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Lead Optimization of Benzoxepin-Type Selective Estrogen Receptor (ER) Modulators and Downregulators with Subtype-Specific ERα and ERβ Activity

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Abstract

Estrogen receptor α (ERα) is an important target for the design of drugs such as tamoxifen (**2a**) and fulvestrant (**5**). Three series of ER-ligands based on the benzoxepin scaffold structure were synthesised – series I containing an acrylic acid, series II with an acrylamide and series III with a saturated carboxylic acid substituent. These compounds were shown to be high affinity ligands for the ER with nanomolar IC_{50} binding values. Series I acrylic acid ligands were generally ERα selective. In particular, compound **13e** featuring a phenylpenta-2,4-dienoic acid substituent was shown to be antiproliferative and downregulated ERα and ERβ expression in MCF-7 breast cancer cells. Interestingly, from series III, the phenoxybutyric acid derivative compound **22** was

not antiproliferative and selectively downregulated ERβ. A docking study of the benzoxepin ligands was undertaken. Compound **13e** is a promising lead for development as a clinically relevant SERD, whilst compound **22** will be a useful experimental probe for helping to elucidate the role of ERβ in cancer cells.

Key words:

Antiproliferative activity, Benzoxepin, Breast Cancer, ER binding, Estrogen receptor ligand, Selective Estrogen Receptor Downregulator, SERD

Introduction

The two nuclear estrogen receptors (ERα and ERβ) mediate the biological effects of the estrogen hormones and ERα is an attractive therapeutic target for diseases including breast cancer and osteoporosis.[1] Estrogens including estradiol (**1**, Figure 1) are known to have tissue selective effects, and there is considerable interest in the therapeutic use of selective estrogen receptor modulators (SERMs).[2] A number of SERMs are currently in clinical use,[3] including tamoxifen (**2a**) for treatment of hormone-dependent breast cancer, and raloxifene (**3a**), lasofoxidine and basidoxifene for the prevention of osteoporosis (Figure 1).[4] The clinical successes of **2a** and **3a** has provided the driving force to discover new, multifunctional SERMs. There is ongoing debate about the role of ERβ in cancer. It is generally thought that expression of ERβ has antiproliferative effects in breast cancer cells.[5, 6] In prostate cancer, its role is still unclear and there is some evidence that certain isoforms of ERβ are oncogenic.[7, 8] ERβ expression has been reported have a potentially protective effect in normal cells on ERα promoted hyperproliferation.[9] There is much research being undertaken to fully elucidate the effects of ERβ in cancers.

The selective ER downregulator (SERD) **5** is in clinical use for the treatment of postmenopausal women with ER-positive, locally advanced or metastatic breast cancer for disease relapse on or after adjuvant anti-estrogen therapy, or disease progression on therapy with an anti-estrogen (Figure 1). The acrylic acid GW5638 (**4a**) and its presumed metabolite GW7604 (**4b**, Figure 1) have also been identified as SERDs mechanistically different to **2a** and **3a**, with full agonist activity in bone and in the cardiovascular system, and antagonistic activity in human endometrial adenocarcinoma Ishikawa cells and in the rat uterus with minimal residual agonist activity.[10, 11,

[12] Breast cancers resistant to **2a** are not cross-resistant to **4a** indicating that this type of SERD has potential as a therapeutic agent. The SERD action of **4b** causes a decrease in cellular ERα levels.[13] Related compounds showed low stimulation of uterine cell proliferation with good ERα and ERβ binding affinities.[14] Acrylic acid-substituted quinoline,[14] naphthalene,[15] tetrahydroisoquinoline,[16] bicyclo[3.3.1]nonane,[17] coumarin,[18] benzopyranobenzoxepanes[19] and benzosuberone scaffolds have also been investigated as SERMs.[15, 20, 21] The 1*H*-pyrido[3,4-*b*]indol-1-ylphenylacrylic acid AZD9496 (**4c**),[22] coumarin,[23] and the indazole ERα modulator GDC-0810 (**4d**)[24] were recently reported as potent and orally bioavailable SERDs and antagonists (Figure 1).

We have previously identified novel SERMs [e.g. compound **3b** (Figure 1)] which demonstrated potential as ER binding ligands,[25] and now we report the development of this chemical template for nonsteroidal SERDs where the ER degradation was optimised. The conformationally restricted benzoxepin template has been elaborated to incorporate substitution with acrylic acids, and related structural modifications. An overview of the three distinct series of compounds described in this study is provided in Figure 2. The evaluation of antiproliferative activity and relative binding affinity of these ligands for ERα and ERβ together with their stimulatory effect on uterine tissue is examined. A molecular modelling study was investigated to rationalise the binding selectivity of these ligands for the ERα and ERβ receptors.

Chemistry

The benzoxepins synthesised in this study are arranged in three different structural classes (Series I, II and III; Figure 2) to investigate the response of the ER ligand-binding domain to

targeted structural alterations (Schemes 1-3). The first group (Series I, compounds **11a-11d** and related alkenes **13a-c** and **13e**, Scheme 1) are ring-fused analogues of **4a**/**4b**, with variation in substitution at the C-8 position of the benzoxepin or benzothiepin scaffold structure. The fluorine substituent at C-8 of the benzoxepin and benzothiepin structure would be expected to increase the lipophilicity of these compounds and also block expected metabolic inactivation, contributing to a longer plasma half-life. It has been reported that the inclusion of a fluorine contributes to the SERM activity of ER ligands such as oxachrysenol[26] and we have observed a similar effect for compound **5**.[25] The second group of compounds investigated (Series II, **14a-14i**, Scheme 2) contain amide-modified derivatives of the core acrylic acid compound **11c**. Compounds **19a**, **19b** and **22** (Series III, Scheme 3) are distinguished by the inclusion of the 4-oxyacetic and 4-oxybutyric acid substituent in place of the acrylic acid in Ring B of **11c**.

The synthetic route that produced the required products **11a-11d** (series I) most efficiently is shown in Scheme 1. The benzoxepin-4-ones **6a-d** were treated with trifluoromethanesulfonic anhydride to afford the intermediate triflates which were then coupled with the arylboronic ester to afford the aldehydes **7a-d** in good yield (steps *i* and *ii*). Vinyl bromination of the alkenes **7a-d** with pyridinium tribromide gave the bromides **8a-d** (Scheme 1, step *iii*). Bromides **8a-c** were then coupled with 4-hydroxyboronic acid in a second Suzuki reaction to afford the phenolic substituted benzoxepins **9a-c** (Scheme 1, step *iv*). A Wittig-Horner reaction of **9a-c** with (ethoxycarbonyl methylene)triphenylphosphorane, followed by saponification of the esters **10a-c** afforded the required acids **11a-c** in good yield (Scheme 1, steps *v* and *vi*). The benzothiepin compound **11d** was obtained in an alternative reaction sequence from the bromide **8d**. Initial Wittig-Horner reaction with (ethoxycarbonylmethylene)triphenylphosphorane followed by

hydrolysis of the ester *in situ* afforded the acrylic acid **12**. Suzuki coupling of the acid **12** with 4-hydroxyphenylboronic acid gave the required product **11d**. The related alkene and α,β-unsaturated ketone products **13a-c** were also synthesised by similar Wittig type reactions from **9a-9c** (Scheme 1, step *vii*). The extended unsaturated acid compound **13e** was obtained from aldehyde **9c** by treatment with (*E*)-ethyl 4-(diethoxyphosphoryl)but-2-enoate (prepared from ethyl 4-bromocrotonate and triethylphosphite) followed by saponification of the ester **13d** (Scheme 1, steps *vii* and *viii*).

Series II consisting of eight α,β-unsaturated amides **14a-h** was obtained from the unsaturated acid **11c** by coupling with various amines in the presence of HOBt, using EDCI as the coupling agent (Scheme 2, step *i*). Surprisingly, the coupling of **11c** with aniline proved to be difficult. An alternative method to obtain the desired product **14i** required the initial preparation of the phosphonate **15** (Scheme 2). 2-Bromo-N-phenylacetamide (prepared from aniline and acetylbromide) was treated with triphenylphosphite to afford the corresponding phosphonate **15**,[27] which was reacted with aldehyde **9c** to afford the required product **14i** in 83% yield (Scheme 2, step *ii*). An alternative reaction of **9c** with the phosphonium bromide prepared from 2-bromo-N-phenylacetamide and triphenylphosphine was unsuccessful.

Related compounds **19a** and **19b** (series III) containing an 8-oxyacetic acid side chain were obtained by O-alkylation of the vinyl bromides **16a** and **16b** (prepared as previously reported)[25] with ethyl bromoacetate to afford esters **17a** and **17b** (Scheme 3, step *i*). Subsequent Suzuki coupling with 4-hydroxyphenylboronic acid followed by hydrolysis of esters **18a** and **18b** afforded the desired acids **19a** and **19b** (Scheme 3, steps *ii* and *iii*). A similar alkylation reaction

of **16a** with ethyl bromobutyrate afforded the ester product **20**, which was subsequently arylated with 4-hydroxyphenylboronic acid to yield **21** (Scheme 3, steps *i* and *ii*). Hydrolysis of ester **21** gave the product **22** containing the required oxybutyric acid side chain (Scheme 3, step *iii*).

Acrylic acid **4b**, required for comparative biochemical studies, was obtained in a novel three-step route by initial McMurray coupling of ketone **23** and propiophenone to afford the iodo-substituted triphenylethylene **24** as the major product (Scheme 4, step *i*), which could be separately converted into either the acrylic acid **25** (94%) or the acrylate ester **26** (93%) by Heck-type reactions with acrylic acid and ethyl acrylate respectively in the presence of palladium acetate (Scheme 4, steps *ii* and *iii* respectively). Deprotection of **25** with boron tribromide directly afforded **4b** (Scheme 4, step *iv*; 28% overall yield) as the major *Z* isomer was obtained (*E/Z* mixture: 1:3.5), while demethylation of **26** followed by *in situ* hydrolysis of intermediate **27** also yielded acrylic acid **4b** (22% overall yield) (Scheme 4, steps *iv* and *v*). Previous synthetic routes to this compound and analogues have relied on Friedel-Crafts acylation, Grignard or Suzuki coupling reactions to generate the triphenylethylene structure,[10, 13, 28] followed by a Wittig or Horner-Emmons reaction for introduction of the acrylate side chain [15% overall yield (Friedel-Crafts, Grignard and Wittig for synthesis of **4b**)] and 48% overall yield (Suzuki coupling followed by Horner-Emmons reaction for synthesis of **4a**)].

X-Ray Crystallography

An X-Ray crystallography study of two of the benzoxepins, ester **18a** and acid **22**, was undertaken to confirm the structural assignments and also to explore potentially important structural features for potent ER activity. The 7-membered ring displays a puckered

conformation in both structures (Figure 3). This results in a molecular arrangement in which the three aromatic rings attached to the 7-membered ring are not coplanar. Calculation of the ring plane and torsional angles between the aromatic rings of these compounds further demonstrates that these rings are arranged out-of-plane with respect to each other (Table 1). For example, the ring angle between the planes of ring A and ring B of compound **22** is 64.3°, and the torsional angle is 42°. Bond lengths between C8 and C18 (compound **18a**) and C8 and C19 (compound **22**) of 1.345 Å and 1.350 Å, respectively, indicate the position of the double bond in the 7-membered ring.

Biochemical Results: Antiproliferative activity in MCF-7 breast cancer cells. The antiproliferative activity of the benzoxepin compounds was firstly evaluated using the ER-expressing (ER-dependent) MCF-7 human breast cancer cell line. Compound **2a** (IC_{50} = 4.1 μM) and **4b** (IC_{50} = 1.3 μM) were used as positive controls and the IC_{50} values obtained are in agreement with previously reported values for these compounds (Table 2).[29, 10, 13, 30]

In the initial series of acrylic acids **11a-11d** the presence of a fluorine at C-8 in benzoxepin compound **11c**, together with the sulfur-containing ring in compound **11d**, resulted in a marked improvement in antiproliferative activity (IC_{50} values of 0.26 and 0.095 μM respectively, Table 2) when compared with non-fluorinated compound **11a** (IC_{50} = 21 μM). The α,β-unsaturated ketones **13a** and **13c** also retained sub-micromolar activity (IC_{50} = 0.89 and 0.97 μM respectively). Compound **13e** with an extended penta-2,4-dienoic acid substituent in Ring B, retains moderate antiproliferative activity (IC_{50} = 1.6 μM).

Introduction of the amide modification (series II) resulted in a decrease in the antiproliferative activity of the fluorinated products **14a-i** when compared with acrylic acid compound **11c**. Piperidine-substituted amides **14e** and **14g** proved to be the most active, together with **14h** (IC_{50} = 0.48, 1.05 and 0.70 μM respectively). Oxyacetic analogues of **2a** had been reported to act as estrogen antagonists in MCF-7 cells.[31, 32] In series III, compounds **19a** and **19b**, containing a 4-oxyacetic acid substituent in place of the acrylic acid in **11b** and **11c**, along with compound **22**, did not have antiproliferative activity at concentrations up to 20 μM (as was observed for the oxyacetic analogue of **2a**[32]).

Cytotoxicity was evaluated by use of the standard LDH assay to establish that the observed antiproliferative effects were attributable to cytostasis rather than cellular necrosis (Table 2). The majority of the compounds demonstrated minimal cytotoxicity (<5% at 10 μM concentration), considerably below that obtained for **2a** (13.4%). Compounds **13e** and **22** were also assessed for toxicity using the non-tumorigenic MCF-10a mammary epithelial cell line. Compound **22** did not have any effect on the viability of MCF-10a cells at concentrations of 1 and 10 μM over 24 and 48 h periods (Figure 4). No effects were observed for compound **13e** at 1 and 2 μM; however, MCF-10a cell viability was reduced at higher concentrations of 5 and 10 μM, with reduction of 50% at 5 μM over 24 h. As the IC_{50} value of **13e** in MCF-7 cells is 1.6 μM, there is a therapeutic window available for this compound at which it may cause antiproliferative effects in breast tumour cells without significant toxicity to non-tumorigenic cells.

Biochemical Results: ER Binding Studies. ER-binding studies were carried out with both ERα and ERβ to confirm receptor involvement in the observed antiproliferative effect. A

fluorescence polarisation procedure was employed for this competitive binding assay which measures the displacement of fluorescein-labelled estradiol (fluoromone) from the human recombinant full length ERα and ERβ. All ER-binding values are expressed in nM. The relative binding affinity (RBA) of ER ligands is often reported. Compound **1** is typically used as the reference ligand and is taken as the 100 % binding value. Using the reported reference IC_{50} values obtained for **1** in ERα (5.7 nM)[33] and ERβ (5.6 nM)[34], the RBAs of the selected conjugates were calculated (Table 2). Values greater than 100 % indicate a greater affinity for the ER than **1**; values less than 100 % indicate a diminished affinity for the ER.

All compounds (with the exception of **13b**) displayed potent ERα and ERβ binding activities, with the majority of the more potent compounds showing selectivity for ERβ. Introduction of the 8-fluoro substituent in series I, as in (**11c**), and also the benzothiepin ring scaffold (**11d**) gave increased ER binding activity for both ERα and ERβ. As an example of the series, compound **11d** exhibited potent binding to both ERα and ERβ with IC_{50} = 4.1 nM (ERα) and 3.1 nM (ERβ). Compound **13e**, containing the extended penta-2,4-dienoic acid substituent in Ring B, was found to display potent ER-binding activity with IC_{50} = 71.6 nM (ERα) and 0.55 nM (ERβ), equivalent to 129-fold ERβ selectivity. Compound **4a** and related amides have previously been reported as SERMs with antagonist activity in rat uterus and acting as agonists in bone.[10] In our acrylamide series II (compounds **14a-i**) compound **14i** demonstrated the most effective binding activity [IC_{50} = 11.7 nM (ERα) and 0.94 nM (ERβ)], with 11-fold ERβ selectivity. Acrylamide **14b**, possessing moderate antiproliferative activity, demonstrated good ER-binding activity (IC_{50} = 67 nM for ERα and 2.4 nM for ERβ, with 27-fold ERβ selectivity).

Triarylethylene oxyalkanoic acid modifications of **2a** have been reported as bone selective estrogen mimetics.[31] Of our compounds in series III, **19a** and **19b** had high IC_{50} binding values indicative of poor affinity for both ERα and ERβ, perhaps explaining their poor antiproliferative activity. The related compound **22**, containing the 4-oxybutyric acid substituent, demonstrated extremely interesting ER-binding properties with IC_{50} = 147 nM (ERα) and 1.23 nM (ERβ), which is 117-fold selectivity for ERβ, despite its lack of antiproliferative potency. This binding value for **22** is an improvement on reported values for the corresponding oxybutyric acid analogue of **2a**.[32]

In order to assist us in the rationalisation of the α/β selectivity observed for the ER binding results obtained for these benzoxepin compounds, the ER binding effects of the core structure **9c** was determined as IC_{50} = 407 nM for ERα and 395 nM for ERβ. This indicates that ER binding is related to the presence of 4-hydroxyphenyl at C-4 or C-5 of the 4,5-diaryl-8-fluorobenzoxepin scaffold. ER selectivity is conferred by the specific type of acidic side chain substituent present at C-4 of Ring B. Small modifications to this side chain lead to dramatic differences in ER-binding affinity, subtype selectivity and antiproliferative potency.

Biochemical Results: Estrogenic Stimulation. The estrogenic stimulation and antagonistic properties of a number of compounds were measured in an in vitro assay using stimulation of alkaline phosphatase (AlkP) in Ishikawa human endometrial adenocarcinoma cells (Table 3).[35] Compounds **11a**-**11d** and **13a** from series I were investigated alongside a representative example from series II (**14a**) and series III (**22**). Antiestrogenic (IC_{50} = 0.0016 μM) and estrogenic activity (4 % stimulation at 1 μM) have been reported for **4b** in Ishikawa cells.[14] Compound **11d**

possessed an improved potency over **2a** as an ER antagonist (IC_{50} = 0.18 μM) while compounds **11a, 11c**, **13a** and **22** also displayed good antiestrogenic activity, with IC_{50} values in the range 0.43 - 1.39 μM. In the acrylamide series, compound **14a** was found to be extremely potent as an estrogen antagonist with an IC_{50} value of 0.0098 μM, comparable to the value reported for **4b**.[14] This compound does not demonstrate significant antiproliferative activity in the estrogen dependent MCF-7 cells (IC_{50} = 13 μM) which is 10-fold less potent than **4b** (IC_{50} = 1.3 μM); ER binding is also less potent than observed for **4b**. This may be attributed to the differences in interaction of **14a** with the ER on binding due to replacement of carboxylic acid of **4b**, **11c** and **11d** with the more lipophilic *N*,*N*-diethylamide substituent. The estrogenic stimulatory effect is determined by measuring AlkP stimulation in the absence of **1** (Table 3). Compounds **11c**, **11d** and **22**, which were among the most potent ER-binding compounds, demonstrated an absence of or very low stimulatory effects (0 %, 1.6 % and 3.2 % at concentrations of 1 μM, 10 μM and 1 μM respectively), when compared with **4b** (4%)[14] and **2a** (10%). The most potent antagonist compound, acrylamide **14a** (evaluated for stimulatory activity at a 100-fold higher concentration of 100 μM, as 1 μM had no effect), together with the ketone **13a** (1 μM), and acrylic acid **11a** (1 μM), showed relatively low stimulatory values of 10 %, 9.6 % and 4.6 % respectively. Selection of the optimal structural features for antiestrogenic activity for benzoxepin and benzothiepin ring scaffolds, without adverse estrogenic effects on tissues such as the uterus, is possible by reference to the results of these AlkP assays.

