# New scaffolds for the design of selective estrogen receptor modulators

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In the present work we report the synthesis of four new ER ligands which can be used as scaffolds for the introduction of the basic side chains necessary for antiestrogenic activity. Affinities and agonist/antagonist characterization of the ligands for both ERα and ERβ have been determined in a competitive radioligand assay, and in an *in vitro* coactivator recruitment functional assay, respectively. Molecular modelling techniques have been used in order to rationalize the experimental results. Compound **2** is reported as a novel ERβ-agonist/ERα-antagonist. Two compounds show an interesting antitumour profile towards two pancreatic cancer cell lines and have been selected for *in vivo* assays.

## Introduction

The estrogen receptor (ER) is a member of the nuclear receptor gene family binding the steroid hormone estradiol. Two subtypes of ER (ERα and ERβ) which have different tissue distribution, are known and they are thought to regulate different estrogen responses.[1] While ERα is an important receptor in mammary gland and uterus, and is mainly involved in reproductive events, ERβ is a more generally expressed ER, and its role seems to be relevant in the central nervous system, bone, lung, cardiovascular system, ovary, testis, urogenital tract, kidney, and colon.[2] Selective estrogen receptor modulators (SERMs) are a therapeutically important class of ER ligands, which show tissue-dependent agonistic or antagonistic behavior, and are used as first line treatment for estrogen-responsive breast cancer and postmenopausal related disorders.[3–5]

The SERM tamoxifen has remained the antihormonal therapy of choice for the treatment of ER positive breast cancer for the last 30 years (Fig. 1).[6] For anti-osteoporotic therapy, SERM raloxifene has a favourable balance of agonist activities in certain tissues (bone, liver, vasculature)[7] and antagonist activities in other tissues, such as uterus and breast, leading to its use as chemo preventive for breast cancer.[8] A number of ER ligands have been synthesized over the years.[9] They include steroidal and nonsteroidal compounds. Conformationally restricted analogues of raloxifene are among the most potent SERMs described, maintaining the biological structure–activity profile reported previously for raloxifene.[10] On the other hand, since the discovery of ERβ in 1996, compounds that are selective in activating or inhibiting these two ER subtypes are intensively sought after.[1] In particular, ERβ agonists (Fig. 2) constitute potential new drug candidates for diseases such as those related to inflammation, prostate dysfunction, immune system disorders, and depression.[11]

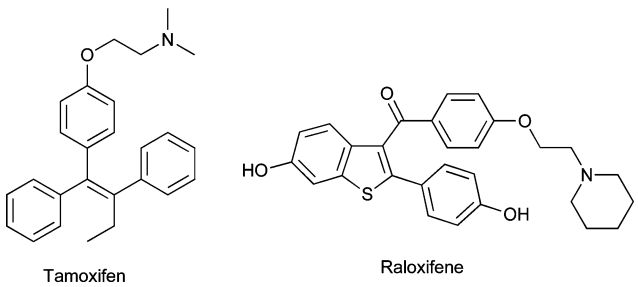


**Fig. 1** Chemical structures of tamoxifen and raloxifene.

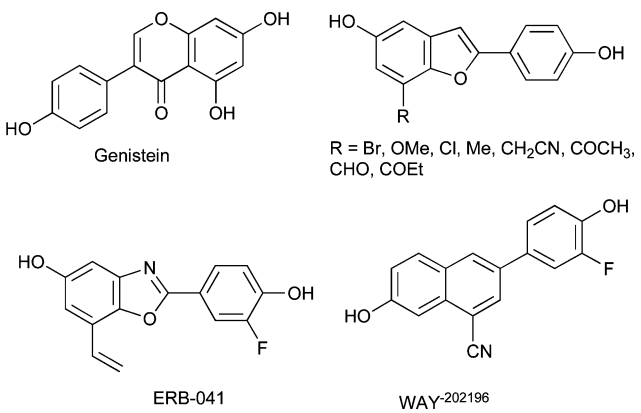


**Fig. 2** Structures of some known ERβ-selective agonists.

The low expression level of ERβ in reproductive tissues such as the uterus, suggests that a selective ERβ agonist may maintain the beneficial effects of estrogen, without the increased risk of breast and endometrial cancer.[12] Genistein is among the first compounds detected showing a 20-fold greater affinity for ERβ but a number of more selective ligands have since been identified.[13] ERβ selective agonist ERB-041 (226-fold selective for β) has been

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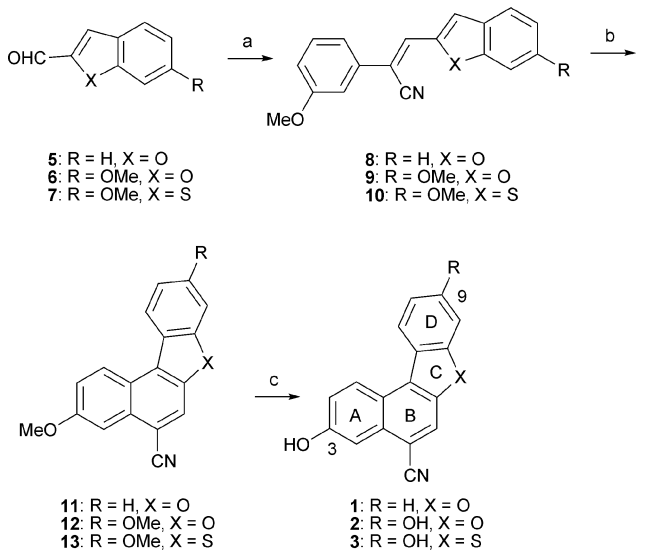
used to demonstrate that this receptor may be a useful target for certain inflammatory processes.[14] Recently, genistein has been reported to display strong inhibition in pancreatic cancer cell lines at doses as low as 20 nM.[15] Other estrogenic agents such as 2-methoxyestradiol[16] and estradiol (E2)[17] have shown antitumour benefits against pancreatic cancer *in vivo.*

Here we report the design, synthesis, evaluation of their affinity for both receptors ERα and ERβ, and antiproliferative activity of new scaffolds for the synthesis of potential SERMs (compounds **1–4**). These compounds contain a cyano group which allows the introduction of the basic side chain present in most SERMs, as has already been demonstrated for compound **24**. A computational study, that has allowed us to rationalize our results, is also presented.

