer chromatography (CH_2Cl_2/EtOAc/MeOH; 55:44:6) (21%). IR ν_{max} (film) 2955–2917, 2849 cm^{-1}. ^1H NMR (400 Mz, $CDCl_3$) δ 1.73 (4H, m, CH_2CH_2), 2.08 (2H, m, CH_2), 2.19 (3H, s, CH_3), 2.82 (4H, m, CH_2CH_2), 2.89 (2H, m, NCH_2), 3.67 (2H, s, CH_2), 4.12 (2H, m, CH_2O), 4.19 (2H, m, CH_2), 6.54–6.55 (2H, d, J = 4.52 Hz, ArH), 6.81–7.56 (10H, m, ArH). HRMS (ESI) calculated for $C_{30}H_{33}NO_2$ 439.2511 (M^+); found: 439.2511.

5.53. 1-(2-{4-[4-(4-Methylbenzyl)-2,3-dihydro-benzo[*b*]oxepin-5-yl]-phenoxy}-ethyl)-piperidine (41d)

This compound was prepared from **40** using a procedure similar to that described for **12a**. The product was obtained as an oil by preparative thin layer chromatography (CH_2Cl_2/EtOAc/MeOH; 50:60:8), (31%). IR ν_{max} (film) 3093–2993, 2976–2828, 1607 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 1.62 (6H, m, CH_2, CH_2–CH_2), 2.29 (2H, t, J = 6.26 Hz, CH_2), 2.34 (3H, s, CH_3), 2.54 (4H, m, CH_2, CH_2), 2.80 (2H, t, J = 6.02 Hz, NCH_2), 3.63 (2H, s, CH_2), 4.13 (2H, t, J = 6.04 Hz, CH_2O), 4.32 (2H, t, J = 6.02 Hz, CH_2), 6.84–6.87 (2H, dd, J = 10.56, 2.00 Hz, ArH), 6.97–7.25 (10H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 22.5, 25.4, 31.9, 40.0, 54.5, 57.5, 70.7, 80.7, 113.6, 121.4, 122.8, 127.3, 128.3, 128.7, 130.4, 130.8, 135.1, 136.0, 136.1, 157.2; HRMS (ESI): calculated for $C_{31}H_{35}NO_2$ (M^+) 453.2660; found: 453.2668.

5.54. 4-(2-{4-[4-(4-Methylbenzyl)-2,3-dihydro-benzo[*b*]oxepin-5-yl]-phenoxy}-ethyl)-morpholine (41e)

This compound was prepared from **40** using a procedure similar to that described for **12a**. The product was purified as an oil by preparative thin layer chromatography (CH_2Cl_2/tOAc/eOH; 50:44:6), (19%). IR ν_{max} (film) 2955–2917, 2849, 1604 cm^{-1}. ^1H NMR (400 Mz, $CDCl_3$) δ 2.03 (2H, m, CH_2), 2.34 (3H, s, CH_3), 2.64 (2H, m, NCH_2), 2.86 (4H, m, CH_2–CH_2), 3.67 (2H, s, CH_2), 3.76 (4H, m, CH_2–CH_2), 4.18 (2H, m, CH_2O), 4.34 (2H, m, CH_2), 6.63–6.66 (2H, dd, J = 7.52, 1.50 Hz, ArH), 6.73–7.11 (10H, m, ArH); ^{13}C NMR (101 Mz, $CDC1_3$) δ 22.6, 31.9, 39.5, 54.1, 57.6, 67.0, 69.0, 113.3, 121.6, 128.8, 130.9, 132.4, 136.4, 137.5; HRMS (ESI): calculated for $C_{30}H_{33}NO_3$ (M^+) 455.2464; found: 455.2460.