Biochemical Results: Effect of compounds 13e and 22 on the expression levels of ERα and ERβ in MCF-7 cells

The expression of ERα and ERβ in MCF-7 cells was examined by Western blotting (Figure 5). Compounds **13e** (series I) and **22** (series III) were chosen for further biochemical evaluation; compound **22** showed an interesting profile of activity due to its lack of antiproliferative effect whilst having an extremely selective effect on ERβ. Compound **13e** has potent antiproliferative activity in MCF-7 cells and good potency against both ERα and ERβ (Table 2). The pure estrogen antagonist **5**, a known SERD, downregulates the expression of ERα and was used as a positive control. Compound **5** causes rapid proteasomal degradation of ERα via ubiquitinylation, resulting in shutdown of the estrogen-signaling process and thus inducing proliferation arrest and apoptosis of estrogen-dependent breast cancer cells.[36] In contrast, SERMs such as 4-hydroxytamoxifen (**2b**, Figure 1) bind to ERα as antagonists or partial agonists depending on the target tissue. MCF-7 breast cancer cells were treated with compounds **13e** and **22** (10 μM), and after 24 h whole cell lysates were prepared and analysed by SDS-PAGE and Western blotting for expression levels of ERα or ERβ. The known SERD **5** reduced ERα protein levels, with little or no effect on ERβ (Figure 5). Compound **13e**, which possessed good antiproliferative activity, was found to downregulate both ERα and ERβ (Figure 5). Compound **22** selectively downregulated ERβ in MCF-7 cells, with little effect on the expression of ERα. This result is consistent with the ER-binding assay, in which compound **22** was ERβ selective.

Compound **22** is the first reported ERβ-selective SERD. It does not have antiproliferative effects in either MCF-7 or MCF-10a cells, despite possessing antiestrogenic activity (Table 2 and Figure 4). There is debate about the role of ERβ in cancer; in prostate cancer, there is some evidence that certain isoforms of ERβ are oncogenic[7, 8] whilst generally thought that expression of ERβ

has antiproliferative effects in breast cancer cells.[5, 6] Due to its unique combination of cellular effects, compound **22** is a useful tool for investigation of the role of ERβ in cancer cells.

Molecular Modelling of Benzoxepins in ERα and ERβ

A number of structurally related benzoxepins were discovered to have diverse effects on ERα and ERβ, in particular compounds **13e** and **22**. Hence, a molecular modelling study of **11c**, **11d**, **13e**, **14b**, **14i** and **22** was carried out to investigate their potential interactions with ERα and ERβ. The two ER subtypes differ significantly in size: 595 amino acids in ERα compared to 485 amino acids (ERβ). The conservation of amino acid sequence in the ligand binding sites of ERα and ERβ is only 59%, with the most notable differences being replacement of Met412 and Leu384 in ERα with Ile and Met in ERβ. The 3ERT X-ray structure of hERα co-crystallised with **2b**[37] was downloaded from the PDB website. For ERβ the 1NDE X-ray structure co-crystallised with a triazine modulator was used.[38] After validating the docking protocol and determining receiver operating characteristic (ROC) values of 0.896 and 0.819 for the ERα and ERβ haystack docking respectively, we undertook a more in-depth binding analysis on compounds **13e** and **22** as they demonstrated the optimal ERα:ERβ binding ratio in favour of ERβ. In addition to 1125 inactive compounds, the ERα and ERβ haystacks contained 39 and 32 known active compounds respectively. Amino acid numbering corresponds to AA(#ERα)/(#ERβ) unless specified in the text. All of our compounds had top-ranked binding poses for both ERα and ERβ, excluding **22** in ERβ, placed the ring A fluorine atom at the same location as the hydroxyl group of **2b**. The comparative ranking of the most potent compounds in ERα and ERβ for the docking analysis demonstrates a high degree of correlation with the experimental binding affinity, particularly for ERα (Table 4). All the new compounds described

in this study ranked in the top 5% of the ordered hit list and are comparable to the known active compounds.

In ERβ, the acid **22** has a 180 degree flipped orientation compared to other compounds in this study, in that the phenolic hydroxyl group mimics the position adopted by **2b** (Figure 7). The fluorine-containing ring no longer clashed with Leu525 of ERα, as this sidechain has rotated 180° and is directed outside the LBP, but is ideally positioned to accept a HB from His475 of ERβ. The *ortho* hydrogen of the ether appended phenyl ring B is located adjacent to the co-crystallised water molecule thereby enabling a HBD interaction. The oxygen atom of the benzoxepin ring is adjacent to the Met421 of ERα which protrudes into the pocket and would lead to electrostatic repulsion but in ERβ this residue is replaced by the Ile373 and moves to the side of the pocket to form a hydrophobic layer below the ligand's fluorinated phenyl group. In ERα, **22** overlays well on the core structure of **2b** except that the ring A fluorophenyl fluorine maps to the hydroxyl group of **2b** (Figure 7). The carboxylic acid side chain is not oriented towards the Asp351 group but this may result from only generating 50 conformers of each ligand as the side-chain has many degrees of freedom. This series of LBP amino acid positional changes between ERα and ERβ all favour binding of **22** to ERβ over ERα which is experimentally reflected in the IC_{50} ER binding values and in the effects on ER expression demonstrated in the western blot in MCF-7 cells.

The 180 degree rotated orientation of a compound's binding pose was previously highlighted by our group[39] and is reminiscent of earlier studies on **3a**. In an influential co-crystal structure of **3a** with ERα (1ERR) the phenolic group of the benzothiophene is interacting with Glu353 and

Arg394.[40] However, a later 2002 X-ray structure of the aroylbenzothiophene core of **3a** with no sidechain (1GWQ)[41] places the A ring in this position and the phenolic moiety interacts with Glu353 and Arg394 which is analogous to the interactions made by **2b** (3ERT). This illustrates the possible symmetry of binding mode of the core scaffold which can rotate 180 degrees depending on the nature of the appended side-chain. While the ability of fluorine to act as a HBA is still debated there is evidence that it is possible, although to a lesser extent than oxygen.[42] Crystallographic evidence in the case of ER binding to fluorinated A ring ligands is not available. However, a very recent publication detailing the X-ray structure of 4,4-dichlorodiphenyltrichloroethane (DDT) in ER (5KRA) places a different halogen, chlorine, in position to partly mimic the Ring A phenolic groups' interactions with Glu353 and Arg394.[43] In addition to the capacity to accept hydrogen bonds, chlorine can also interact with Glu353 through a halogen bond.[44] This data adds confidence to our postulated binding modes wherein the fluorine atom can occupy the traditional "Ring A" hydroxyl group position and engage in HBA or dipole interactions with Arg394 and a bridging water molecule.

For both ERα and ERβ, **13e** co-locates the ring A fluorinated phenyl group adjacent to the 4-hydroxy group of **2b** and is positioned to accept HBs from Arg394/346 (Figure 6). The flipped orientation as observed with **22** docked in ERβ is not possible in this case due to the linear delocalised nature of the "antagonistic" sidechain which would clash with Asp351/303. For the ERβ binding pose of **13e**, the phenolic ring C clashes with Leu525 of ERα which is involved with generating a lipophilic hole with Leu384 (Figure 6). In ERβ, Leu384 is replaced with Met336 which locks a co-crystallised water in place for a HBA interaction with the acyclic secondary amine of the 1NDE co-crystallised piperazinyl-1,3,5-triazine. Leu476 of ERβ can

therefore relocate enabling the phenol group to occupy the pocket and act as a HBD with Met295, in addition to potentially acting as a HBA with His475. In ERβ, the *ortho* hydrogen atoms of both the phenol C ring and phenyl B ring are adjacent to the co-crystallised water molecule, bridging from Met336, thereby enabling HBD interactions.

Consistent with previously reported X-Ray studies of **4a**[12] and **4c**[22] in ERα, the acrylic acid moiety of **13e** is located adjacent to Asp351/303 in both the ERα and ERβ docked structures, demonstrating an unusual acid−acid interaction which causes helix 12 to adopt an unexpected conformation and has been proposed to be important for achieving a downregulator-antagonist profile for these acrylic acid ligands. With these studies in mind, the docked compounds were not deprotonated. This observation is also consistent with the X-Ray structure of **4a** in the LBP of ERα which has revealed that the compound occupies an orientation similar to **2b** in the ligand binding pocket.[12] The unionized carboxylic acid groups of **4a** and Asp351 form a hydrogen bond observed in the crystal structure at pH 5.6. The acrylic acid substituent induces a conformation of H12 in which it is displaced from the hydrophobic cleft, and stabilized by the formation of hydrogen bonds from the carboxylic acid of **4a** to the amide backbone NH groups of Leu536 and Try537 at the N terminus of H12. This relocation causes a significant increase (27%) in the exposed hydrophobic surface of H12 and induces destabilization and ERα protein degradation.

Discussion

In this work, we have identified novel structures based on the benzoxepin scaffold which have a distinct profile of activity compared to **2a**, implying that they may be useful in the treatment of ER-positive breast cancer tumours which may have developed resistance to conventional hormonal treatments.[45, 46] Breast tumorigenesis can be associated with an increase in ERα expression and a decrease in ERβ. Thus, it is hypothesized that ERβ plays a role as a tumor suppressor in breast cancer. Breast tumours that become resistant to one antiestrogen class often continue to maintain sensitivity to another class of antiestrogen. SERDs can be considered as a further development in the design of antiestrogen type treatments for breast cancer, and agents such as **5** have been shown to be effective against ERα-positive, **2a**-resistant breast cancers.[47] Compounds that induce ERα degradation could be used to increase the period of time that patients can be successfully treated with antiestrogen therapies.[48]

There is currently much interest in the discovery of novel molecular scaffolds with SERM or SERD profile properties which could be suitable for development of new therapies for the treatment of breast cancer, osteoporosis, and related hormone-dependent conditions. The profound effect of the acrylic acid H-bonding with the H-12 residues observed in the X-Ray structure of **4a** prompted us to investigate the effect of a number of conformationally-constrained benzoxepin templates on the ER affinity and antiproliferative effects in MCF-7 ER-positive breast cancer cells. In the design of the compounds we have included functional groups such as acrylic acids, acrylamides, methyl ketones, oxyacetic acids, oxybutyric acids and extended pentadienoic acids, which can form HBs to Helix 12 Asp351, and also interact with the backbone Helix12 amide groups. The recently reported benzopyranobenzoxepanes were identified as

potent SERMs for treatment of postmenopausal symptoms, behaving as antagonists in the uterus, whilst exhibiting potent agonist activity in bone and plasma lipids.[19] We have identified a number of fluoro-substituted benzoxepin compounds containing an acrylic acid side chain which demonstrate potent antiproliferative activity against the MCF-7 human breast cancer cell line (Table 2). The presence of the fluorine substituent at C-8 in Ring A (Figure 2) is expected to block metabolism of these compounds, ensuring a longer plasma half-life. These ER-targeting benzoxepins are designed to circumvent the metabolic complications introduced by *E*, *Z* isomerisation of the **2a**-type triarylethylene antiestrogen structures. These compounds were demonstrated to be high affinity ligands for the ER with the majority of IC_{50} values in the nanomolar range. The acrylic acid ligands in series I were generally ERα selective, while the phenylpenta-2,4-dienoic acid derivative **13e**, acrylamide **14i** and the phenoxybutyric acid derivative **22** demonstrated selectivity for ERβ of 11-, 117- and 129-fold respectively, with low Ishikawa cell stimulation. In series III, it was apparent that two–carbon homologation of the oxyacetic side chain of **19a** to produce compound **22** resulted in a notable improvement in the ER affinity and reduced estrogenic stimulatory effect as demonstrated in the Ishikawa cell assay. Compound **13e** was shown to be a downregulator of ERα expression in MCF-7 breast cancer cells, while compound **22** was non-antiproliferative and selectively downregulated expression of ERβ. The compounds demonstrated low cytotoxicity in both the LDH assay and in the MCF-10a non-tumorigenic cell line. The receptor selectivity for ERβ for the most potent compounds **13e** and **22** was examined in docking studies and suggest a correlation between slight change in binding site amino acids between the two isoforms and enhanced ERβ binding for this benzoxepin ring template. The downregulation effect in ERα of **13e** may be attributed to the additional hydrogen bonding of the carboxyl group with the Leu536, Tyr537 (via a water

molecule) and the expected interaction with Asp 351. The novel SERDs **13e** and **22** based on the high-affinity benzoxepin ligand core structure with phenoxybutyric acid and penta-2,4-dienoic acid substituents respectively resulted in differing profiles of downregulation of the ER, and are potentially useful scaffolds for future development.

Molecular modelling studies confirm that inclusion of the benzoxepin scaffold in compounds **13e** and **22** facilitates a number of key interactions with the ER LBD residues. The low oral bioavailability of **5** indicates the potential for the development of potent orally available SERDs.[49] Further biochemical studies are necessary to determine the effects of these novel analogues on estrogen response element (ERE) transcription and ERα stability in MCF-7 cells, and to determine the mechanistic differences between their activity and that of **2a**. Molecular modifications which facilitate varied interactions between the ligand and the receptor residues, resulting in possible unfolding of the LBD and increasing its hydrophobicity are seen to result in the discovery of new types of antiestrogens with potential clinical applications.

Conclusions

This study demonstrates that an alternative ligand core structure, i.e. the benzoxepin scaffold, can be used in place of the triarylethylene core characteristic of **4a** and **4b**. This study extends our current understanding of the pharmacophore requirements for SERD activity, and probes the effect of the benzoxepin ligand core structure and the amido, phenylacrylic acid, phenoxyacetic acid, phenoxybutyric acid and penta-2,4-dienoic acid substituents on the activity of the ER. Specific structural modifications which facilitate additional interactions between the ligand and the ER have been shown to be key factors in the design of novel SERDs which have useful clinical applications. Compound **22** shows significant potential for utility in probing the role of ERβ in the development of cancer. Compound **13e**, which features the phenylpenta-2,4-dienoic

acid substituent on the benzoxepin core, was antiproliferative and effectively downregulated ERα in MCF-7 breast cancer cells. These novel ligands can be used to probe the size, shape and flexibility of the ERα and ERβ ligand-binding domains and will be investigated further as potential drugs.

Experimental Section

Materials and methods: Chemistry

All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous DCM was obtained by distillation from calcium hydride, and anhydrous THF by distillation from benzophenone-sodium, both in inert atmospheres immediately before use. IR spectra were recorded as thin films on NaCl plates or as KBr discs on a Perkin-Elmer Paragon 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX 400 instrument at 20 ^{o}C, 400.13 MHz for ^{1}H spectra, 100.61 MHz for ^{13}C spectra, or 376 MHz for ^{19}F spectra, in either $CDCl_3$ or CD_3OD (internal standard tetramethylsilane). High resolution molecular ion determinations (HRMS) (mass measurement accuracies of $< \pm 5$ ppm) were obtained at the Mass Spectrometry Laboratory, Centre for Synthesis and Chemical Biology (CSCB), University College Dublin, by Dr. Dilip Rai. Additional mass measurements were obtained by Dr. Martin Feeney at the HRMS Laboratory in the Department of Chemistry, Trinity College Dublin. TLC was performed using Merck Silica gel 60 TLC aluminium sheets with fluorescent indicator visualizing with UV light at 254nm. Flash chromatography was carried out using standard silica gel 60 (230-400 mesh) from Merck. All products isolated were homogenous on TLC. Samples were tested for purity using reversed phase HPLC (Waters Alliance system) at 254 nm using a Phenomenex column (4 μ micron, 250 x 4.60 mm) using a mobile phase of acetonitrile: water (0.1 % TFA) (70:30) delivered at a flow rate of 1.0 mL/min. Final compounds had a purity of ≥ 95%. Compounds **6b**, **16a** and **16b** were synthesized and characterized as previously described by us.[25]

General Method I. Preparation of 6a, 6c, 6d. A mixture of appropriately substituted butyric acid (27.8 mmol) and polyphosphoric acid (51 g) was heated at 70 °C for 4 h. The brown syrup was poured into ice-water (100 mL) and the aqueous layer extracted with ethyl acetate (4 × 200 mL). The combined organic layers were washed with 10% NaOH (100 mL), brine (100 mL), dried over sodium sulfate and the solvent removed under reduced pressure. Compounds **6a** (waxy white solid, 35% yield),[50] **6c** (yellow oil, 31% yield)[51] and **6d** (yellow oil, 77% yield)[52] were prepared and characterized as previously reported.

General Method II: Synthesis of 7a-7d. Trifluoromethanesulfonic anhydride (4.38 mmol) in dichloromethane (20 mL) was added to a suspension of **6a-6d** (2.19 mmol) and sodium sulphate (4.38 mmol) in dichloromethane (20 mL) at 0 °C. The suspension was stirred at rt for 18 h, filtered and the filtrate washed with water (50 mL), brine (50 mL), dried over sodium sulfate and the solvent removed under reduced pressure. The residue was dissolved in THF (10 mL), and 4-formylphenylboronic acid (7.2 mmol) and Na_2CO_3 (2M aq., 2.93 mL) were added. The mixture was stirred under nitrogen for 10 min. $Pd(PPh_3)_4$ (138 mg) was added and the reaction refluxed at 85 °C for 6 h. The solution was cooled to 20 °C and acidified with HCl (2M). The aqueous layer was extracted with dichloromethane (3 × 30 mL) 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.

General Method III: Preparation of 8a-8d. Pyridinium tribromide (5.46 mmol, 90% grade) was added portionwise to a solution of **7a-7d** (3.64 mmol) in dichloromethane (10 mL) at 0 °C. The solution was warmed to rt and stirred for 18 h. $NaHCO_3$ (10%, 20 mL) was added and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers

were washed with water (2 × 20 mL), and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography.

General method IV: Preparation of 9a-9c, 18a-18b and 21. To a solution of **8a-8c**, **17a**, **17b** or **20** (2.63 mmol) in THF (20 mL) was added 4-hydroxyphenylboronic acid (3.94 mmol), sodium carbonate (2M, 6.58 mL, 13.2 mmol), and $Pd(PPh_3)_4$ (0.05 g) and the solution refluxed at 90 °C for 22 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.

General Method V. Preparation of 10a-10c. A solution of **9a-9c** (2.05 mmol) and (ethoxycarbonylmethylene)triphenylphosphorane (2.67 mmol) in dry CH_2Cl_2 (20 mL) was refluxed for 6 h. The solution was cooled to rt and the organic layer diluted with dichloromethane (20 mL), washed with water (30 mL) and brine (30 mL), dried over sodium sulfate and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, 5% ethyl acetate/95% hexane) to remove triphenylphosphine oxide.

General Method VI: Preparation of 11a-11c. To a solution of **10a-10c** (1.64 mmol) in methanol (10 mL) was added NaOH (2M aq., 10 mL) and the mixture was refluxed for 3 h, cooled to rt and then acidified with conc. HCl. The aqueous layer was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were washed with water, brine, dried over sodium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 5% ethyl acetate/hexane).

General Method VII: Preparation of 13a-13d. A solution of the appropriate bromoketone or bromoester (10 mmol) and triethylphosphite (11.4 mmol) was refluxed at 120-130 °C for 2 h.

The product was isolated as a colourless oil (100%) and used in the following reaction. To a suspension of sodium hydride (1.5 mmol, 60% dispersion) in THF (10 mL) at 0 °C was added the appropriate phosphorane or triphenylphosphonium salt (1.5 mmol) and the solution stirred for 10 min. Compound **9c** (1.0 mmol) was added to this mixture at 0 °C and the solution warmed to room temperature and stirred overnight. Water (30 mL) was added and the aqueous layer extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate and the solvent removed under reduced pressure.

General Method VIII: Synthesis of 14a-14h. To a solution of **11c** (0.62 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added HOBt (1.10 mmol), EDCI (1.10 mmol) and triethylamine (1.36 mmol) and the mixture was stirred for 10 min. The appropriate amine (1.10 mmol) was added and the mixture stirred overnight at rt. Water (30 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate and the solvent removed under reduced pressure.

General Method IX: Preparation of 13e, 19a, 19b, and 22. A solution of **13d**, **18a**, **18b** or **21** (100 mg) was refluxed in ethanol (5 mL) with NaOH (aq., 1M, 2 mL) for 1 h. The solution was cooled, acidified and the aqueous layer extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent removed under reduced pressure. The product was purified by flash column chromatography (1:1 ethyl acetate/hexane).

***(E)*-3-{4-[(*E/Z*)-1-(4-Hydroxyphenyl)-2-phenylbut-1-enyl]phenyl}acrylic acid (4b)**

Method 1: A solution of acid **25** (0.35 mmol, 1 eq.) was stirred in anhydrous DCM (5 mL) and the reaction mixture was cooled to -78 °C. Boron tribromide solution (1.0 M, 1.40 mmol, 4 eq)

was added slowly to the reaction mixture. The reaction was stirred at -78 °C for 45 min then at rt for a further three h. The reaction was quenched by addition of methanol (5 mL). The mixture was evaporated to dryness *in vacuo* and the residue was purified *via* flash chromatography (DCM:MeOH) to afford **4b** as a resin (60%).[28] *Method 2:* A solution of the ester **26** (0.38 mmol) was stirred in anhydrous DCM (5 mL) and the reaction mixture was cooled to -78 °C. Boron tribromide solution (1.0 M, 1.52 mmol, 4 eq.) was added slowly to the solution. The reaction was stirred at -78 °C for 45 min then at rt for a further three h. The reaction was quenched by addition of methanol (5 mL). The mixture was evaporated to dryness *in vacuo*. The residual product (**27**) was dissolved in THF (10 mL) and 0.1 M NaOH solution was added. The mixture was refluxed for 1 h., then diluted with ethyl acetate and the solution was acidified with HCl (10 %). The aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was purified *via* flash chromatography (DCM:MeOH) to afford **4b** as a resin (50%, *Z/E* = 3.5/1). IR: ν_{max} (KBr) cm^{-1}: 3396, 3189, 2965, 1685, 1635, 1603, 1511, 1441. ^1H NMR ($CDCl_3$): δ 10.84 (1H, bs), 7.56 - 7.60 (1H d, *J* = 16 Hz), 6.56 – 7.38 (13H, m), 6.42 – 6.48 (1H, d, *J* = 16 Hz), 3.02 (1H, bs), 2.53 – 2.59 (2H, m), 0.96 – 1.00 (3H, m). ^{13}C NMR ($CDCl_3$): δ 167.7, 157.2, 146.7, 145.1, 143.4, 143.0, 139.1, 134.9, 132.7, 132.6, 132.0, 130.7, 130.4, 128.9, 128.7, 128.6, 128.0, 127.1, 126.9, 118.8, 118.3, 115.9, 115.2, 30.3, 29.6, 13.7. HRMS (ESI): Found 369.1490 $(M–H)^+$, $C_{25}H_{21}O_3$ requires 369.1491.

4-(2,3-Dihydrobenzo[b]oxepin-5-yl)benzaldehyde (7a) was prepared from **6a** by general method II to give a yellow oil, which was used without further purification (83%). ^1H NMR ($CDCl_3$): δ 10.03 (1H, s), 7.97 (2H, d, *J* = 8.04 Hz), 7.41 (2H, d, *J* = 8.04 Hz), 7.21 (1H, t, *J* =

7.78 Hz), 7.09 (1H, d, *J* = 8.00 Hz), 6.91 (1H, t, *J* = 7.54 Hz), 6.67 (1H, d, *J* = 7.52 Hz), 5.28 (1H, s), 4.56 (2H, t, *J* = 5.76 Hz), 3.06 (2H, t, *J* = 5.78 Hz)

4-(8-Methoxy-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (7b) was prepared from **6b** by general method II. The product was purified by flash column chromatography (silica gel, 5% diethyl ether/hexane) to give the product as a yellow oil (40%) which was used without further purification in the following reaction. ^{1}H NMR ($CDCl_3$): δ 10.03 (1H, s), 7.86 (2H, d, *J* = 8.00 Hz), 7.46 (2H, d, *J* = 8.00 Hz), 6.81 (1H, d, *J* = 8.52 Hz), 6.69 (1H, d, *J* = 2.48 Hz), 6.59 (1H, d,d, *J* = 2.50 Hz, 6.04 Hz), 6.27 (1H, t, *J* = 6.02 Hz, CH), 4.50 (2H, t, *J* = 5.78 Hz, OCH_2), 3.82 (3H, s, OCH_3), 2.59 (2H, q, *J* = 6.01 Hz, OCH_2CH_2); ^{13}C NMR ($CDCl_3$): δ 191.5, 159.6, 158.9, 149.1, 139.7, 134.7, 131.3, 129.3, 128.8, 128.5, 123.1, 109.1, 106.5, 76.0, 54.9, 30.3.

4-(8-Fluoro-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (7c) was prepared from **6c** by general method II. The product was purified by flash column chromatography (silica, 2.5% methanol/dichloromethane) to give the product as yellow oil (80%). IR ν_{max}(KBr): 1698, 1601, 1151 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 10.05 (1H, s), 7.91 (2H, d, *J* = 8 Hz), 7.40 (2H, d, *J* = 8 Hz), 6.82 (1H, d, *J* = 8 Hz), 6.65 (2H, d, *J* = 8 Hz), 4.60 (2H, t, *J* = 6.0 Hz), 3.10 (2H, t, *J* = 6.0 Hz); ^{13}C (101 MHz, $CDCl_3$): δ 191.5, 162.0 (J_F = 250 Hz), 158.8 (J_F = 12 Hz), 148.4, 139.5, 134.8, 131.6 (J_F = 10 Hz), 129.6, 129.4, 128.7, 127.1, 110.2 (J_F = 21 Hz), 109.0 (J_F = 23 Hz), 107.4 (J_F = 23 Hz), 76.8, 29.8; ^{19}F NMR ($CDCl_3$): δ -112.7. HRMS (ESI) Found 269.0987$(M+H)^+$; $C_{17}H_{14}O_2F$ requires 269.0978.

4-(8-Fluoro-2,3-dihydrobenzo[b]thiepin-5-yl)benzaldehyde (7d) was prepared from **6d** by general method II. The product was purified by flash column chromatography (silica gel, 5% diethyl ether/hexane) to give the product as a yellow solid (75%). Mp 140 °C; IR ν_{max}(KBr): 3420.3, 1695.9, 1600.8, 1211.2 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.99 (1H, s), 7.80 (2H, d, *J* = 8.52

Hz), 7.42 (1H, q (d,d), *J* = 2.52 Hz, 6.00 Hz), 7.36 (2H, d, *J* = 8.52 Hz), 7.02 – 6.98 (2H, m), 6.69 (1H, t, *J* = 7.78 Hz), 3.49 (2H, t, *J* = 6.52 Hz), 2.35 (2H, q, *J* = 7.03 Hz); ^{13}C NMR ($CDCl_3$): δ 191.4, 161.9, 147.2, 142.0, 139.8, 136.4, 134.9, 131.7, 131.6, 131.4, 129.4, 128.0, 121.3, 121.0, 115.2, 115.0; ^{19}F NMR ($CDCl_3$): δ -113.593; HRMS (ESI) Found 283.0584$(M-H)^+$; $C_{17}H_{12}FOS$ requires 283.0593.

4-(4-Bromo-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (8a) was prepared from **7a** by general method III. The crude product purified by flash column chromatography (silica gel, 5% diethyl ether/hexane) to give **8a** as a brown oil (74%) which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 10.01 (1H, s), 7.89 (2H, d, *J* = 8.0 Hz), 7.40 (2H, d, *J* = 8.0 Hz), 7.22-7.13 (2H, m), 6.90 (1H, d, *J* = 7.5 Hz), 6.66 (1H, d, *J* = 7.5 Hz), 4.54 (2H, t, *J* = 6.0 Hz), 3.05 (2H, t, *J* = 5.8 Hz).

4-(4-Bromo-8-methoxy-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (8b) was prepared from **7b** by general method III. The crude product was obtained as a dark brown solid (93%) which was used without further purification; Mp 94 °C; ^{1}H NMR ($CDCl_3$): δ 10.06 (1H, s), 7.93 (2H, d, *J* = 8.0 Hz), 7.42 (2H, d, *J* = 8.0 Hz), 6.65 (1H, d, *J* = 2.5 Hz), 6.57 (1H, d, *J* = 9.0 Hz), 6.50 (1H, dd, *J* = 2.5 Hz, 6.5 Hz), 4.57 (2H, t, *J* = 5.7 Hz), 3.79 (3H, s), 3.15 (2H, t, *J* = 5.7 Hz); ^{13}C NMR ($CDCl_3$): δ 191.4, 159.8, 157.8, 148.7, 137.9, 134.8, 131.8, 130.2, 129.3, 123.3, 121.6, 109.3, 106.5, 75.3, 54.9, 41.7.

4-(4-Bromo-8-fluoro-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (8c) was prepared from **7c** by general method III. The crude product was purified by flash column chromatography (silica, 5% diethyl ether/hexane) to give **8c** as a white solid (91%). Mp 96 °C; IR ν_{max} (KBr): 1698, 1601, 1151 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 10.05 (1H, s), 7.91 (2H, d, *J* = 8.5 Hz), 7.40 (2H, d, *J* = 8.0 Hz), 6.82 (1H, m), 6.65 (2H, q, *J* = 3.2 Hz), 4.60 (2H, t, *J* = 6.0 Hz), 3.10 (2H, t, *J* = 6.0

Hz); ^{13}C NMR ($CDCl_3$): δ 191.4, 162.1 (d, J_F = 251 Hz), 157.5 (d, J_F = 10 Hz), 148.1, 137.6, 134.9, 132.0, 129.8, 127.5 (J_F = 10 Hz) 122.9, 110.4 (J_F = 20 Hz), 109.2, 108.9, 76.2, 41.2; ^{19}F NMR ($CDCl_3$): δ -111.71. HRMS (ESI): Found; 347.0076 $(M+H)^+$; $C_{17}H_{13}O_2BrF$ requires 347.0083.

4-(4-Bromo-8-fluoro-2,3-dihydrobenzo[b]thiepin-5-yl)benzaldehyde (8d) was prepared from **7d** by general method III. The crude product was purified by chromatography (silica gel, 10% diethyl ether/hexane) to give 8d as a yellow oil (76%). ^{1}H NMR ($CDCl_3$): δ 10.02 (1H, s), 7.86 (2H, d, *J* = 8.5 Hz), 7.42-7.38 (3H, m), 6.95-6.91 (1H, m), 6.84 (1H, q (d,d), *J* = 5.5 Hz, 3.0 Hz), 3.49 (2H, t, *J* = 6.5 Hz), 2.88 (2H, t, *J* = 6.3 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ: 191.3, 162.0, 147.2, 140.8, 140.7, 135.1, 135.0, 134.9, 131.7, 131.6, 130.0, 129.2, 124.8, 121.6, 121.3, 115.4, 115.2; ^{19}F ($CDCl_3$): -112.599 ppm; HRMS (ESI): Found; 362.9877$(M+H)^+$; $C_{17}H_{13}OFSBr$ requires 362.9855.

4-(4-(4-Hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (9a) was prepared from **8a** by general method IV. The crude product was purified by flash column chromatography (silica gel, 5% diethyl ether/hexane) to give **9a** as a bright yellow powder (85%). Mp 164 °C; which was used without further purification in the subsequent reaction. IR ν_{max}(KBr): 3334, 1675, 1610, 1598, 1190, 1169 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.90 (1H, s), 7.67 (2H, d, *J* = 8.0 Hz), 7.23-7.17 (4H, m), 7.03 (2H, d, *J* = 8.0 Hz), 6.88 (2H, d, *J* = 8.5 Hz), 6.82 (1H, d, *J* = 7.6 Hz), 6.70 (2H, d, *J* = 8.5 Hz), 4.66 (2H, t, *J* = 6.0), 3.82 (2H, t, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$): δ 192.3, 163.6, 161.1, 157.1, 155.1, 148.5, 140.5, 135.0, 134.2, 133.3, 131.7, 131.6, 130.8, 129.3, 115.2, 111.1, 110.9, 109.8, 109.6, 80.6, 35.8.

4-(4-(4-Hydroxyphenyl)-8-methoxy-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (9b) was prepared from **8b** by general method IV. The crude product was purified by flash column

chromatography (silica gel, 50% diethyl ether/hexane) to give **9b** as a yellow solid (54 %). IR ν_{max} (KBr) 3394, 1691, 1598, 1215, 1144 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.75-7.67 (2H, m), 7.57 (2 H, d, *J* = 8.6 Hz), 7.55-7.48 (1H, m), 7.26 (2H, d, *J* = 8.0 Hz), 6.65 (1H, d, *J* = 2.5 Hz), 6.62 (1H, s), 6.52-6.46 (2H, m), 4.56 (2H, t, *J* = 5.8 Hz), 3.79 (3H, s), 3.11 (2H, t, *J* = 5.8 Hz); ^{13}C NMR ($CDCl_3$): δ 192.2, 160.0, 157.2, 154.7, 148.8, 139.4, 135.8, 134.2, 134.0, 132.1, 131.4, 130.9, 129.2, 128.4, 115.1, 110.0, 107.4, 80.2, 55.4, 36.1; HRMS (ESI): Found 395.1265$(M+Na)^+$ $C_{24}H_{20}O_4Na$ requires 395.1259.

4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)benzaldehyde (9c) was prepared from **8c** by general method IV. The crude product was purified by flash column chromatography (silica gel, 10% diethyl ether/hexane) to give **9c** as a yellow solid (100%). Mp 141 °C; IR ν_{max}(KBr): 3394, 1691, 1598, 1215, 1144 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.89 (1H, s), 7.64 (2H, d, *J* = 8.5 Hz), 7.14 (2 H, d, *J* = 8.0 Hz), 6.98 (2H, d, *J* = 8.6 Hz), 6.88 (1H, d, *J* = 9.0 Hz), 6.75 (2H, d, *J* = 7.0 Hz), 6.66 (2H, d, *J* = 8.5 Hz), 4.65 (2H, t, *J* = 6.0 Hz), 2.73 (2H, t, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 191.9, 163.2, 160.7, 156.6, 154.7, 148.1, 140.1, 134.6, 133.8, 132.9, 131.6, 131.3, 130.4, 128.9, 114.8, 110.7, 110.5, 109.4, 109.2, 80.2, 35.4; ^{19}F NMR ($CDCl_3$): δ -112.59. HRMS (ESI): Found 359.1086$(M-H)^+$; $C_{23}H_{16}O_3F$ requires 359.1083.

Ethyl (*E*)-3-(4-(4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylate (10a) was prepared from **9a** by general method V. The crude product was used in the next reaction without any further purification (80%). ^1H NMR ($CDCl_3$): δ 7.61 (1H, d, *J* = 16.0 Hz), 7.27-7.22 (3H, m), 7.14 (1 H, d, *J* = 7.5 Hz), 6.99 (5H, t, *J* = 8.0 Hz), 6.82 (1H, d, *J* = 5.5 Hz), 6.65 (2H, d, *J* = 8.6 Hz), 6.37 (1H, d, *J* = 16.1 Hz), 4.63 (2H, t, *J* = 6.0 Hz), 4.24 (2H, q, *J* = 7.2 Hz), 2.69 (2H, t, *J* = 6.0 Hz), 1.32 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$): δ 166.9, 155.5, 154.4,

144.1, 143.6, 138.9, 136.3, 135.7, 133.6, 131.8, 131.5, 130.4, 128.9, 128.1, 127.1, 123.3, 121.7, 117.1, 114.6, 80.0, 60.1, 35.4, 13.9.

Ethyl (*E*)-3-(4-(4-(4-hydroxyphenyl)-8-methoxy-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylate (10b) was prepared from **9b** by general method V as a yellow solid (96%). Mp 198 °C; IR ν_{max} (KBr): 3033, 1681, 1602, 1219, 1145 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.60 (1H, d, *J* = 15.6 Hz), 7.28 (2H, t, *J* = 4.3 Hz), 7.03 (4H, q, *J* = 4.0 Hz), 3.79-6.71 (2H, m), 6.67 (2H, d, *J* = 8.5 Hz), 6.63 (1H, dd, *J* = 2.5 Hz, 6.0 Hz), 4.64 (2H, t, *J* = 5.8 Hz), 4.28 (2H, q, *J* = 7.0 Hz), 3.84 (3H, s), 2.74 (2H, t, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 171.5, 154.4, 147.0, 142.8, 141.3, 138.9, 135.6, 134.2, 133.8, 131.8, 130.8, 126.8, 125.7, 119.7, 115.0, 110.9, 109.7, 80.7, 59.8, 55.1, 35.7, 15.1; HRMS (ESI): Found 465.1697 $(M+Na)^+$; $C_{28}H_{26}O_5Na$ requires 465.1678.

Ethyl (*E*)-3-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylate (10c) was prepared from **9c** by general method V as a yellow solid (98%); Mp 146 °C. ^1H NMR ($CDCl_3$): δ 7.62 (1H, d, *J* = 16.1 Hz), 7.29 (2H, d, *J* = 8.4 Hz), 7.02-6.97 (4H, m), 6.88 (1H, q(dd), *J* = 2.6 Hz, 6.9 Hz), 6.82-6.73 (2H, m), 6.65 (2H, d, *J* = 8.8 Hz), 6.37 (1H, d, *J* = 15.7 Hz), 4.64 (2H, t, *J* = 6.0 Hz), 4.27 (3H, q, *J* = 7.1 Hz), 2.73 (2H, t, *J* = 6.0 Hz), 1.34 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$): δ 167.4, 162.3 (d, J_F = 241 Hz), 157.1 (J_F = 10 Hz), 154.7, 144.4, 139.2, 134.0, 132.4, 131.8, 131.7, 131.0, 130.8, 129.6, 127.60, 119.3, 117.1, 115.1, 110.9 (d, J_F = 20 Hz), 109.6 (d, J_F = 20 Hz), 80.7, 60.6, 35.8, 14.3; ^{19}F NMR ($CDCl_3$): δ -112.556. HRMS (ESI): Found 431.1648$(M+H)^+$; $C_{27}H_{24}O_4F$ requires 431.1659.

(*E*)-3-(4-(4-(4-Hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylic acid (11a) was prepared from **10a** by general method VI as bright yellow crystals (86%); Mp 239 °C; IR ν_{max}(KBr): 3393, 1677, 1631, 1211 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.64 (1H, d, *J* = 16.08 Hz), 7.25 (2H, d, *J* = 8.04 Hz), 7.18 (2 H, d, *J* = 7.5 Hz), 7.08 (1H, d,d, *J* = 1.0 Hz, 7.0 Hz), 6.98 (4H, m),

6.76 (1H, dd, *J* = 1.5 Hz, 6.0 Hz), 6.59 (2H, d, *J* = 8.5 Hz), 6.31 (1H, d, *J* = 16.0 Hz), 4.56 (2H, t, *J* = 6.1 Hz), 2.64 (2H, t, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 171.6, 157.1, 154.4, 146.8, 144.8, 138.3, 136.0, 134.4, 132.0, 131.9, 131.5, 130.9, 128.7, 127.8, 116.5, 115.1, 109.9, 107.3, 80.4, 36.0; HRMS (ESI) Found 407.1253$(M+Na)^+$; $C_{25}H_{20}O_4Na$ requires 407.1259.