5.55. Biochemical evaluation of activity

5.55.1. 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 tumor cell line MCF-7 was cultured in Eagle's minimum essential medium in a 95%O_2/ 5% CO_2 atmosphere with 10% fetal calf serum. 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 (FBS), 2 mM L-glutamine and 100 μg/mL penicillin/streptomycin (complete medium). Cells were trypsinised and seeded at a density of 1.5×10^4 into a 96-well plate and incubated at 37 °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 °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 color 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.

5.56. Cytotoxicity studies

Human MCF-7 breast cancer cells were plated at a density of 1.5×10^4 per well in a 96-well plate, then incubated at 37 °C, 95%O_2/5% CO_2 atmosphere for 24 h. Cells were treated with the compound of choice at varying concentrations (1 nM–100 μM), then incubated for a further 72 h. Following incubation 50 μL aliquots of medium were removed to a fresh 96-well plate. Cytotoxicity was determined using and LDH assay kit obtained from Promega, following the manufacturer's instructions for use. A 50 μL per well LDH substrate mixture was added and the plate left in darkness at room temperature for equilibration. Stop solution (50 μL) was added to all wells before reading the absorbance at 490 nm. A control of 100% lysis was determined for a set of untreated cells which were lysed through the addition of 20 μL lysis solution to the media 45 min prior to harvesting. Data were pre-

sented following calculation, as percentage cell lysis versus concentration of subject compound.

5.57. Estrogen receptor binding assay

ERα and ERβ fluorescence polarization based competitor assay kits were obtained from Panvera at Invitrogen Life Technologies. The recombinant ER (insect expressed, full length, untagged human ER obtained from recombinant baculovirus-infected insect cells) and the fluorescent estrogen ligand were removed from the −80 °C freezer and thawed on ice for 1 h prior to use. The fluorescent estrogen ligand (2 nM) was added to the ER (30 nM for ERα and 20 nM for ERβ) and screening buffer (100 mM potassium phosphate (pH 7.4), 100 μg/mL BGG, 0.02%NaN_3 was added to make up to a final volume that was dependant on the number of tubes used (number of tubes (e.g., 50) × volume of complex in each tube (50 μL) = total volume (e.g., 2500 μL). Test compound (1 μL, concentration range 1 nM) to 100 μM) was added to 49 μL screening buffer in each borosilicate tube (6mm diameter). To this 50 μL of the fluorescent estrogen/ER complex was added to make up a final volume of 100 μL and final concentration range for the test compound of 0.01 nM to 1 μM. A vehicle control contained 1% (v/v) of ethanol and a negative control contained 50 μL screening buffer and 50 μL fluorescent estrogen/ER complex. The negative control was used to determine the polarisation value when no competitor was present (theoretical maximum polarization). The tubes were incubated in the dark at room temperature for 2 h and were mixed by shaking on a plate shaker. The polarization values were read on a Beacon single-tube fluorescent polarization instrument with 485 nm excitation and 530 nm emission interference filters. For ERα and ERβ, graphs of anisotropy (mA) versus competitor concentration were obtained for determination of IC_{50} values.