(*E*)-3-(4-(4-(4-Hydroxyphenyl)-8-methoxy-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylic acid (11b) was prepared from **10b** by general method VI as an orange powder (83%); Mp 125 °C; IR ν_{max} (KBr): 3033, 1681, 1602, 1219, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.72 (1H, d, *J* = 15.8 Hz), 7.32 (2H, d, *J* = 8.0 Hz), 7.04 (4H, d, *J* = 8.3 Hz), 6.75 (2H, d, *J* = 9.0 Hz), 6.73-6.60 (3H, m), 6.39 (2H, d, *J* = 15.8 Hz), 4.64 (2H, t, *J* = 6.0 Hz), 3.85 (3H, s), 2.75 (2H, t, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 171.6, 159.9, 157.1, 154.4, 146.8, 144.8, 138.3, 136.0, 134.4, 132.0, 131.9, 131.5, 130.9, 128.7, 127.8, 116.5, 115.1, 109.9, 107.3, 80.4, 55.4, 36.0; HRMS (ESI) Found 437.1354$(M+Na)^+$; $C_{26}H_{22}O_5Na$ requires 437.1365.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylic acid (11c) was prepared from **10c** by general method VI to give a pale yellow solid (96%); Mp 130 °C; IR ν_{max} (KBr): 3033, 1681, 1602, 1219, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.70 (1H, d, *J* = 16.6 Hz), 7.32 (2H, d, *J* = 8.8 Hz), 7.03 (4H, m), 6.88 (1H, q(d,d), *J* = 2.4 Hz, 6.8 Hz), 6.74 (2H, m), 6.65 (2H, d, *J* = 8.8 Hz), 6.37 (1H, d, *J* = 16.6 Hz), 4.63 (2H, t, *J* = 5.9 Hz), 2.62 (2H, t, *J* = 5.9 Hz); ^{13}C NMR ($CDCl_3$): δ 171.5, 154.4, 147.0, 142.8, 141.3, 138.9, 135.6, 134.2, 133.8, 131.8, 130.8, 126.8, 125.7, 119.7, 115.0, 110.9, 109.7, 80.7, 35.7; ^{19}F NMR ($CDCl_3$): δ -112.939; HRMS (ESI): Found 425.1180 $(M+Na)^+$; $C_{25}H_{19}O_4FNa$ requires 425.1165.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]thiepin-5-yl)phenyl)acrylic acid (11d) was prepared from **12** by general method IV as a yellow solid (100%) and was used in the following reaction without further characterization. IR ν_{max} (KBr): 3402, 1606, 1571,

1246, 1176 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.49-7.43 (4H, m), 7.18-7.14 (1H, m), 6.99 (2H, d, *J* = 8.5 Hz), 6.87 (1H, q, (d,d), *J* = 6.0 Hz, 2.5 Hz), 6.83 (2H, d, *J* = 8.5 Hz), 6.59 (2H, d, *J* = 8.5 Hz), 6.41 (1H, d, *J* = 16.1 Hz), 3.40 (2H, m), 2.58 (2H, m); ^{13}C NMR ($CDCl_3$): δ 167.6, 161.5, 156.5, 144.0, 143.3, 142.6, 135.8, 132.7, 132.6, 132.1, 131.3, 131.1, 130.5, 127.7, 118.9, 116.0, 115.0; ^{19}F NMR ($CDCl_3$): δ -114.444.

(*E*)-3-(4-(4-Bromo-8-fluoro-2,3-dihydrobenzo[b]thiepin-5-yl)phenyl)acrylic acid (12). To a solution of **8d** in dry dichloromethane (10 mL) was added (ethoxycarbonylmethylene)triphenylphosphorane (2.03 mmol) and the mixture was refluxed for 6 h. The solution was cooled to rt and the organic layer diluted with dichloromethane and washed with water, brine, dried over sodium sulfate and the solvent removed under reduced pressure. The crude ester product (*E*)-3-[4-(4-bromo-8-fluoro-2,3-dihydro-1-benzothiepin-5-yl)-phenyl]-acrylic acid ethyl ester [(92% yield), 1.15 mmol] was then treated with NaOH (11.5 mmol) in ethanol (2 mL) and refluxed for 3 h. The aqueous layer was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were washed with water, brine, dried over sodium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 10% ethyl acetate/hexane) to give the product as an off-white solid (27%), which was used directly in the subsequent reaction without further purification. IR ν_{max} (KBr): 3454, 2903, 2855, 1682, 1629, 12112 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.79 (1H, d, *J* = 15.8 Hz), 7.54 (2H, d, *J* = 8.2 Hz), 7.40 (1H, dd, *J* = 2.6 Hz, 5.8 Hz), 7.29 (2H, d, *J* = 8.2 Hz), 6.97-6.88 (2H, m), 6.47 (1H, d, *J* = 15.8 Hz), 3.66 (2H, t, *J* = 6.4 Hz), 2.89 (2H, t, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$): δ 171.9, 161.9, 146.0, 143.5, 141.1, 141.0, 139.9, 135.1, 132.9, 131.8, 131.7, 129.8, 127.7, 124.2, 121.4, 121.2. 117.2, 115.3, 115.1; ^{19}F NMR ($CDCl_3$): δ -112.917.

(*E*)-Ethyl 4-(diethoxyphosphoryl)but-2-enoate. A solution of ethyl (*E*)-4-bromobut-2-enoate (10 mmol) and triethylphosphite (11.4 mmol) was refluxed at 120-130 °C for 2 h. The product was obtained as a colourless oil (100%). ^{1}H NMR ($CDCl_3$): δ 6.77 (1H, m), 5.88 (1H, dd,), 4.15-3.86 (6H, m), 2.62 (2H, m), 1.22 (9H, m); ^{13}C NMR ($CDCl_3$): δ 165.3, 137.2, 125.5, 62.0, 60.2, 30.9, 29.5, 16.1, 13.9; ^{31}P NMR ($CDCl_3$): δ 25.580; HRMS (ESI): Found 273.0860 $(M+Na)^+$; $C_{10}H_{19}O_5NaP$ requires 273.0868.

(*E*)-4-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)but-3-en-2-one (13a) was prepared from diethyl (2-oxopropyl)phosphonateand **9c** by general method VII. The residue was purified by flash column chromatography (silica gel, 20% ethyl acetate/hexane) to give the product as a yellow solid (50%); Mp 188 °C; IR ν_{max}(KBr): 3250, 1634, 1599, 1270 cm^{-1}; ^{1}H (*d*-DMSO): δ 7.48 (1H, d, *J* = 16.6 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.04-7.00 (4H, m), 6.87 (1H, d, *J* = 9.5 Hz), 6.82-6.65 (5H, m), 4.65 (2H, t, *J* = 5.8 Hz), 2.74 (2H, t, *J* = 5.8 Hz), 2.38 (3H, s); ^{13}C NMR ($CDCl_3$): δ 198.5, 163.1, 160.6, 156.7, 154.4, 143.8, 143.2, 139.0, 134.9, 133.4, 131.9, 131.5, 131.4, 131.3, 130.4, 127.4, 126.2, 114.7, 110.6, 110.4, 109.3, 109.1, 80.3, 35.3, 27.0; ^{19}F NMR ($CDCl_3$): δ -112.976. HRMS (ESI): Found 401.1537$(M+H)^+$; $C_{26}H_{22}FO_3$ requires 401.1553.

(*E*)-4-(8-Fluoro-5-(4-styrylphenyl)-2,3-dihydrobenzo[b]oxepin-4-yl)phenol (13b) was prepared from **9c** by general method VII. To a solution of **9c** (250 μmol) in benzene (4 mL) was added benzyltriphenylphosphonium bromide 250 μmol) and benzoic acid (250 μmol) and the solution was refluxed for 5 h and then cooled to rt. The crude residue was purified by flash column chromatography (silica gel, 15% ethyl acetate in hexane) to give the product as a brown gel (65%). IR ν_{max} (film): 3454, 2918, 2845, 1609, 1581 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.51 (1H, d, *J* = 7.6 Hz), 7.37 (1H, t, *J* = 7.54 Hz), 7.31 (1H, s), 7.22 (3H, s), 7.03-7.08 (4H, m), 6.98 (1H, d,

J = 8.0 Hz), 6.88-6.83 (3H, m), 6.79 (1H, t,d, *J* = 2.2 Hz, 6.0 Hz), 6.68 (2H, d, *J* = 8.0 Hz), 6.55 (1H, d, *J* = 2.0 Hz), 4.86 (1H, bs, OH), 4.64 (2H, t, *J* = 6.0 Hz), 2.72 (2H, t, *J* = 6.3 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 153.8, 139.9, 137.8, 136.7, 135.6, 134.9, 134.1, 131.5, 131.4, 131.2, 130.7, 130.4, 129.7, 129.4, 128.4, 128.2, 127.8, 127.6, 127.1, 126.6, 125.5, 114.5, 114.4, 110.4, 110.2, 109.1, 108.9, 80.3, 35.2; ^{19}F NMR ($CDCl_3$): δ -113.461; HRMS (ESI) Found 433.1596$(M-H)^+$; $C_{30}H_{22}FO_2$ requires 433.1604.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-1-phenylprop-2-en-1-one (13c) was prepared from **9c** by general method VII. To a solution of **9c** (90 mg, 250 μmol) in benzene (4 mL) was added 1-phenyl-2-(triphenylphosphoranylidene)ethanone (95 mg, 250 μmol) and benzoic acid (30.5 mg, 250 μmol) and the solution was refluxed for 5 h and then cooled to rt. The crude residue was purified by flash column chromatography (15% ethyl acetate in hexane) to give the product as a yellow solid (67%); Mp 201°C; IR ν_{max} (KBr): 3256, 1650, 1576, 1597, 1588, 1217 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.98 (2H, d, *J* = 8.5 Hz), 7.72 (2H, d, *J* = 16.1 Hz), 7.59 (2H, m), 7.56 – 7.49 (2H, m), 7.39 (2H, d, *J* = 8.0 Hz), 7.02 – 6.97 (4H, m), 6.87 (1H, dd, *J* = 9.5 Hz, 2.5 Hz), 6.82 – 6.73 (2H, m), 6.67 (2H, d, *J* = 8.5 Hz), 4.64 (2H, t, *J* = 6.0 Hz), 2.72 (2H, t, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$): δ 192.1, 190.8, 163.5, 161.0, 157.1, 156.9, 154.8, 144.8, 144.3, 139.4, 138.1, 135.4, 133.9, 132.8, 132.5, 131.8, 131.6, 130.8, 129.3, 128.5, 128.0, 121.5, 115.0, 111.0, 109.7, 109.5, 80.7, 35.8; ^{19}F NMR ($CDCl_3$): δ -112.953; HRMS (ESI) Found 485.1546$(M+Na)^+$; $C_{31}H_{23}FO_3Na$ requires 485.1529.

Ethyl (2*E*,4*E*)-5-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)penta-2,4-dienoate (13d) was prepared from **9c** and (*E*)-ethyl 4-(diethoxyphosphoryl)but-2-enoate, generated from ethyl 4-bromocrotonate and triethylphosphite,

by general method VII as yellow crystals (96%); Mp 88 °C; IR ν_{max}(KBr): 3436, 1682, 1623, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.44 (1H, m), 7.24 (2H, d, *J* = 8.5 Hz), 7.04 (2 H, d, *J* = 8.5 Hz), 6.98 (2H, d, *J* = 8.0 Hz), 6.89 (1H, q (dd), *J* = 2.5 Hz, 7.0 Hz), 6.80 (3H, m), 6.77 (1H, q (dd), *J* = 2.5 Hz, 5.5 Hz) 6.69 (2H, d, *J* = 8.5 Hz), 5.98 (1H, d, *J* = 15.6 Hz), 4.66 (2H, t, *J* = 6.0 Hz), 4.26 (2H, *J* = 5.9 Hz), 2.74 (2H, t, *J* = 6.0 Hz), 1.33 (3H, t, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 167.2, 161.5, 160.0, 154.4, 144.6, 142.5, 142.0, 138.8, 135.7, 134.3, 134.1, 132.7, 131.8, 130.8, 126.7, 126.0, 121.0, 115.2, 110.9, 110.8, 109.7, 109.5, 80.7, 60.4, 35.8, 14.3; ^{19}F NMR ($CDCl_3$): δ -113.094. HRMS (ESI): Found 457.1821$(M+H)^+$; $C_{29}H_{26}O_4F$ requires 457.1815.

(2*E*,4*E*)-5-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)penta-2,4-dienoic acid (13e) was prepared from **13d** by general method IX as a yellow solid (86%); Mp 156 °C; IR ν_{max}(KBr): 3436, 1684, 1621, 1513 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.52 (1H, ddd, *J* =15.3, 8.5, 1.9 Hz), 7.26 (2H, d, *J* = 8.0 Hz), 7.04 (2 H, d, *J* = 8.5 Hz), 6.98 (2H, d, *J* = 8.5 Hz), 6.90 (1H, q (dd), *J* = 2.5 Hz, 7.0 Hz), 6.82 (3H, m), 6.77 (1H, q (dd), *J* = 2.5 Hz, 6.0 Hz) 6.67 (2H, d, *J* = 8.5 Hz), 5.98 (1H, d, *J* = 15.5 Hz), 4.65 (2H, t, *J* = 5.8 Hz), 2.74 (2H, t, *J* = 5.8 Hz); ^{13}C NMR ($CDCl_3$): δ 170.9, 161.3, 157.0, 154.3, 146.8, 142.7, 141.2, 138.8, 135.5, 134.2, 133.8, 132.4, 131.8, 131.7, 130.7, 129.1, 126.7, 125.6, 119.5, 114.9, 110.8, 110.6, 109.5, 109.3, 80.5, 35.6; ^{19}F NMR ($CDCl_3$): δ -113. HRMS (ESI): Found 427.1352$(M-H)^+$ $C_{27}H_{20}FO_4$ requires 427.1346.

(*E*)-N,N-Diethyl-3-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylamide (14a) was prepared from diethylamine by general method VIII.

The residue was purified by flash column chromatography (silica gel, 5% ethyl acetate/hexane) to give **14a** as a yellow solid (60%); Mp 239 °C; IR ν_{max} (KBr): 3379, 1685, 1599, 1510, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.99 (1H, s), 7.55 (1H, d, *J* = 15.5 Hz), 7.18 (2H, d, *J* = 8.0 Hz), 7.02-

6.90 (4H, m,), 6.86 (1H, q(d,d), *J* = 2.5 Hz, 7.00 Hz), 6.80-6.69 (5H, m) 4.63 (2H, t, *J* =6.0 Hz), 3.47 (4H, m), 2.70 (2H, t, *J* = 6.0 Hz), 1.25 (3H, t, *J* = 7.0 Hz), 1.17 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$): δ 166.3, 163.4, 157.0, 156.9, 155.9, 143.4, 142.8, 139.4, 134.9, 133.0, 132.9, 132.7, 131.8, 131.7, 131.4, 130.6, 127.3, 116.6, 115.3, 110.9, 110.7, 109.6, 109.4, 80.8, 42.5, 41.3, 35.7, 14.9, 13.1; ^{19}F NMR ($CDCl_3$): δ -113.328. HRMS (ESI) Found 458.2109$(M+H)^+$; $C_{29}H_{29}FNO_3$ requires 458.2131.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-1-(pyrrolidin-1-yl)prop-2-en-1-one (14b) was prepared from pyrrolidine by general method VIII. The residue was purified by flash column chromatography (silica, 5% ethyl acetate/hexane) to give **14b** as a yellow solid (61%); Mp 140 °C. IR ν_{max}(KBr): 3405, 1646, 1578, 1215, 1143 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.57 (1H, d, *J* = 15.2 Hz), 7.25 (2H, d, *J* = 8.2 Hz), 6.99 (4H, q (d,d) *J* = 2.1 Hz, 6.40 Hz), 6.87 (1H, q (d,d), *J* = 2.6 Hz, 7.0 Hz), 6.72- 6.81 (4H, m), 6.67 (1H, d, 15.2 Hz), 4.63 (2H, t, *J* = 5.8 Hz), 3.63-3.57 (4H, m), 2.72 (2H, t, *J* = 5.8 Hz), 2.02 (2H, q, *J* = 6.6 Hz), 1.89 (2H, q, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$): δ 192.3, 163.3, 160.8, 157.0, 156.9, 156.1, 143.5, 142.2, 139.5, 134.8, 132.7, 131.7, 131.5, 130.6, 127.6, 127.3, 117.6, 115.3, 115.0, 110.8, 110.6, 109.6, 109.4, 80.8, 46.8, 46.2, 35.7, 25.9, 24.2; ^{19}F NMR ($CDCl_3$): δ -113.308; HRMS (ESI) Found 478.1811$(M+Na)^+$; $C_{29}H_{26}NO_3FNa$ requires 478.1794.

(*E*)-N-(2-(Diethylamino)ethyl)-3-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylamide (14c) was prepared from 2-diethylaminoethylamine by general method VIII. The residue was purified by flash column chromatography (silica gel, 10% methanol/dichloromethane) to give **14c** as a yellow oil (18%). IR ν_{max}(KBr): 3394 (OH and NH), 1657 (C=O), 1608, 1511 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.47 (1H, d, *J* = 15.6 Hz), 7.15 (2H, d, *J* = 8.5 Hz), 6.96 (4H, q (d,d) *J* = 8.3 Hz, 4.8 Hz), 6.88 (1H, q

(d,d), *J* = 2.3 Hz, 7.5 Hz), 6.82 - 6.71 (2H, m), 6.67 (2H, d, *J* = 8.5 Hz), 6.30 (2H, d, *J* = 15.5 Hz), 4.62 (2H, t, *J* = 6.0 Hz), 3.48 (2H, d, *J* = 5.0 Hz), 2.68 (8 H, m), 1.08 (6H, t, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 166.2, 160.5, 156.6, 156.5, 155.4, 142.9, 140.3, 138.9, 134.7, 132.6, 132.2, 131.4, 131.3, 130.3, 126.9, 119.6, 114.9, 110.5, 110.3, 109.2, 108.9, 80.4, 51.1, 46.3, 36.2, 35.3, 10.3; ^{19}F NMR ($CDCl_3$): δ -113.284. HRMS (ESI) Found 501.2541; $C_{31}H_{34}N_2O_3F(M+H)^+$ requires 501.2553.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-1-(piperazin-1-yl)prop-2-en-1-one (14d) was prepared from piperazine by general method VIII. The crude residue was purified by flash column chromatography (silica, 25% methanol/dichloromethane) to afford **14d** as a yellow solid (59%); Mp 188 °C; IR ν_{max}(KBr): 3392 (OH and NH), 1641 (C=O), 1603, 1509; ^{1}H NMR (CD_3OD): δ 7.53 (1H, d, *J* = 15.6 Hz), 7.46 (2H, d, *J* = 8.0 Hz), 7.15 (1H, d, *J* = 15.6 Hz), 7.01 – 6.96 (4H, q (d,d), *J* = 8.5 Hz), 6.91-6.79 (3H, m), 6.68 (2H, d, *J* = 8.5 Hz), 4.62 (2H, t, *J* = 5.8 Hz), 3.67 (3H, br d, *J* = 30.1 Hz), 2.74 (2H, t, *J* = 5.8 Hz), 2.08 (6H, m); ^{13}C NMR (CD_3OD): δ 164.1, 156.1, 142.8, 140.7, 139.5, 134.3, 133.3, 132.3, 131.4, 131.3, 131.1, 130.2, 126.9, 117.3, 114.5, 110.0, 109.8, 108.9, 108.6, 80.0, 35.2; ^{19}F NMR (CD_3OD): δ -115.054; HRMS (ESI): Found 493.1906 $(M+Na)^+$; $C_{29}H_{27}N_2O_3FNa$ requires 493.1903.

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-1-(4-methylpiperazin-1-yl)prop-2-en-1-one (14e) was prepared from methylpiperazine by general method VIII. The crude residue was purified by flash column chromatography (silica gel, 2.5% methanol/dichloromethane) to give **14e** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.55 (1H, d, *J* = 15.6 Hz), 7.22 (2H, d, *J* = 8.0 Hz), 6.97 (4H, m), 6.81 (5H, m), 6.65 (2H, d, *J* = 8.5 Hz), 4.61 (2H, t, *J* = 6.0 Hz), 3.72 (4H, br d), 2.72 (2H, t, *J* = 5.8 Hz), 2.46 (4H, m), 2.32 (3H, s); ^{13}C NMR

($CDCl_3$): δ 165.4, 162.9, 156.6, 155.3, 143.0, 142.6, 138.9, 134.7, 132.7, 132.5, 131.3, 130.30, 126.9, 117.2, 115.7, 114.8, 110.5, 110.3, 109.2, 109.0, 80.4, 54.7, 45.4, 45.2, 35.3; ^{19}F NMR ($CDCl_3$): δ -113.228. M^+ 485.3. HRMS (ESI): Found 485.2242 (M+H); $C_{30}H_{30}FN_2O_3$ requires 485.2240.

(*E*)-1-(4-Benzylpiperazin-1-yl)-3-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)prop-2-en-1-one (14f) was prepared from 1-benzylpiperazine by general method VIII. The crude residue was purified by flash column chromatography (silica gel, 10% methanol/dichloromethane) to give **14f** as a yellow solid (92%); Mp 130 °C; IR ν_{max}(KBr): 3240 (OH), 1643 (C=O), 1602, 1580, 1511, 1439 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.57 (1H, d, *J* = 15.6 Hz), 7.22 (2H, d, *J* = 8.0 Hz), 6.99 (4H, d, *J* = 6.5 Hz), 6.88 (1H, q (d,d), *J* = 2.3 Hz, 7.0 Hz), 6.83-6.75 (3H, m), 6.69 (2H, d, *J* = 8.5 Hz), 4.64 (2H, t, *J* = 5.8 Hz), 3.72 (4H, br d), 3.56 (2H, s), 2.73 (2H, t, *J* = 5.8 Hz), 2.50 (4H, t, *J* = 4.5 Hz), 2.27 (3H, s); ^{13}C NMR ($CDCl_3$): δ 165.4, 162.9, 156.6, 155.4, 143.0, 142.6, 139.0, 136.7, 134.6, 132.5, 132.5, 132.3, 131.4, 130.4, 130.3, 129.7, 128.8, 127.9, 126.9, 126.9, 116.4, 115.7, 114.9, 110.5, 110.3, 109.2, 109.0, 80.4, 62.4, 52.8, 45.4, 35.6; ^{19}F NMR ($CDCl_3$): δ -113.186; HRMS (ESI) Found 561.2528$(M+H)^+$; $C_{36}H_{34}FN_2O_3$ requires 561.2553

(*E*)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-1-(4-(4-methylphenyl)piperazin-1-yl)prop-2-en-1-one (14g) was prepared from *p*-tolylpiperazine dihydrochloride by general method VIII. The crude residue was purified by flash column chromatography (silica gel, 35% ethyl acetate/hexane) to give **14g** as a yellow solid (58%); Mp 160 °C; IR ν_{max}(KBr): 3234 (OH and NH), 1642 (C=O), 1600, 1579, 1222, 1143 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.57 (1H, d, *J* = 15.0 Hz), 7.21 (2H, d, *J* = 8.0 Hz), 7.09 (2H, d, *J* = 8.0 Hz), 6.97 (4H, m), 6.86 (6H, m), 6.69 (2H, d, *J* = 8.0 Hz), 4.62 (2H, t, *J* = 6.5 Hz), 3.82 (3H, br d), 3.13

(4H, s), 2.70 (2H, t, *J* = 6.5 Hz), 2.27 (3H, s); ^{13}C NMR ($CDCl_3$): δ 171.3, 165.9, 157.1, 155.6, 148.6, 143.5, 143.3, 139.4, 135.1, 133.2, 132.9, 131.8, 130.7, 130.3, 129.8, 127.3, 117.0, 115.9, 115.2, 110.9, 110.7, 109.6, 109.4, 80.8, 60.4, 50.4, 49.9, 45.9, 42.2, 35.7, 21.0, 20.4 ; ^{19}F NMR ($CDCl_3$): δ -113.168; HRMS (ESI): Found 561.2560$(M+H)^+$; $C_{36}H_{34}FN_2O_3$ requires 561.2553

(*E*)-N-Ethyl-3-(4-(8-fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)acrylamide (14h) was prepared from ethylamine hydrochloride by general method VIII. The crude residue was purified by flash column chromatography (silica gel, 10% methanol/dichloromethane) to give **14h** as a yellow solid (40%); Mp 251 °C; IR ν_{max}(film): 3279 cm^{-1}(OH and NH), 1650 (C=O), 1602 cm^{-1}; ^{1}H NMR (*d*-DMSO): δ 7.36 (2H, d, *J* = 7.6 Hz), 7.29 (1H, s), 7.00-6.97 (1H, q, *J* = 2.8 Hz, 7 Hz), 6.94-6.88 (5H, m), 6.76-6.72 (1H, t, *J* = 7.8 Hz) 6.60-6.58 (2H, d, *J* =8.5 Hz), 6.56-6.52 (1H, d, *J* = 15.6 Hz), 4.56-4.53 (2H, t, *J* = 5.8 Hz), 3.18 (2H, m), 2.66-2.63 (3H, t, *J* = 5.8 Hz), 1.08-1.04 (2H, t, *J* = 7.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 176.4, 164.7, 159.9, 156.4, 139.6, 137.8, 134.0, 132.9, 131.7, 131.3, 130.4, 127.1, 122.2, 118.2, 114.9, 111.3, 109.1, 81.0, 35.3, 33.5, 14.7; ^{19}F NMR ($CDCl_3$): δ -113.510; HRMS (ESI): Found 430.1805 $(M+H)^+$; $C_{27}H_{25}FNO_3$ requires 430.1818.

(E)-3-(4-(8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[b]oxepin-5-yl)phenyl)-N-phenylacrylamide (14i). To a suspension of sodium hydride (375 μmol) in THF (10 mL) at 0 °C was added **15** (375 μmol) and the solution was stirred for 30 min. The aldehyde **9c** (250 μmol) in THF (10 mL) was added and the solution was refluxed overnight. Water (30 mL) was added and the aqueous layer extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, 1:1 diethyl ether/hexane) to give the product as a solid (83%); Mp 235 °C; IR

ν_{max}(KBr): 3517, 1663, 1597, 1512, 1257, 1172 cm^{-1}; ^{1}H NMR (*d*-DMSO): δ 7.67 (2H, d, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 15.6 Hz), 7.43 (2H, d, *J* = 8.0 Hz), 7.33 (2H, t, *J* = 8.0 Hz), 7.08-6.92 (7 H, m), 6.79-6.74 (2H, m), 6.60 (2H, d, *J* = 8.5 Hz), 4.55 (2H, t, *J* = 5.5 Hz), 2.65 (2H, t, *J* = 5.8 Hz); ^{13}C NMR (*d*-DMSO): δ 163.5, 160.2, 156.9, 143.1, 139.8, 139.6, 139.3, 134.0, 132.7, 131.8, 131.5, 130.4, 128.8, 127.4, 123.3, 122.1, 119.2, 114.9, 111.0, 110.8, 109.5, 109.3, 80.4, 35.5; ^{19}F NMR ($CDCl_3$): δ -113.439; HRMS (ESI): Found 500.1645$(M+Na)^+$ $C_{31}H_{24}NO_3FNa$ requires 500.1638.

Phenylcarbamoylmethylphosphonic acid diethyl ester (15). Bromoacetyl bromide (10 mmol) in diethyl ether (9 mL) was added dropwise to a solution of aniline (10 mmol) and NaOH (1 M, 4.55 mL) at 0 °C, and the solution stirred overnight at rt. The solution was diluted with water and the aqueous layer extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent removed under reduced pressure. The crude 2-bromo-N-phenylacetamide product was purified by recrystallisation to give the required product as a white solid (51%). A solution of 2-bromo-N-phenylacetamide (4.8 mmol) and triethylphosphite (5.26 mmol) was refluxed in toluene (10 mL) at 120°C for 4 h and cooled to rt. The toluene was removed under reduced pressure and the residue purified by flash column chromatography (silica gel, methanol/dichloromethane/hexane 5:50:150) to afford **15** as a colourless oil (89 %)[27] which was used in the following reaction without further purification.

[4-(4-Bromo-8-fluoro-2,3-dihydrobenzo[*b*]oxepin-5-yl)phenoxy]acetic acid ethyl ester (17a). Potassium carbonate (4.20 mmol) was added to a solution of **16a** (0.84 mmol) in acetone (50 mL). The suspension was stirred for 15 min and ethyl bromoacetate (1.10 mmol) was added and the mixture refluxed for 8 h. The solution was cooled, filtered and the solvent removed

under reduced pressure. The residue was purified by chromatography (silica gel, 5% diethyl ther/hexane) to give the product as a colourless oil (70%) which was used without further purification in the subsequent reaction. ^{1}H NMR ($CDCl_3$): δ 7.14 (2H, d, *J* = 8.5 Hz), 6.99 (2H, d, *J* = 8.6 Hz), 6.87-6.85 (1H, m), 6.80-6.78 (2H, q, *J* = 1.5 Hz, 6.5 Hz), 4.77 (1H, s), 4.62-4.59 (2H, t, *J* = 5.8 Hz), 4.31-4.25 (2H, q, *J* = 7.0 Hz, 7.5 Hz), 3.06-3.03 (2H, t, *J* = 6.0 Hz), 1.37-1.33 (3H, t, *J* = 7.0 Hz).

Ethyl 2-[4-(4-bromo-2,3-dihydro-8-methoxybenzo[*b*]oxepin-5-yl)phenoxy]acetate (17b) was prepared from **16b** (1.86 mmol) and ethyl bromoacetate (2.23 mmol) using a procedure similar to that described for **17a**. The crude residue was purified by chromatography (silica, 10% diethyl ether/hexane) to give the product as a brown solid (77%) which was used without further purification in the subsequent reaction. Mp 94 °C; IR ν_{max} (KBr) 3445, 1695, 1607, 1198 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.14 (2H, d, *J* = 8.5 Hz), 6.93 (2H, d, *J* = 8.6 Hz,), 6.65-6.70 (2H, m), 6.55 (1H, q, *J* = 2.8 Hz, 6.0 Hz), 4.64 (2H, t, *J* = 5.0 Hz), 4.58-4.53 (2H, m), 4.31-4.26 (2H, m), 3.79 (3H, s), 3.05 (2H, t, *J* = 5.8 Hz), 1.32 (3H, t, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 168.9, 159.9, 157.8, 157.2, 156.9, 138.7, 136.2, 135.3, 134.9, 132.4, 131.1, 129.7, 126.4, 125.1, 120.6, 114.4, 114.2, 109.5, 106.8, 105.7, 105.5, 65.4, 61.4, 56.3, 41.6, 14.1.

{4-[8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]oxepin-5-yl]phenoxy}acetic acid ethyl ester (18a) was prepared from **17a** by general method IV. The crude product was purified by flash column chromatography (silica gel, 25% ethyl acetate/hexane) to give **17a** as a white solid (74%); Mp 181 °C; IR ν_{max}(KBr): 3431, 1731 (C=O), 1211 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.98 (2H, d, *J* = 6.5 Hz), 6.89 (4H, m), 6.76 (1H, m), 6.64 (4H, m), 4.61 (2H, t, *J* = 6.0 Hz), 4.55 (2H, s, C*H*$_2$), 4.26 (2H, q, *J* = 7.0 Hz), 2.68 (2H, t, *J* = 6.0 Hz), 1.27 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$): δ 169.1, 156.3, 154.2, 137.6, 135.4, 134.9, 134.4, 133.1, 132.5, 131.9, 130.8, 130.5,

128.1, 116.1, 114.9, 113.9, 110.8, 110.6, 109.5, 109.3, 80.9, 65.3, 61.4, 35.5, 14.1; ^{19}F NMR ($CDCl_3$): δ -113.577; HRMS (ESI): Found 457.1406 $(M+Na)^+$; $C_{26}H_{21}O_5FNa$ requires 457.1427.

{4-[8-Methoxy-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]oxepin-5-yl]phenoxy}acetic acid ethyl ester (18b) was prepared from **17b** by general method IV. The crude product was purified by flash column chromatography (silica, 5% ethyl acetate/hexane) to give the product as a beige solid (74%); Mp 138 °C, which was used without further purification in the subsequent reaction. IR ν_{max} (KBr): 3437, 1735 (C=O), 1218 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.69-7.66 (2H, m), 7.58 (1H, d,d, *J* = 1.6 Hz, 5.7 Hz), 7.50-7.48 (2H, m), 7.02 (2H, d, *J* = 8.6 Hz), 6.93 (2H, d, *J* = 8.8 Hz), 6.67 (2H, q, *J* = 2.2 Hz), 6.62 (1H, dd, *J* = 2.7 Hz, 5.7 Hz), 4.64 (2H, t, *J* = 6.0 Hz), 4.58 (2H, s), 4.29 (2H, q, *J* = 7.1 Hz), 3.84 (3H, s), 2.71 (2H, t, *J* = 6.0 Hz), 1.32 (3H, t, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$): δ 169.1, 159.7, 157.1, 156.2, 154.4, 135.3, 132.6, 131.6, 130.8, 129.4, 128.7, 128.6, 114.9, 114.8, 113.8, 109.8, 107.2, 80.5, 65.4, 61.4, 55.4, 35.8, 14.1.

{4-[8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]oxepin-5-yl]phenoxy}acetic acid (19a) was prepared from **18a** by general method IX as a brown solid (25%); Mp 245 °C; IR ν_{max}(KBr): 3379, 1607 cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.00 (2H, d, *J* = 8.6 Hz), 6.88-6.86 (2H, m), 6.83-6.79 (2H, m) 6.71 (1H, s), 6.65 (4H, t, *J* = 8.5 Hz), 4.61 (2H, t, *J* = 6.0 Hz), 4.57 (2H, s), 2.68 (2H, t, *J* = 6.0 Hz); ^{13}C NMR (*d*-DMSO): δ 172.3, 160.0, 157.2, 156.8, 156.6, 156.2, 137.8, 134.1, 133.5, 133.1, 132.1, 131.6, 130.2, 114.9, 113.9, 110.6, 110.4, 109.3, 109.1, 80.6, 67.2, 35.2; ^{19}F NMR (376 MHz, *d*-DMSO): δ -113.9375; HRMS (ESI): Found 429.1126 $(M+Na)^+$; $C_{24}H_{19}O_5FNa$ requires 429.1114.

{4-[8-Methoxy-4-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]oxepin-5-yl]phenoxy}acetic acid (19b) was prepared from **18b** by general method IX as a brown solid in quantitative yield; Mp 201 °C; IR ν_{max}(KBr): 3402, 1735, 1607 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.62-7.57 (2H, m), 7.47 (1H,

dd, J = 1.5 Hz, 6.0 Hz), 7.41-7.38 (2H, m), 6.89 (2H, d, J = 9.8 Hz), 6.82 (2H, d, J = 9.0 Hz), 6.67 (2H, d, J = 11.6 Hz), 6.60-6.55 (1H, m), 4.53 (2H, t, J = 6.0 Hz), 4.50 (2H, s), 3.74 (3H, s), 2.62 (2H, t, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 171.4, 159.6, 157.0, 155.8, 154.3, 136.8, 135.9, 135.6, 134.5, 132.6, 132.5, 132.1, 131.9, 131.7, 130.8, 129.3, 128.6, 114.9, 113.9, 109.8, 107.2, 80.5, 64.9, 55.4, 35.7; HRMS (ESI): Found 441.1304 $(M+Na)^+$; $C_{25}H_{22}O_6Na$ requires 441.1314.

4-[4-(4-Bromo-8-fluoro-2,3-dihydro-1-benzoxepin-5-yl)phenoxy]butyric acid ethyl ester (20) was prepared from **16a** (1.86 mmol) and ethyl bromobutyrate (2.23 mmol) using a procedure similar to that described for **17a**, using ethyl 4-bromobutyrate in place of ethyl bromoacetate. The residue was purified by flash column chromatography (10% ethyl acetate: 90% hexane) to give **20** as a white solid (68%); Mp 89 °C; IR ν_{max}(KBr): 2981, 1759, 1605, 1199 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.14 (2H, d, J = 8.0 Hz), 6.88 (2H, d, J = 8.0 Hz) , 6.80-6.72 (2H, m), 6.66-6.62 (1H, m), 4.60 (2H, t, J = 5.8 Hz), 4.15 (2H, q, J = 7.2 Hz), 4.03 (2H, t, J = 6.0 Hz), 3.01 (2H, t, J = 5.7 Hz), 2.53 (2H, t, 7.3 Hz), 2.15 (2H, m), 1.26 (3H, t, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 173.2, 162.5 (J_F = 250 Hz), 158.1, 157.6, 138.7, 134.6, 132.6, 132.5, 131.0, 129.3 (J_F = 3 Hz), 121.4, 113.9, 110.5 (J_F = 21 Hz), 109.2 (J_F = 22 Hz), 77.4, 66.5, 60.4, 41.0, 30.8, 24.6, 14.2; ^{19}F NMR ($CDCl_3$): δ -112.6492. HRMS (ESI): Found 449.0748$(M+H)^+$; $C_{22}H_{23}BrFO_4$ requires 449.0764.

4-{4-[8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin-5-yl]phenoxy}-butyric acid ethyl ester (21) was prepared from **20** by general method IV. The crude product was purified by column chromatography (silica, hexane:diethyl ether 1:1) to give the required product as a white solid (118 mg, 86%) which was used in the subsequent reaction without further purification. ^{1}H NMR ($CDCl_3$): δ 6.98 (2H, d, J = 6.52 Hz), 6.86-6.80 (4H, m), 6.75-6.70 (1H, m), 6.65-6.61 (4H, m) 4.61 (2H, t, J = 6.0 Hz), 4.15 (2H, q, J = 7.2 Hz), 3.92 (2H, t, J = 6.0 Hz), 2.67 (2H, t, J

= 6.0 Hz), 2.49 (2H, t, *J* = 7.3 Hz), 2.09-2.02 (2H, m), 1.25 (3H, t, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 173.7, 163.3, 160.8, 157.2, 156.8, 145.3, 137.2, 135.6, 134.4, 133.9, 133.2, 132.4, 131.8, 130.7, 114.9, 113.6, 110.8, 110.6, 109.5, 109.3, 80.9, 66.5, 60.6, 35.5, 30.9, 24.6, 14.2; ^{19}F NMR ($CDCl_3$, Me_4Si): δ -113.6567.

4-{4-[8-Fluoro-4-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin-5-yl]-phenoxy}-butyric acid (22) was prepared from **21** by general method IX as a pale brown solid (69%); Mp 95 °C; IR ν_{max}(KBr): 1702 (C=O), 3368 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.98 (2H, d, *J* = 8.5 Hz), 6.85-6.80 (4H, m), 6.75-6.69 (1H, m), 6.63 (4H, d, *J* = 8.6 Hz), 4.61 (2H, t, *J* = 6.0 Hz), 3.94 (2H, t, *J* = 6.0 Hz), 2.67 (2H, t, *J* = 6.0 Hz), 2.54 (2H, t, *J* = 7.3 Hz), 2.07 (2H, t, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$): δ 179.2, 163.3, 160.8, 157.1, 156.8, 156.7, 154.1, 137.2, 135.6, 134.5, 133.3, 133.2, 132.4, 131.8, 130.9, 114.9, 113.7, 110.9, 110.7, 109.5, 109.3, 80.9, 66.3, 35.5, 30.5, 24.36; ^{19}F NMR ($CDCl_3$): δ -113.5651; HRMS(ESI): Found 457.1407$(M+Na)^+$; $C_{26}H_{23}O_5FNa$ requires 457.1427.

1-Iodo-4-(1-(4-methoxyphenyl)-2-phenylbut-1-enyl)benzene (24). To a suspension of zinc dust (45.0 mmol) in dry THF (30 mL) was added titanium tetrachloride (422.5 mmol) *via* a syringe and the mixture was then refluxed for 2 h in darkness under nitrogen. A mixture of 4-iodophenyl-4-methoxyphenylmethanone (**23**)[53] (5.00 mmol, 1 eq.) and propiophenone (15.0 mmol, 3 eq.) in anhydrous THF (40 mL) was added to the Zn/$TiCl_4$ mixture *via* syringe. The mixture was then refluxed for a further 3 h. Afterwards, the mixture was allowed to cool then diluted with ethyl acetate (100 mL) and washed with 10 % potassium carbonate solution (50 mL). After filtration, the organic layer was separated out and the aqueous layer was extracted with ethyl acetate (100 mL × 3). The combined organic layers were washed with 10 % potassium carbonate solution (50 mL), water (50 mL) and brine solution (50 mL) then dried over

anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield crude product as an amber oil. The material was purified *via* flash chromatography (hexane:diethyl ether) to afford the product as a light green oil which later solidified into an off-white waxy resin[54] which was used without further purification in the following reaction. (48%, *Z/E* = 3.5/1). IR: ν_{max} (KBr) cm^{-1}: 3436, 2966, 1605, 1508, 1480, 1461, 1441, 1288, 1246, 1172, 1029, 1006; ^{1}H NMR ($CDCl_3$): δ 6.68 – 7.81 (13H, m), 3.91 (0.66H, s), 3.75 (2.34H, s), 2.58 – 2.66 (2H, m), 1.01 – 1.13 (3H, m). ^{13}C NMR ($CDCl_3$): δ 157.3, 143.1, 141.8, 141.5, 136.9, 136.2, 134.5, 132.5, 131.6, 131.2, 130.3, 129.4, 129.3, 129.2, 127.8, 127.7, 127.6, 125.9, 125.8, 113.3, 112.6, 112.4, 54.9, 54.7, 54.6, 28.8, 13.4.