5.58. Estrogenic activity: alkaline phosphatase assay

Following the procedure of Littlefield et al.[38], human Ishikawa cells were maintained in Eagle's minimum essential medium (MEM containing 10% vol/vol fetal bovine serum (FBS) and supplemented with 100 U/mL penicillin and 10 μg/mL streptomycin, 2 mM glutamine, and 1 mM sodium pyruvate. Twenty four hours before the start of the experiment, near confluent cells were changed to an estrogen-free medium (EFBM), A 1:1 mixture of phenol-free Ham's F-12 and Dulbecco's modified Eagle's medium, together with the supplements listed above, and 5% calf serum, stripped of endogenous estrogens with dextran coated charcoal. On the day of the experiment, cells were harvested with 0.25% trypsin and plated in 96-well flat bottomed microtitre plates in EFBM at a density of 2.5×10^4 cells/well. Test compounds were dissolved in ethanol at 10^{-3} M, diluted with EFBM (final concentration of ethanol 0.1%) and filter sterilised. After addition of the test compounds, (plated in 50 μL, added estradiol in 50 μL, and blank medium to give a final volume 150 μL) the cells were incubated at 37 C in a humidified atmosphere containing 95%O_2/5% CO_2 for 72 h. All experimental values were obtained in triplicate. The microtitre plates were then inverted and the growth medium removed. The plates were then rinsed by gentle immersion and swirling in 2 L of PBS (0.15 M NaCl, 10 mM sodium phosphate, pH 7.4). The plates were removed from the container, the residual saline in the plate was not removed, and the wash was repeated. The buffered saline was then shaken out, and the plate blotted on paper towel. The covers were replaced and the plates were placed at −80 °C for at least 15 min then thawed at room temperature for 5–10 min. The plates were then placed on ice and 50 μL ice cold solution containing 50 mM *p*-nitrophenyl phosphate, 0.24 mM $MgCl_2$ and 1 M diethanolamine (pH 9.8) was added. The plates were warmed to room temperature (time zero), and the yellow color from the production of *p*-nitrophenol was allowed to develop. The plates were monitored at 405 nm until maximum stimulation of the cells showed an absorbance of approximately 1.2.

5.59. Computational details

5.59.1. QSAR model

5.59.1.1. Data set. A data set of 27 compounds was selected (see Supplementary information, Fig. 6) utilizing known ER active ligands extracted from literature including three ligands with similar structures to the benzoxepin scaffolds of this series (benzothiepin-derived SERMs) previously published by our group.[39] The criteria for selection were 2-fold: (1) accuracy of the chemical structure and (2) of the determined IC_{50} of the compounds. The ratio of selectivity ER β/α was used as input for the prediction of selectivity of the benzoxepin series. This data was represented in the form of the natural logarithm for convenience. The original data set was divided several times into a training set of 20 ligands and an external test set of seven ligands, respectively. Cross-validation procedure was implemented by using leave-one-out (LOO) cross-validation procedure implying exclusion of each compound of the training set and the prediction of its activity by the model developed using the remaining compounds of the training set.

5.59.1.2. Descriptor set. A standard set of 2D descriptors (e.g., log*P*, topological indices, BCUT and GCUT descriptors, TPSA), the ratio of those descriptor values to the number of heavy atoms in the molecule, including substructural ones (numbers of occurrences of fragments), were generated. Two software packages, one developed 'in-house' (Quasi-fragmental descriptors implemented through Pipeline Pilot) and the QSAR module in MOE 2006.08[24] package, respectively, permitted calculation of the set.

The first, Quasi-Fragmental descriptors, as described previously so only a brief discussion of changes to the implementation will be detailed.[46,47] Four classification methods are available to the user, the first classification level corresponds to the general type of atom; the second level corresponds to a type of chemical element. Atoms on the third and fourth classification levels characterize type of hybridization, bond environment for each atom, atomic formal charge, and the number of hydrogen neighbours. The fourth level of classification was employed with a maximum fragment size of 15 heavy (non-hydrogen) atoms.

5.59.1.3. Model building. Our modeling was based on two applications of statistical methods—multiple linear regression (MLR) and partial least squares (PLS) method. The MLR with implemented genetic algorithm (GA) was used to converge on those descriptors not strongly correlated with one another. A number of different initial descriptor sets, including only substructural, 2D-MOE, normalized 2D-MOE descriptors, and different combinations of all, were chosen to analyze their impact on studied activity. At this stage, to introduce some nonlinearity, and to increase the predictability of the linear models, some nonlinear modifications, such as the square, square root, and common logarithm (log D_i, calculated only for those fragments that were found in all structures contained in the database), were applied to the initial descriptors. The top subsets consisting of 4–5 descriptors and based on MLR modeling for each partition were used for further modeling. The main part of our modeling was performed using the PLS method with LOO cross-validation. For each model, the LOO cross-validated predictions were examined and outliers were identified. The PLS procedure was subsequently repeated without cross-validation inputting the correct components contributing to the final model.