***(E)*-3-{4-[(*E/Z*)-1-(4-Methoxyphenyl)-2-phenylbut-1-enyl]phenyl}acrylic acid (25)**. A suspension of sodium hydrogen carbonate (1.25 mmol, 2.5 eq), *n*-butylammonium hydrogensulfate (0.50 mmol, 1 eq) and crushed 4A molecular sieves (0.20 g) in DMF (2 mL) was stirred for 15 min under a nitrogen atmosphere. Iodo triarylethylene (**24**) (0.50 mmol, 1 eq) and acrylic acid (1.00 mmol, 2 eq) were added and stirred for a further 15 min before the addition of palladium (II) acetate (0.03 mmol, 0.05 eq). The mixture was stirred at 60 °C for 4 h, then allowed to cool. Water (20 mL) was added, followed by 30 mL of ethyl acetate. The palladium and insolubles were filtered off. The aqueous phase was extracted with 3 × 30 mL ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo.* The crude mixture was purified *via* flash chromatography (ethyl acetate) to afford the desired product as an off-white resin (94%, *Z/E* = 3.5/1)[55]. IR: ν_{max} (KBr) cm^{-1}: 3396, 2929, 1702, 1631, 1508, 1439; ^{1}H-NMR ($CDCl_3$): δ 6.26 – 7.56 (15H, m), 3.85 (0.66H, s), 3.65 (2.34H, s), 2.43 – 2.52 (2H, m), 0.92 (3H, t , *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 169.8, 162.5, 157.1, 145.8, 145.1, 144.9, 141.6, 141.5, 137.1, 134.5, 132.2, 131.4,

130.9, 130.2, 129.6, 129.1, 128.3, 127.6, 127.5, 127.4, 126.9, 125.9, 125.7, 117.3, 116.7, 113.2, 112.4, 54.7, 54.5, 28.6, 13.1. HRMS (EI): Found 407.1634 $(M+Na)^+$, $C_{26}H_{24}O_3Na$ requires 407.1623.

***(E)*-3-{4-[(*E/Z*)-1-(4-Methoxyphenyl)-2-phenylbut-1-enyl]phenyl}acrylic acid methyl ester (26)** was prepared from **24** (0.5 mmol, 1 eq) and methyl acrylate (1.0 mmol, 2 eq) using a procedure similar to that described for **25**. The crude mixture was purified *via* flash chromatography (ethyl acetate) to afford the product as an off-white resin (93%). IR: ν_{max} (KBr) cm^{-1}: 3442, 2961, 1721, 1640, 1606, 1510, 1443. ^1H NMR ($CDCl_3$): δ 6.46 – 7.56 (13H, m), 3.85 (3H, s), 3.71 (3H, s), 2.51 (2H, q, J = 7.0 Hz), 0.98 (3H, t, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 167.1, 157.2, 145.8, 144.2, 141.7, 141.6, 137.1, 134.5, 132.2, 131.5, 129.6, 129.2, 127.5, 125.8, 116.9, 112.4, 54.5, 51.3, 28.6, 13.2. HRMS (EI): Found 421.1784$(M+Na)^+$, $C_{27}H_{26}O_3Na$ requires 421.1780.

X-Ray Crystallography

Data for **18a** were collected on a Bruker D8 QUEST Eco using Mo Kα radiation (λ = 0.71073 Å); data for **22** were collected on a Bruker Apex DUO using Cu Kα radiation (λ = 1.54178 Å) using a Mitegen cryoloop and at 100(2) K (Oxford Cryostream, Oxford Cobra Cryosystem respectively). Bruker APEX software was used to collect, correct (Lorentz and polarization) and reduce data, determine the space group, solve and refine the structure.[56] Absorption corrections were applied using SADABS 2014.[57] Bruker APEX software was used to determine the space group, solve and refine the structure. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model with appropriately fixed isotropic thermal parameters.

Compound 18a: Crystal Data for $C_{26}H_{23}FO_5$ (M =434.44 g/mol): monoclinic, space group Cc (no. 9), a = 5.3145(2) Å, b = 18.5230(8) Å, c = 22.2510(9) Å, β = 93.9217(14)°, V = 2185.27(15) Å^3, Z = 4, T = 100.0 K, μ(MoKα) = 0.097 mm^{-1}, *Dcalc* = 1.320 g/cm^3, 17459 reflections measured (5.728° ≤ 2Θ ≤ 58.35°), 5689 unique (R_{int} = 0.0515, R_{sigma} = 0.0560) which were used in all calculations. The final R_1 was 0.0471 (I > 2σ(I)) and wR_2 was 0.0932 (all data). CCDC deposition number: 1498827.

Compound 22: Crystal Data for $C_{27}H_{27}FO_6$ (M =466.48 g/mol): monoclinic, space group P21/c (no. 14), a = 17.1268(6) Å, b = 13.2593(5) Å, c = 10.1147(4) Å, β = 93.5261(19)°, V = 2292.59(15) Å^3, Z = 4, T = 99.97 K, μ(CuKα) = 0.832 mm^{-1}, Dcalc = 1.352 g/cm^3, 31392 reflections measured (5.17° ≤ 2Θ ≤ 136.948°), 4189 unique (R_{int} = 0.0612, R_{sigma} = 0.0370) which were used in all calculations. The final R_1 was 0.0613 (I > 2σ(I)) and wR_2 was 0.1715 (all data). CCDC deposition number: 1498828.

*$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Biochemical evaluation of activity. MCF-7 cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC) and were cultured in Minimum Essential Media with GlutaMAX™-I (Gibco) supplemented with heat-inactivated fetal bovine serum (10%)(Gibco), penicillin/streptomycin 5000 U/mL (1%)(Gibco) and non-essential amino acids (1%)(Sigma). MCF-10a cells were obtained as a kind gift from Dr. Susan McDonnell, University College Dublin School of Chemical and Bioprocess Engineering, and were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12; Gibco) supplemented with 5% horse serum (Invitrogen), 20 ng/mL epidermal growth factor (Merck Millipore), 0.5 μg/mL hydrocortisone (Sigma), 100 ng/mL cholera toxin (Sigma), 10 μg/mL insulin (Sigma), and

penicillin/streptomycin 5000 U/mL (1%)(Gibco). All biochemical experiments were performed in triplicate.

Cell viability studies. Cells were seeded in 96-well plates at a density of 2.5×10^4 cells/mL (200 μL/well). After 24 h, cells were treated with a vehicle control [1% (v/v) ethanol] or a range of drug concentrations, followed by incubation for 72 h at 37 °C in 5% CO_2. The growth medium was removed (50 μL of which was reserved for cytotoxicity studies), cells were washed with 100 μL phosphate buffered saline (PBS), and 50 μL MTT was added (final concentration of 1 mg/mL MTT). Cells were incubated for 2 h in darkness at 37 ^oC, after which they were solubilized by addition of DMSO (200 μL) at rt in darkness for 20 min. The absorbance value of control cells (with no added compound or solvent) was set to 100% cell viability and, from this, graphs of cell viability (%) versus compound concentration were prepared using GraphPad Prism version 5.[58]

Cytotoxicity studies. 50 μL Aliquots of growth medium (see Cell viability studies, above) were removed to a fresh 96-well plate, and cytotoxicity was determined using CytoTox 96® Non-Radioactive Cytotoxicity Assay obtained from Promega.[59] Lactate dehydrogenase (LDH) substrate mixture (50 μL) was added to each well and the plate left in darkness at rt for equilibration. Stop solution (50 μL) was added to all wells and absorbance at 490 nm was measured. The control (100% cell lysis) was by lysing untreated cells by addition of lysis solution (20 μL) to the media 45 min prior to the assay end-point. Data is presented as cell lysis (% of control) versus compound concentration.

ER Binding Assay. ERα and ERβ fluorescence polarization based competitor assay kits were purchased from Invitrogen Life Technologies.[33, 34] The recombinant ER and the fluorescent estrogen ligand were thawed on ice for 1 hr 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 bovine gamma globulin, 0.02% NaN_3) to give a fluorescent estrogen/ER complex. Test compound (1 μL, at a range of concentrations) was added to screening buffer (49 μL) in each tube. To this 50 μL of the fluorescent estrogen / ER complex was added. A vehicle control contained 1% (v/v) of ethanol; a negative control was used to determine the theoretical maximum polarization (50 μL screening buffer and 50 μL fluorescent estrogen/ER complex). The tubes were incubated in the dark at room temperature for 2 h and were mixed by shaking on a plate-shaker. Polarization values were measured on a Beacon single-tube fluorescent polarization instrument fitted with with 485 nm excitation and 530 nm emission interference filters. For ERα and ERβ, graphs of anisotropy (mA) versus compound concentration were obtained for determination of IC_{50} values.

Estrogenic activity: Alkaline phosphatase assay. Following the procedure of Littlefield,[35] human Ishikawa cells were maintained in Eagle's Minimum Essential Medium (MEM containing 10% (v/v) fetal bovine serum (FBS) and supplemented with 100 U/mL penicillin and 10 μg/mL streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. 24 h before the start of the experiment, this was replaced by an estrogen-free medium (EFBM) consisting of a 1:1 mixture of phenol-free Ham's F-12 and Dulbecco's Modified Eagles Medium (DMEM), 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 (2.5×10^4 cells/well). Test compounds were dissolved in ethanol, diluted with EFBM (final concentration of ethanol 0.1% v/v) and sterile filtered. After addition of the test compounds and **1** (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. Growth medium was removed, cells were then twice rinsed in PBS, and placed at -80 °C for at least 15 min, before thawing at rt for 5-10 min. Plates were then placed on ice and ice-cold solution containing *p*-nitrophenyl phosphate (50 mM), $MgCl_2$ (0.24 mM) and diethanolamine (1M, pH 9.8)(50 μL) was added. The plates were warmed to room temperature (time zero), and the yellow colour 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.

Determination of Expression Levels of ERα and ERβ by Western Blotting

MCF-7 cells were seeded at a density of 1×10^6 cells/mL in T25 flasks, left to adhere overnight and then treated with vehicle control (0.1% v/v ethanol), **5** (1 μM), **13e** (10 μM) or **22** (10 μM) for 24 h. Whole-cell lysates (50 μg) were resolved on 4-20 % Mini-PROTEAN TGX pre-cast gels (Bio-Rad Laboratories Ltd., Hertfordshire, UK) and transferred onto Immobilon-P PVDF membranes. The membranes were blocked with 5% (w/v) milk protein and incubated overnight with ERα mAb (D8H8; Cell Signalling Technology, Danvers, MA, USA), ERβ mAb (Merck Millipore, Darmstadt, Germany), or GAPDH (Calbiochem, Nottingham, UK) as the loading control. The secondary antibodies were coupled to horseradish peroxidase. Membranes were then exposed to ECL (Bio-Rad, Hertfordshire, UK) for 1 min and images detected using the Bio-Rad GelDoc system.

Computational Details: Molecular Docking Study

The 3ERT X-ray structure of hERα co-crystallised with **2b**[37] was downloaded from the PDB website. For ERβ the 1NDE X-ray structure co-crystallised with the triazine ERb modulator 4-(2-{[4-{[3-(4-chlorophenyl)propyl]sulfanyl}-6-(1-piperazinyl)-1,3,5-triazin-2-yl]amino}ethyl)phenol was downloaded.[38] Both were prepared using QuickPrep in MOE 2015.[60] Water molecules in proximity to the ligands were retained. *MAKE_RECEPTOR* 3.0.1. was utilised to define the binding site for subsequent docking studies.[39] A modified version of the DUD derived ERα antagonist haystack set was generated for validation studies.[61] A number of duplicate compounds and compounds containing mis-assigned atom types were discarded leaving 39 actives and 1125 inactives for docking using FRED 3.0.1.[62 63] ERβ antagonists (32 compounds) were obtained from the work of Zhang[64] and merged with the ERα inactive compounds to create the ERβ haystack. Due to similarities between ERα and ERβ ligands the same inactive compound collection was utilised. In-house active compounds were included within each database. OMEGA 2.5.1.4. was used to generate 50 conformers of each compound prior to docking.[65 66] Default parameters were used and the top 1000 docked compounds were retained for subsequent analysis. Chemgauss4 scoring was implemented and ROCS analysis[39] was used to ascertain ranking accuracy within a Pipeline Pilot protocol.[67]

Supporting Information. Three-dimensional computational models of target-ligand complexes in Figures 5 and 6 (as PDB-formatted coordinate files) and molecular formula strings are available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations.

EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; EFBM, estrogen-free basal medium; ER, estrogen receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HB, Hydrogen bond; HBA, Hydrogen bond acceptor; HRMS, high resolution mass spectrometry; HBD,

Hydrogen bond donor; HOBt, Hydroxybenzotriazole; IC, inhibitory concentration; LDH, Lactate dehydrogenase; LBD, ligand-binding domain; LBP, ligand-binding pocket; MEM, Eagle's Minimum Essential Medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, Nuclear Magnetic Resonance; PBS, phosphate buffered saline; SAR, structure-activity relationship; SERD, Selective Estrogen Receptor Downregulator; SERM, Selective Estrogen Receptor Modulator; TMS, tetramethylsilane.

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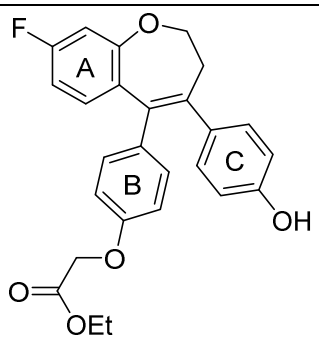
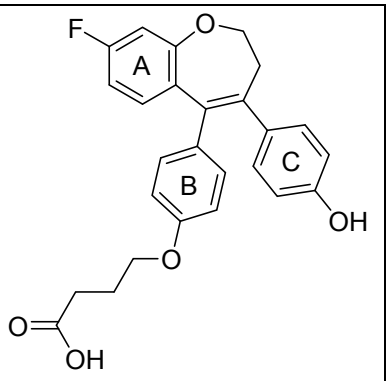
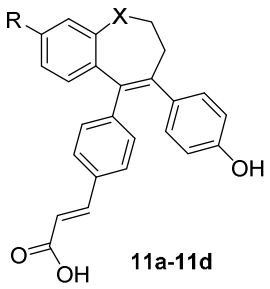
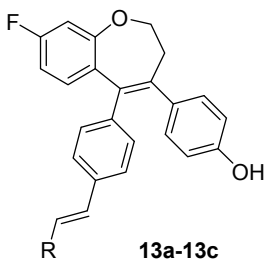
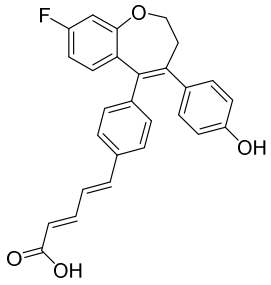
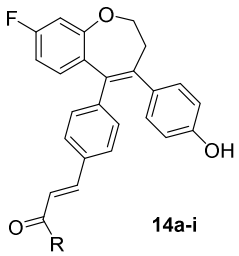
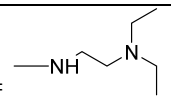
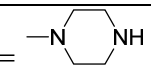
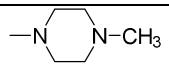
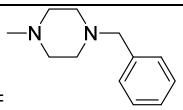
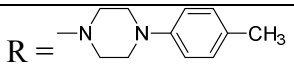
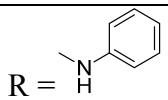
Table 1: Ring Angles from X-ray Crystallographic Data for Benzoxepins **18a** and **22**

		
Compound:	**18a**	**22**
CCDC deposition number:	1498827	1498828
Ring plane AB angle (°)	75.3	64.3
Ring plane BC angle (°)	59.7	64.3
Ring plane AC angle (°)	85.9	73.4
RingAB Torsion (°)[a]	39.5	42.02
RingBC Torsion (°)[b]	9.7	6.08

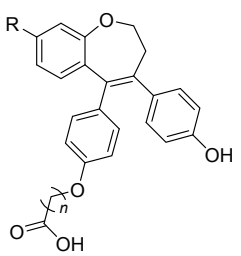
[a]Measured between C16-C17-C18-C19 (**18a**) and C17-C18-C19-C20 (**22**)

[b]Measured between C19-C18-C8-C5 (**18a**) and C20-C19-C8-C5 (**22**). Refer to Figure 3 for numbering.

	Structure	Antiproliferative activity IC_{50} (μM)[a]	Cytotoxicity at 10μM (%)	ERα IC_{50} (nM)[b]	ERβ IC_{50} (nM)[b]	RBA ERα[c]	RBA ERβ[c]	Selectivity (α/β)
Series I  **11a-11d**								
11a	R = H; X = O	21	2.5	21	46	27	12	2.2
11b	R = OCH_3; X = O	> 20	4.5	23	119	25	4.7	5.3
11c	R = F; X = O	0.26	5.0	14	72	41	7.8	5.2
11d	R = F; X = S	0.095	0	4.1	3.1	139	181	0.8
Series I  **13a-13c**								
13a	R = $COCH_3$	0.89	3.7	59	175	9.7	3.2	3.0
13b	R = C_6H_5	12	2.2	22440	14340	0.025	0.039	0.6
13c	R = COC_6H_5	0.97	0	104	447	5.5	1.3	4.2

13e		1.6	0	71.6	0.55	7.9	1018	0.008
Series II  **14a-i**								
14a	R = -N(C_2H_5)$_2$	13	6.2	349	43	1.6	13.0	0.12
14b	R = 	14	2.3	67	2.4	8.5	233	0.038
14c	R = 	8.8	4.0	1023	585	0.56	0.96	0.58
14d	R = 	9.5	6.1	14	25	41	22	1.9
14e	R = 	0.48	2.5	57	354	10	1.6	6.3
14f	R = 	8.4	5.8	74	72	7.7	7.7	1.0
14g	R = 	1.05	9.0	48	203	11.9	2.8	4.3
14h	R = -NHC_2H_5	0.70	4.7	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*
14i	R = 	1.3	0	11.7	0.94	52	596	0.087

Series III



19a	R = F; n = 1	> 20	2.6	634	34	0.89	16.4	0.054
19b	R = OCH_3; n = 1	> 20	0	429	1014	1.3	0.55	2.4
22	R = F; n = 3	> 20	5.3	147	1.23	3.9	455	0.008
Positive Controls								
2a		4.1[d]	13.4	70[g]	170[g]	8.1	3.4	2.4
2b		0.11	0	26.3	26.1	14.3	28	1.9
4b		1.3[e]	Nd[f]	4.4	6.8	130	82	1.6

Table 2: Antiproliferative effects of benzoxpeins **11a-11d**, **13a-13c**, **13e**, **14a-14i**, **19a**, **19b**, and **22** in MCF-7 cells, and ERα and ERβ binding affinities

[a]Experimental values represent the average for two independent experiments performed in triplicate with typical standard errors below 20%. 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 and six replicate experiments for ERβ, with typical standard errors below 20%.

[c]The relative binding affinity (RBA) of estrogen receptor ligands is reported, using IC_{50} values for **1** as the reference ligand [ERα (5.7 nM) and ERβ (5.6 nM); 100 % binding value].[33 34]

[d]IC_{50} value for **2a** is in agreement with the reported IC_{50} value for **2a** in MCF-7 cells (4.4 μM)[29]

[e] IC_{50} value for **4b** is in agreement with the reported IC_{50} inhibition concentration range of 10^{-7}-10^{-6} μM using the MTT assay on human MCF-7 cells[13]

[f] Not determined.

[g] ER-binding values obtained are in agreement with the reported ER IC_{50} binding data for **2a** (Invitrogen references ERα 60.9 nM; ERβ 188 nM).

Table 3: Antiestrogenic and estrogenic activity for compounds 11a-11d, 13a, 14a and 22 in Ishikawa cells

Compound	Antiestrogenic Activity [IC_{50} (μM)][a]	Estrogenic Activity[a, b] (% Stimulation)
11a	0.67	4.6 (1 μM)
11b	> 1[c]	15.8 (0.01 μM)
11c	1.39	0.0 (1 μM)
11d	0.18	1.6 (10 μM)[d]
13a	0.45	9.6 (1 μM)
14a	0.0098	10 (100 μM)[d]
22	0.43	3.2 (1 μM)
2a	0.28	10 (1 μM)
2b	0.01	(1 μM)

[a]Values are an average of at least twelve replicate experiments with typical standard errors below 20%.

[b]Relative initial stimulator activity for compounds at concentrations of 0.01-100 μM, in comparison with **1** (1nM) = 100%.

[c]Did not reach 50% inhibition at concentrations up to 1 μM

[d]0% stimulation activity at 1 μM

Table 4. ***In silico*** **docking comparative ranking**[a]

ERα			**ERβ**		
Compound	**Rank**	**IC_{50} (μM)**	**Compound**	**Rank**	**IC_{50} (μM)**
11d	1	0.0041	**11c**	6	0.0722
11c	3	0.0143	**11d**	10	0.0031
14i	8	0.0117	**13e**	20	0.00094
14b	11	0.067	**14i**	22	0.0024
13e	16	0.0716	**14b**	23	0.00055
22	26	0.147	**22**	58	0.00123

[a]The six active compounds were included in the optimised ERα/β antagonist haystacks, containing approximately 1160 compounds, and after docking the ranked list shows that all the active compounds are found within the top 5% for both ER isoforms.

Figure 1. Structures of β-estradiol and selected ER modulators and ER downregulators

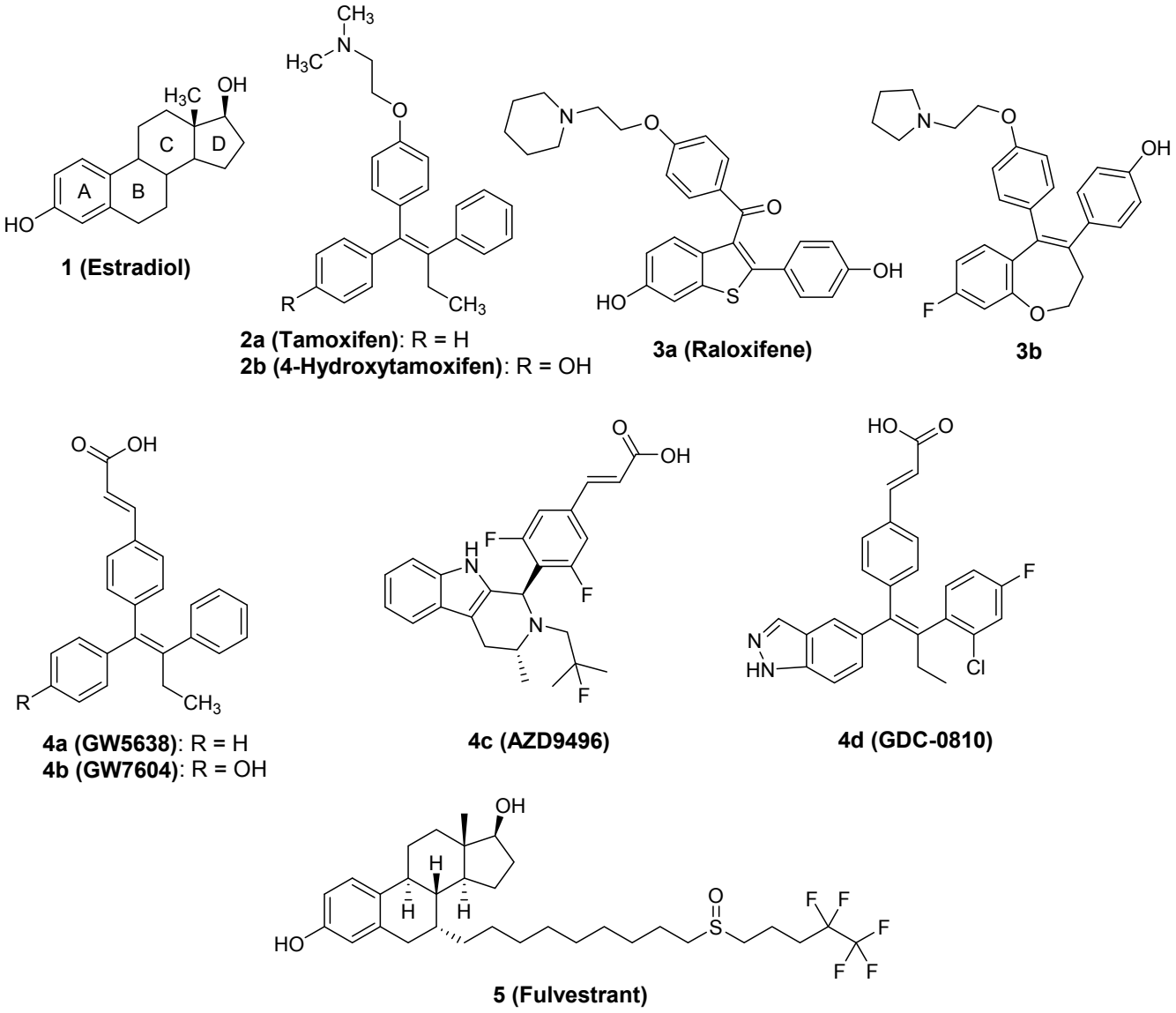
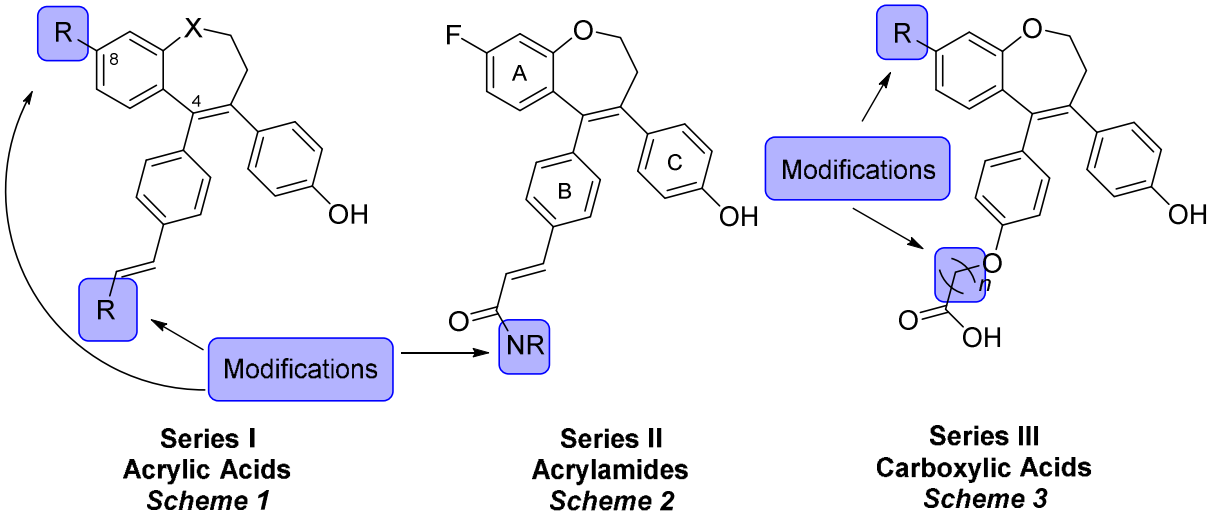


Figure 2. Benzoxepins and benzothiepins designed in this study. Locant positions are indicated on the first structure and the A, B and C rings are indicated on the middle structure.



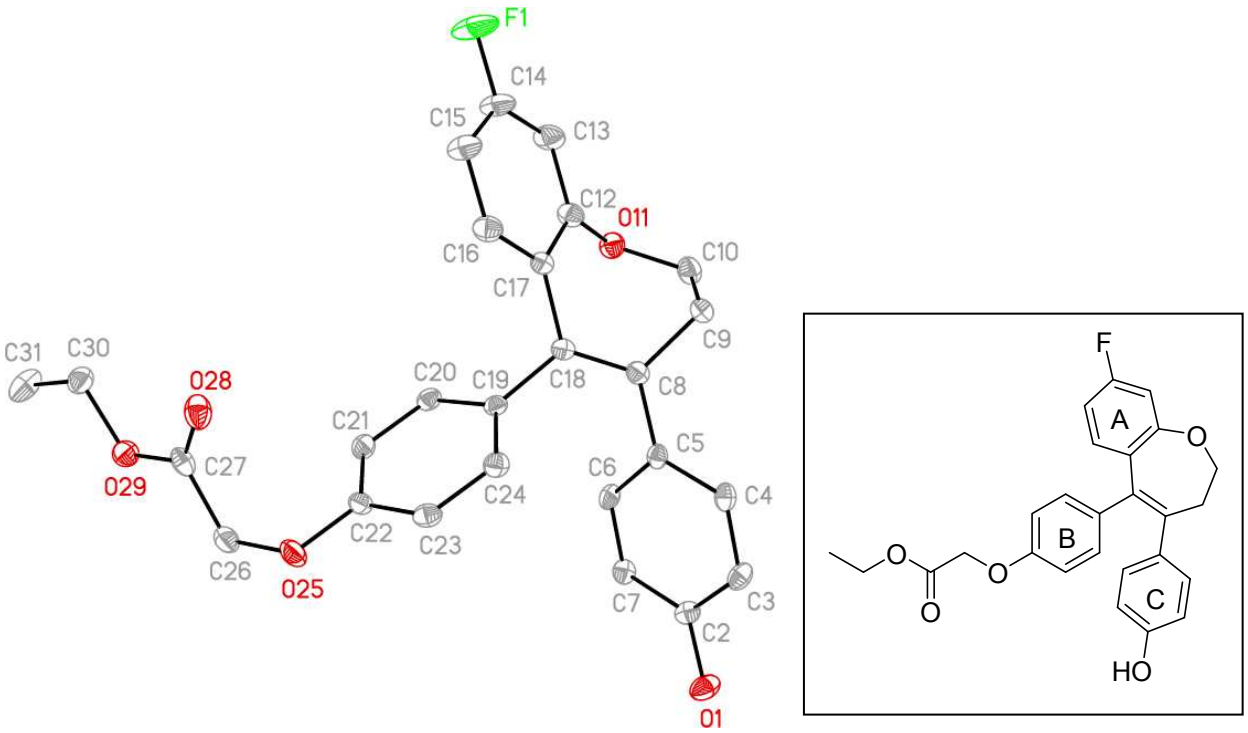
Series I
Acrylic Acids
Scheme 1

Series II
Acrylamides
Scheme 2

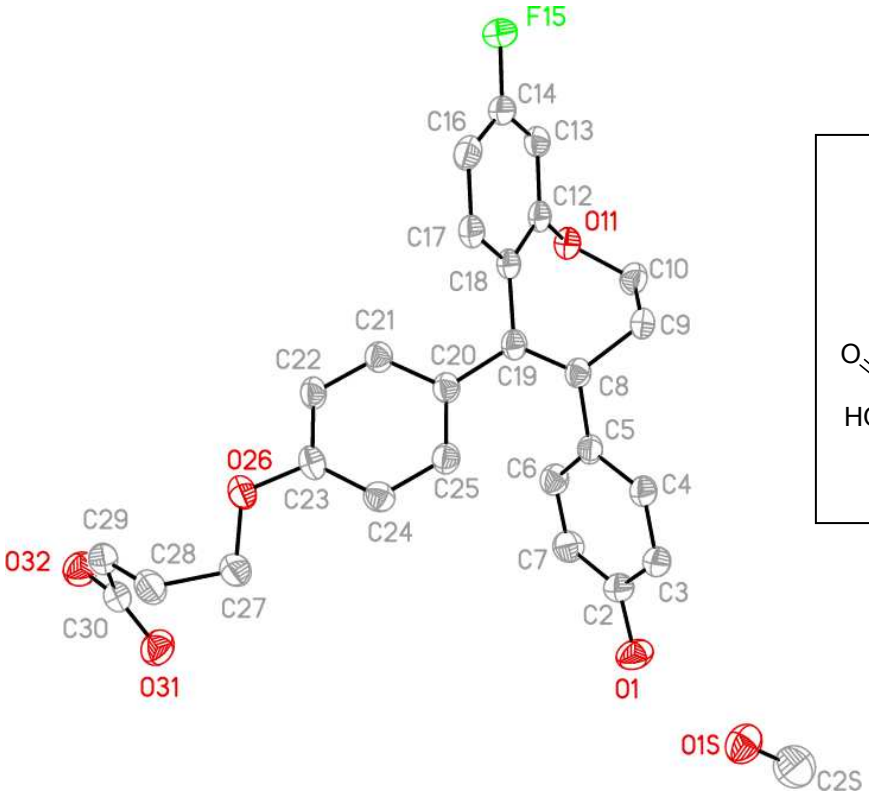
Series III
Carboxylic Acids
Scheme 3

Figure 3. X-Ray crystallographic structures of (a) **18a** and (b) **22** with atomic displacement parameters shown at 50% probability. Hydrogen atoms omitted for clarity. **22** crystallizes as a methanol solvate. CCDC deposition numbers: 1498827 (**18a**) and 1498828 (**22**).

A.



B.


F15
C14
C16
C13
C12
C17
O11
C18
C10
C21
C9
C22
C20
C19
C8
C5
O26
C6
C4
C23
C25
C29
C24
C7
C28
C3
O32
C27
C2
C30
O31
O1
O1S
C2S

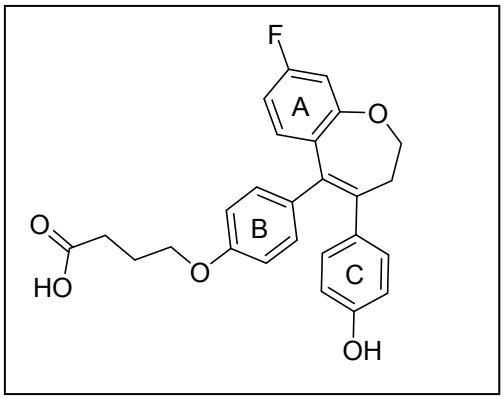

F
A
O
O
B
HO
O
C
OH

Figure 4. Effects of compounds **13e** and **22** on viability of MCF-10a cells.

Non-tumorigenic MCF-10a cells were treated with the indicated concentrations of compound **13e** or **22** for 24 and 48 h. Results are expressed as the average of three independent experiments (± SEM) performed in triplicate.

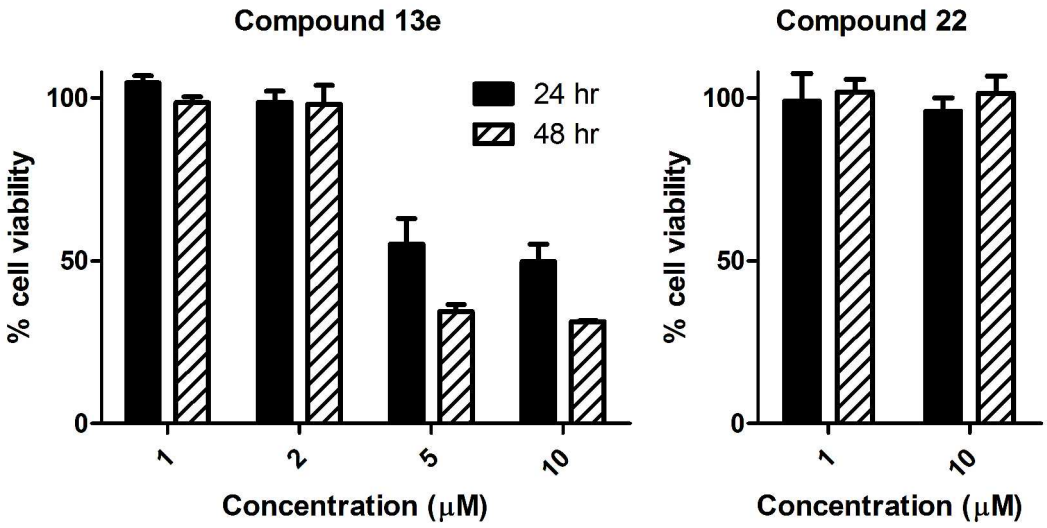


Figure 5. Effects of **5**, **13e** and **22** on expression levels of ERα and ERβ in MCF-7 breast cancer cells.

MCF-7 cells were treated with vehicle control (0.1% v/v ethanol), **5** (1 μM), **13e** (10 μM) or **22** (10 μM) for 24 h. Cells were lysed and separated by SDS-PAGE. The membrane was probed with anti-ERα [1:1000] or anti-ERβ [1:1000] antibodies. GAPDH was used as a loading control [1:1000]. Results are representative of three separate experiments.

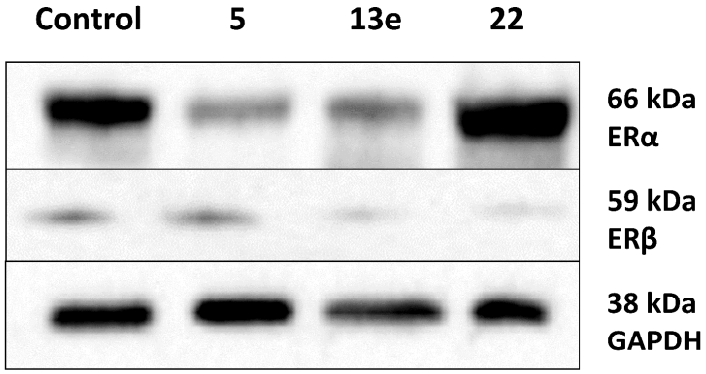


Figure 6. Top ranked poses of **13e** in ERα and ERβ[a]

[a]Carbon atoms of compound **13e** are illustrated in green in ERα and blue in ERβ (oxygen atoms are red; fluorine and water molecules are bright green). PBD structures 3ERT[37] and 1NDE[38] were used for molecular docking.