5.59.2. Docking

5.59.2.1. Ligand preparation. Compound **26b** was drawn using ACD/Chemsketch v10[48] and SMILES strings generated. A single conformer was generated ensuring a final MMFF optimization step for refinement of the compound, using Omega v2.1.[49]

5.59.2.2. Receptor preparation. PDB entries 3ERT and 1QKN were downloaded from the Protein Data Bank (PDB). All waters were retained in both isoforms. Addition and optimization of hydrogen positions for these waters was carried out using MOE 2006.08[24] ensuring all other atom positions remained fixed. The resulting structure was imported into ADT (Autodock Tools)[50] with Geisteger charges and atoms types subsequently added. Importantly, the correct tautomeric position for His524 was chosen, Nε. Flexible residues were selected for the fully flexible docking routine as outlined in the next section.

5.59.2.3. Flexible Residue selection. Ten crystallographic structures displaying a resolution <2.28 Å with a bound antagonist were downloaded from the Protein Data Bank (PDB_ID: 1SJ0, 1UOM, 1XP1, 1XP6, 1XP9, 1XPC, 1XQC, 1YIM, 1YIN, and 3ERT) and a set of 36 estrogen actives with activities ranging from nanomolar to low micromolar potency were docked in all using a rigid docking protocol previously optimized.[51] The receptor which docked the least number of actives was chosen as the template for overlay of the remaining X-ray structures. Superposition of the 10 crystal structures was executed and a svl script (MOE.2006.08) was used to analyze the rmsd differences between all residues of the active site as summarized in Figure 2. From the analysis, the following residues were chosen to be flexible in the docking process—ERα: Met421, Ile424, Phe425, His524, and Leu525. Equivalent residues were also selected by a similar process in ERβ: Ile328, Ile331, Phe332, His430, and Leu431.

5.59.2.4. Docking. Autodock 4 through ADT[52] was employed as the docking tool as it allows both ligand and receptor flexibility to be modelled. A grid parameter file was set up with a grid box of 175,000 grid points per map centered on the calculated center of the co-crystallised ligands of each isoform. Docking was carried out using the Lamarckian genetic algorithm (GALS) and changing only ga_run 50, ga_num_evals 25,00,000, ga_num_generations 5000.

Acknowledgments

We are very grateful to Professor Richard Hochberg at Yale University Medical School, for kindly facilitating the alkaline phosphatase experiments with the generous gift of the Ishikawa cells. 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 (HRD), with additional support for computational facilities from the Wellcome Trust.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.09.035.