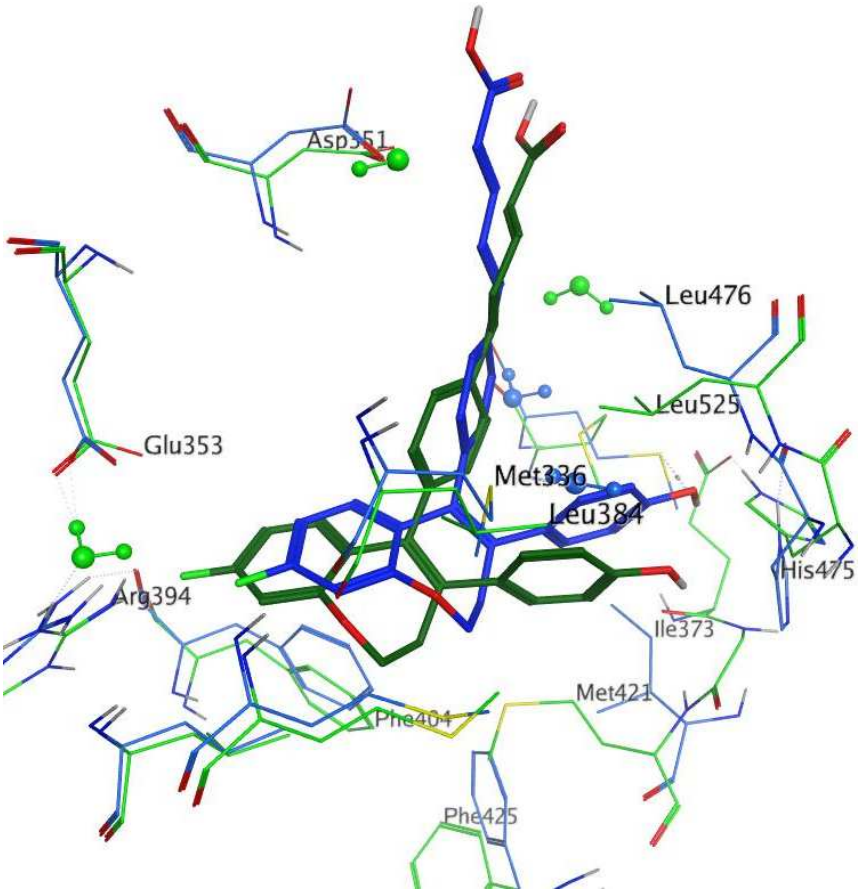
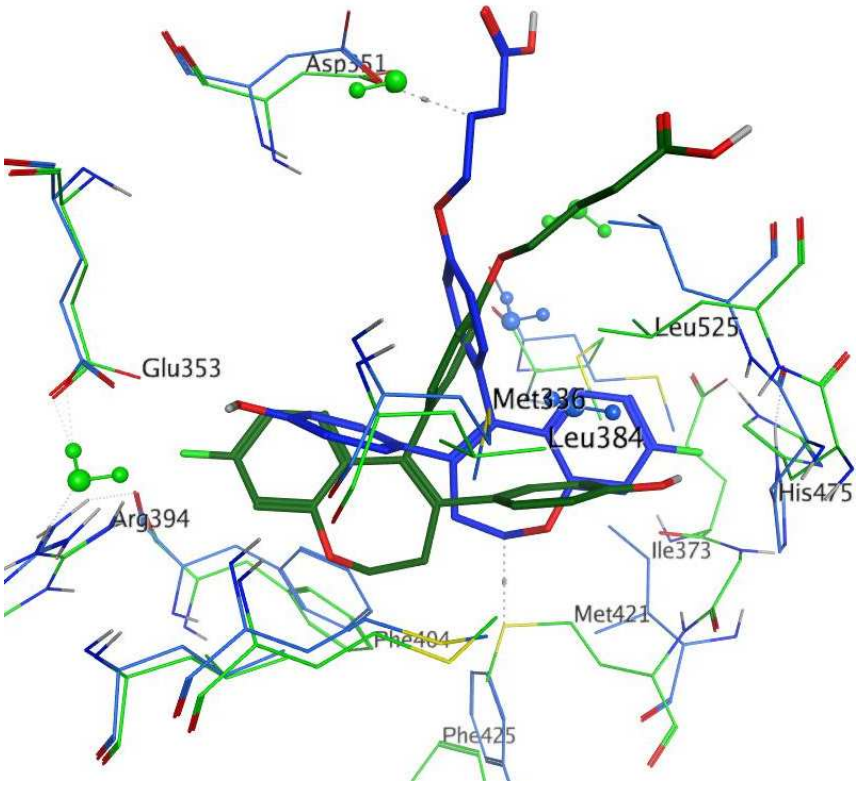
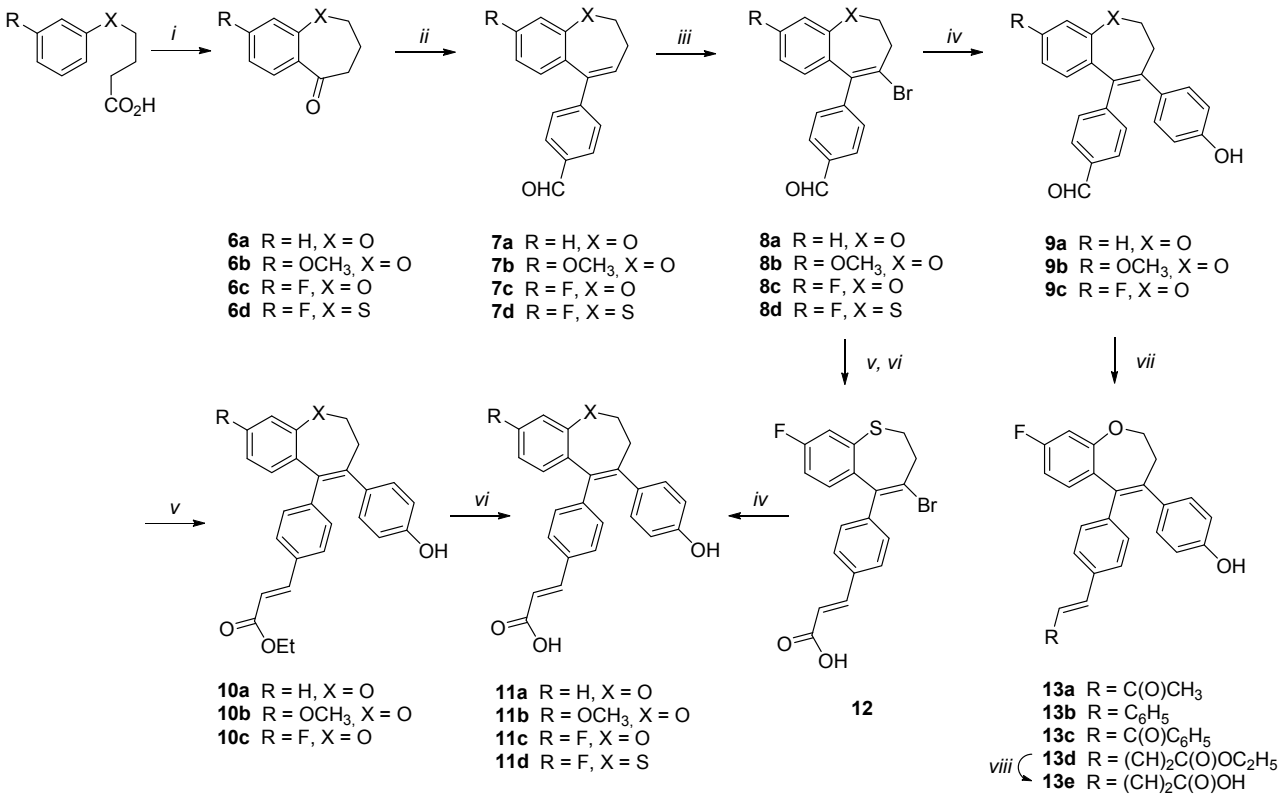


Figure 7. Top ranked poses of **22** in ERα and ERβ[a]

[a]Carbon atoms of compound **22** are illustrated in green in ERα and blue in ERβ (oxygen atoms are red; fluorine and water molecules are bright green). PBD structures 3ERT[37] and 1NDE[38] were used for molecular docking.

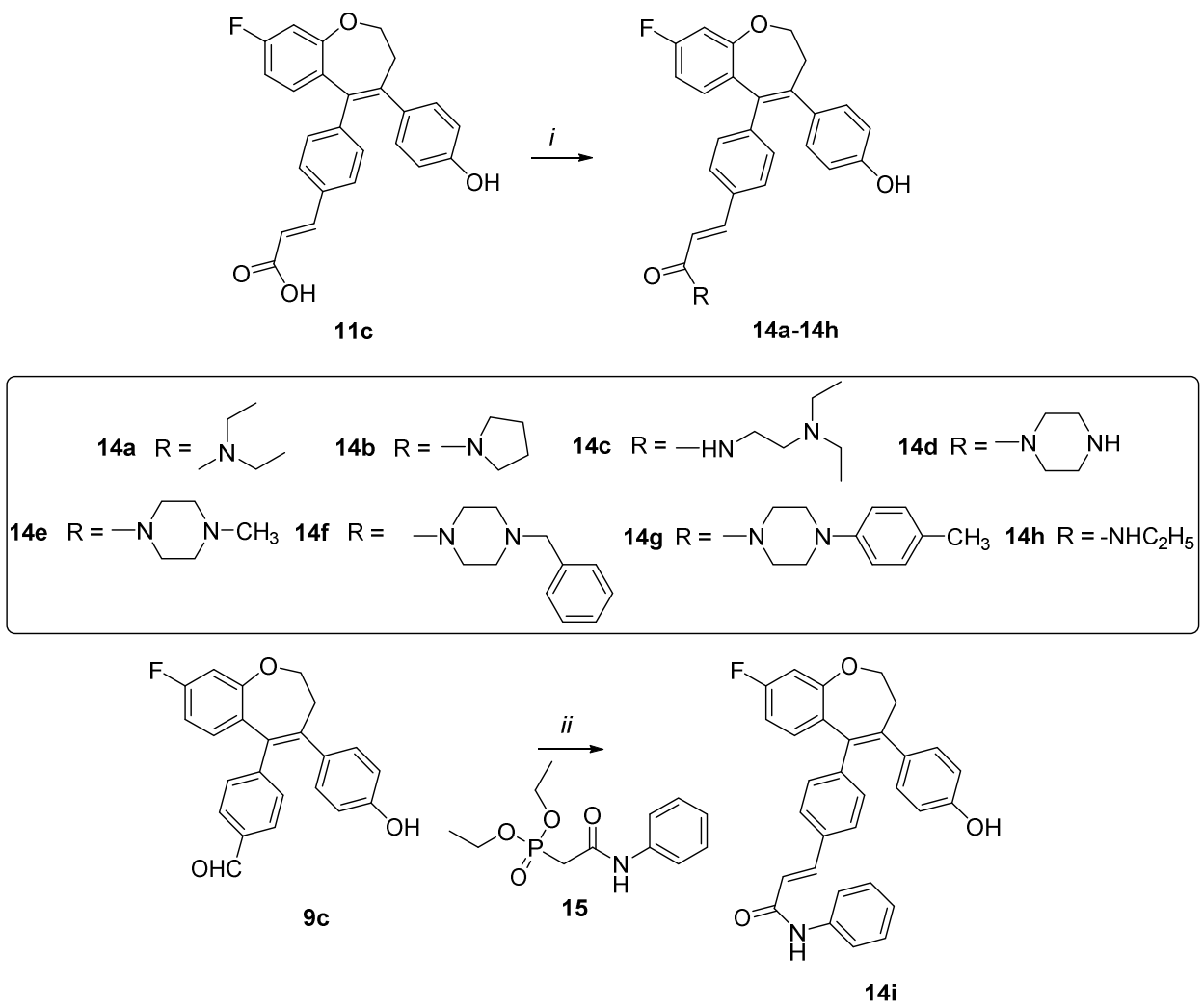


Scheme 1. Synthesis of ER-targeting benzoxepins 11a-11d and 13a-13e[a]



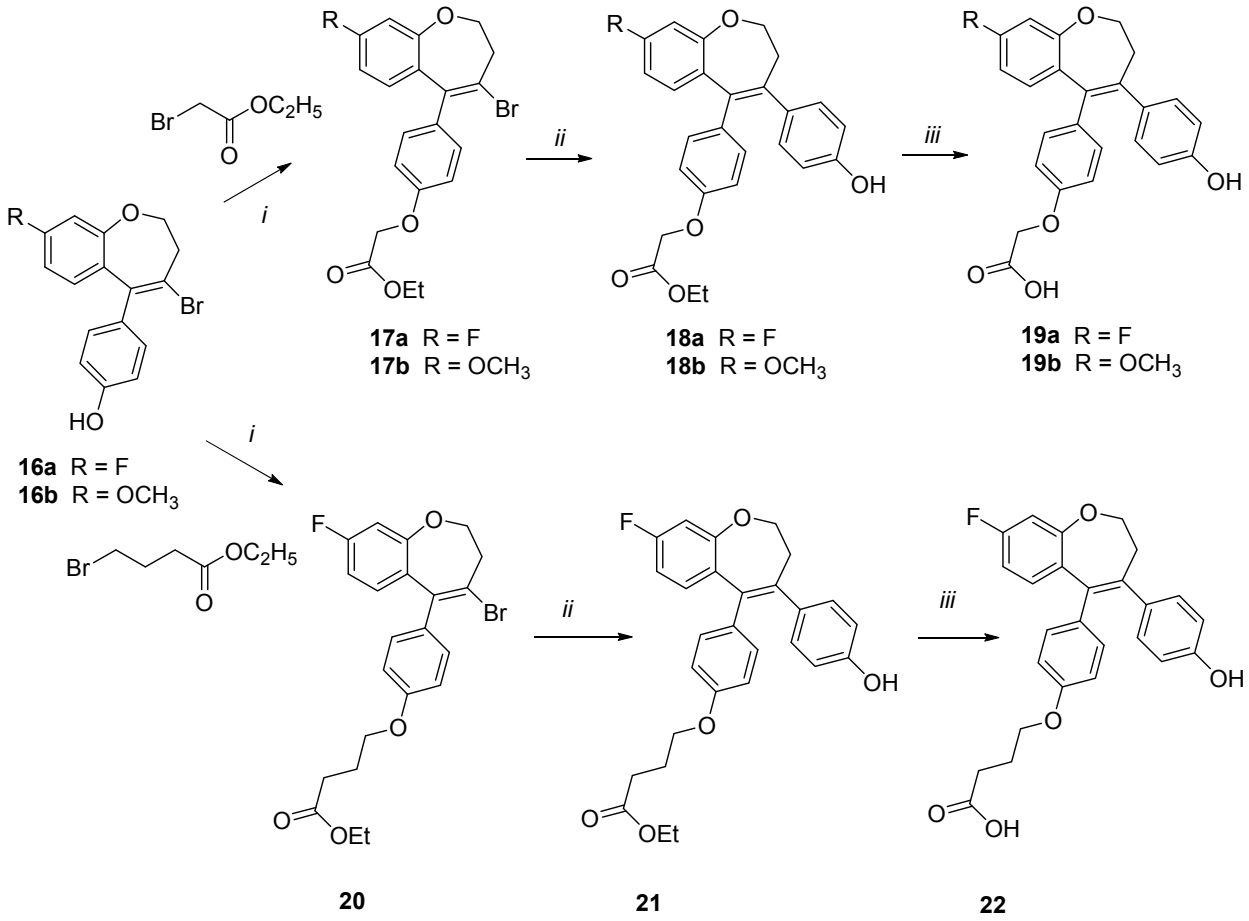
[a]Reagents and conditions: (i) Polyphosphoric acid, 70 °C, 4 h, 29-77%; (ii) $(CF_3SO_2)_2O$, Na_2SO_4, 0 °C → rt, 18 h; then 4-$(CHO)C_6H_4B(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, THF, 85 °C, 6 h, 40-83%; (iii) Pyridinium tribromide, CH_2Cl_2, 0 °C → rt, 18 h, 74-93%; (iv) $Pd(PPh_3)_4$, $HOC_6H_4B(OH)_2$, Na_2CO_3 (2M, aq.), THF, 90 °C, 22 h, 54-100%; (v) $(C_6H_5)_3P{=}CHCO_2C_2H_5$, CH_2Cl_2, reflux, 6 h, 80-98%; (vi) NaOH (2M, aq.), CH_3OH, reflux, 3 h, 83-96%; (vii) $CH_3COCH_2P(O)(OEt)_2$, $C_6H_5COCH_2P(O)(OEt)_2$, $EtO_2CCH{=}CHCH_2P(O)OEt_2$ or $C_6H_5CH_2PPh_3$; reflux, 2 h; NaH, THF, 0 °C → rt, 12 h, 50-67%; (viii) NaOH, EtOH, reflux, 1 h, 86%.

Scheme 2. Synthesis of α,β-unsaturated benzoxepins 14a-14i[a]



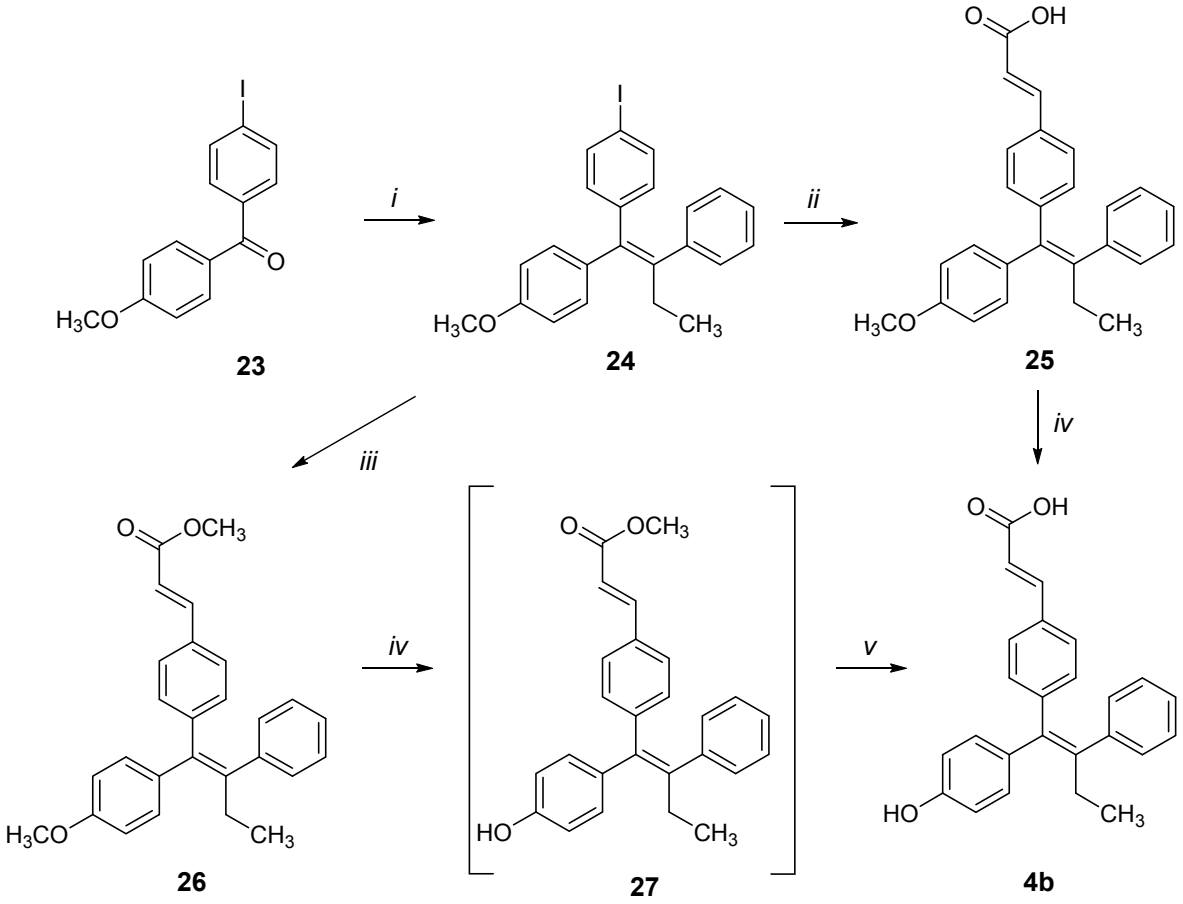
[a]Reagents and conditions: (i) Amine, HOBt, EDCI, Et_3N, CH_2Cl_2, 0 °C → rt, 18 h, 18-92%; (ii) NaH, THF, reflux, 18 h, 83%

Scheme 3. Synthesis of benzoxepins 19a, 19b and 22[a]



[a]Reagents and conditions: (i) K_2CO_3, KI, acetone, reflux, 8 h, 68-77%; (ii) 4-$OHC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, 2M Na_2CO_3, THF, reflux, 6 h, 74-86%; (iii) 1M NaOH, EtOH, reflux, 1 h, 25-69%.

Scheme 4. Synthesis of acrylic acid 4b[a]



[a]Reagents and conditions: (i) $C_6H_5COC_2H_5$, Zn, $TiCl_4$, THF, reflux, 2 h then 3 h, 48%; (ii) $NaHCO_3$, $(nBu)_4NHSO_3$, $Pd(OAc)_2$, $CH_2{=}CHCO_2H$, DMF, 60 °C, 4 h, 94%; (iii) $NaHCO_3$, $(nBu)_4NHSO$, $Pd(OAc)_2$, $CH_2{=}CHCO_2CH_3$, DMF, 60 °C, 4 h, 93%; (iv) BBr_3, CH_2Cl_2, -78 ° C → rt, 3 h, 60%; (v) NaOH (aq), EtOH, reflux, 1 h, 50%.

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