References and notes

1. Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 1081.
2. Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 883.
3. Park, W. C.; Jordan, V. C. *Trends Mol. Med.* **2002**, *8*, 82.
4. Bardin, A.; Boulle, N.; Lazennec, G.; Vignon, F.; Pujol, P. *Endocr. Relat. Cancer* **2004**, *11*, 537.
5. Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. *Nature* **1997**, *389*, 753.
6. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
7. Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J. A.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608.
8. Pike, A. C.; Brzozowski, A. M.; Walton, J.; Hubbard, R. E.; Thorsell, A. G.; Li, Y. L.; Gustafsson, J. A.; Carlquist, M. *Structure* **2001**, *9*, 145.
9. Wu, Y. L.; Yang, X.; Ren, Z.; McDonnell, D. P.; Norris, J. D.; Willson, T. M.; Greene, G. L. *Mol. Cell* **2005**, *18*, 413.
10. Lloyd, D. G.; Buenemann, C. L.; Todorov, N. P.; Manallack, D. T.; Dean, P. M. *J. Med. Chem.* **2004**, *47*, 493.
11. Labrie, F.; Labrie, C.; Belanger, A.; Simard, J.; Gauthier, S.; Luu-The, V.; Merand, Y.; Giguere, V.; Candas, B.; Luo, S.; Martel, C.; Singh, S. M.; Fournier, M.; Coquet, A.; Richard, V.; Charbonneau, R.; Charpenet, G.; Tremblay, A.; Tremblay, G.; Cusan, L.; Veilleux, R. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 51.
12. Zhou, H. B.; Comninos, J. S.; Stossi, F.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2005**, *48*, 7261.
13. Kanbe, Y.; Kim, M. H.; Nishimoto, M.; Ohtake, Y.; Kato, N.; Tsunenari, T.; Taniguchi, K.; Ohizumi, I.; Kaiho, S.; Morikawa, K.; Jo, J. C.; Lim, H. S.; Kim, H. Y. *Bioorg. Med. Chem.* **2006**, *14*, 4803.
14. Jain, N.; Kanojia, R. M.; Xu, J.; Jian-Zhong, G.; Pacia, E.; Lai, M. T.; Du, F.; Musto, A.; Allan, G.; Hahn, D.; Lundeen, S.; Sui, Z. *J. Med. Chem.* **2006**, *49*, 3056.
15. Kim, S.; Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L. Y.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M.; Sharma, N.; Dahllund, J.; Thorsell, A. G.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171.
16. Sibley, R.; Hatoum-Mokdad, H.; Schoenleber, R.; Musza, L.; Stirtan, W.; Marrero, D.; Carley, W.; Xiao, H.; Dumas, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1919.
17. Wallace, O. B.; Lauwers, K. S.; Dodge, J. A.; May, S. A.; Calvin, J. R.; Hinklin, R.; Bryant, H. U.; Shetler, P. K.; Adrian, M. D.; Geiser, A. G.; Sato, M.; Burris, T. P. *J. Med. Chem.* **2006**, *49*, 843.
18. Lloyd, D. G.; Hughes, R. B.; Zisterer, D. M.; Williams, D. C.; Fattorusso, C.; Catalanotti, B.; Campiani, G.; Meegan, M. J. *J. Med. Chem.* **2004**, *47*, 5612.
19. Sarkhel, S.; Sharon, A.; Trivedi, V.; Maulik, P. R.; Singh, M. M.; Venugopalan, P.; Ray, S. *Bioorg. Med. Chem.* **2003**, *11*, 5025.
20. McCague, R.; Leclercq, G.; Jordan, V. C. *J. Med. Chem.* **1988**, *31*, 1285.
21. Platzek, J.; BeckmannW; Geisler, J.; Kirstein, H.; Niedballa, U.; Ottow, E.; Radau, S.; Schulz, C.; Wessa, T. (Schering Aktiengesellschaft, Germany). PCT Int. Appl. WO 2003033461, 2003, 48 pp..
22. Shani, J.; Gazit, A.; Livshitz, T.; Biran, S. *J. Med. Chem.* **1985**, *28*, 1504.
23. Hummel, C. W.; Geiser, A. G.; Bryant, H. U.; Cohen, I. R.; Dally, R. D.; Fong, K. C.; Frank, S. A.; Hinklin, R.; Jones, S. A.; Lewis, G.; McCann, D. J.; Rudmann, D. G.; Shepherd, T. A.; Tian, H.; Wallace, O. B.; Wang, M.; Wang, Y.; Dodge, J. A. *J. Med. Chem.* **2005**, *48*, 6772.
24. Molecular Operating Environment (MOE), developed and distributed by Chemical Computing Group. http://www.chemcomp.com.
25. Hachiya, I.; Moriwaki, M.; Kobayashi, S. *Tetrahedron Lett.* **1995**, *36*, 409.
26. Freedman, J.; Stewart, K. T. *J. Heterocyc. Chem.* **1989**, *26*, 1547.
27. Orlov, V. D.; Mikhed' kina, E. I.; Lavrushin, V. F. *Zhurnal Obshchei Khimii* **1984**, *54*(1), 168.
28. Teo, C. C.; Kon, O. L.; Sim, K. Y.; Ng, S. C. *J. Med. Chem.* **1992**, *35*, 1330.
29. Acton, D.; Hill, G.; Tait, B. S. *J. Med. Chem.* **1983**, *26*, 1131.
30. Catherino, W. H.; Wolf, D. M.; Jordan, V. C. *Mol. Endocrinol.* **1995**, *9*, 1053.
31. Mccague, R. *Tetrahedron: Asymmetry* **1990**, *1*, 97.
32. McCague, R.; Jarman, M.; Leung, O. T.; Foster, A. B.; Leclercq, G.; Stoessel, S. *J. Steroid Biochem.* **1988**, *31*, 545.
33. McCague, R.; Leclercq, G. *J. Med. Chem.* **1987**, *30*, 1761.
34. Grese, T. A.; Cho, S.; Finley, D. R.; Godfrey, A. G.; Jones, C. D.; Lugar, C. W., 3rd; Martin, M. J.; Matsumoto, K.; Pennington, L. D.; Winter, M. A.; Adrian, M. D.; Cole, H. W.; Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Short, L. L.; Glasebrook, A. L.; Bryant, H. U. *J. Med. Chem.* **1997**, *40*, 146.
35. Qin, Z.; Kastrati, I.; Chandrasena, R. E.; Liu, H.; Yao, P.; Petukhov, P. A.; Bolton, J. L.; Thatcher, G. R. *J. Med. Chem.* **2007**, *50*, 2682.
36. Lloyd, D. G.; Smith, H. M.; O' Sullivan, T.; Zisterer, D. M. M. J. M. *Med. Chem.* **2005**, *4*, 335.
37. *C-QSAR; Biobyte Corp., W. t. S., Suite 204, Claremont, CA.*
38. Littlefield, B. A.; Gurpide, E.; Markiewicz, L.; McKinley, B.; Hochberg, R. B. *Endocrinology* **1990**, *127*, 2757.
39. Meegan, M. J.; Barrett, I.; Zimmermann, J.; Knox, A. J.; Zisterer, D. M.; Lloyd, D. G. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 655.
40. Mukherjee, S.; Saha, A.; Roy, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 957.
41. Osterberg, F.; Morris, G. M.; Sanner, M. F.; Olson, A. J.; Goodsell, D. S. *Proteins* **2002**, *46*, 34.
42. Celik, L.; Lund, J. D.; Schiott, B. *Biochemistry* **2007**, *46*, 1743.
43. Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z. B.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A.; Keith, J. C., Jr.; Harris, H. A. *J. Med. Chem.* **2004**, *47*, 5021.
44. Yang, C.; Edsall, R., Jr.; Harris, H. A.; Zhang, X.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2004**, *12*, 2553.
45. (a) Nitikin, K. V.; Andryukhova, N. P. *Can. J. Chem.* **2004**, *82*, 571; (b) Ghosh, A.; Bhattacharya, S.; Raychaudhuri, S. R.; Chatterjee, A. *Indian. J. Chem B.* **1992**, *31*, 299.
46. Artemenko, N. V.; Baskin, I. I.; Palyulin, V. A.; Zefirov, N. S. *Doklady Chem.* **2001**, *381*, 317.
47. Artemenko, N. V.; Palyulin, V. A.; Zefirov, N. S. *Doklady Chem.* **2002**, *386*, 114.
48. ACD/Chemsketch v10, Advanced Chemistry Labs. http://www.acdlabs.com/download/chemsk.html.
49. OMEGA 2.1, distributed by Openeye Scientific Software.
50. Autodock Tools (ADT), developed and distributed by The Scripps Research Institute. http://autodock.scripps.edu/resources/adt.
[51]. Knox, A. J.; Meegan, M. J.; Carta, G.; Lloyd, D. G. *J. Chem. Inf. Model.* **2005**, *45*, 1908.
[52]. Sanner, M. F. *J. Mol. Graph. Model.* **1999**, *17*, 57.