## Results and discussion

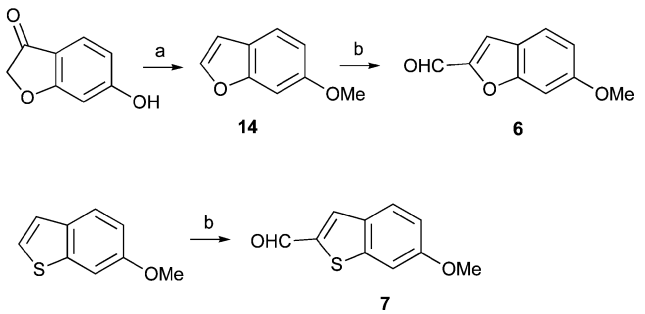
### Chemistry

All target molecules were prepared as depicted in Schemes 1–3. The key step in all syntheses is a Knoevenagel type condensation to give an α-cyanostilbene, followed by an efficient photochemical six electron ring closure which afforded the tetracyclic framework. The synthesis of **1** began with commercially available 1-benzofuran-2-carbaldehyde (**5**), which was condensed with (3-methoxyphenyl)acetonitrile using NaOEt as a base, to give **8**. Irradiation of **8** in the presence of $I_2$ brought about the oxidative photocyclization of the stilbene type system, to yield **11**. The reaction product precipitated in the photochemical reactor, and the pure compound was isolated by filtration. Finally, deprotection was carried out by heating a solution of **11** in DCM with a large excess of $BBr_3$ in a sealed tube for two days.




**Scheme 1** Synthesis of ligands **1–3**. *Reagents and conditions*: (a) (3-methoxyphenyl)acetonitrile, NaOEt–EtOH, 62% for **8**, 80% for **9**, 73% for **10**; b) *hv*, $I_2$, 65% for **11**, 82% for **12**, 84% for **13**; (c) $BBr_3$, DCM, 99% for **1**, 86% for **2**, 60% for **3**.

Compounds **2** and **3** were prepared following the same procedure. The corresponding aldehydes used as starting material are not commercially available, and were prepared as outlined in Scheme 2. Aldehyde **6** was obtained in three steps from 6-hydroxy-3-cumarone, by transformation into 6-methoxybenzofuran (**14**) and subsequent formylation to give **6**. Aldehyde **7** was obtained by formylation of 6-methoxybenzothiophene, which was obtained following a previously described method.[18]




**Scheme 2** Synthesis of aldehydes **6** and **7**. *Reagents and conditions*: (a) (i) $Me_2SO_4$, 65% (ii) $NaBH_4$, MeOH, 70%; (b) n-BuLi, DMF, THF, −78 °C, 61% for **6**, 83% for **7**.

Taking into account that the ER has been reported to have some tolerance for the oxygen–oxygen distance (12.2 Å in genistein, 10.9 Å in estradiol)[13] and that this distance in **2** and **3** is around 10.6 Å, compound **4** (12.9 Å) was designed in order to increase and explore the influence of the oxygen–oxygen distance which, together with the enhanced flexibility of the system, could lead to better interactions with key amino acids in the ligand binding domain of ERβ and, therefore, to an improvement of the affinity for this receptor. Other authors have shown that isoxazole **18** (Fig. 3), with an oxygen–oxygen distance of 13.4 Å has an improved affinity toward ERβ ($IC_{50} = 1.4$ nM) compared to the less bulky analogue **19** (oxygen–oxygen distance of 10.5 Å, $IC_{50} = 54$ nM).[19]

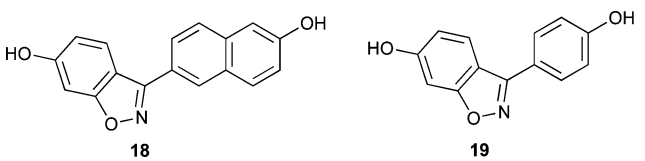
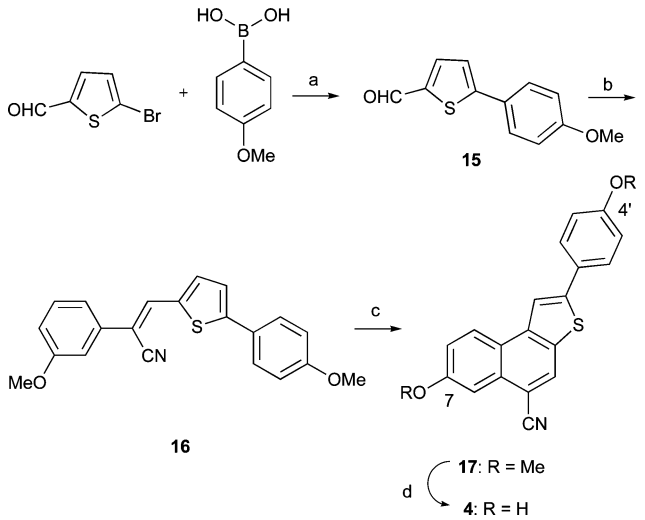


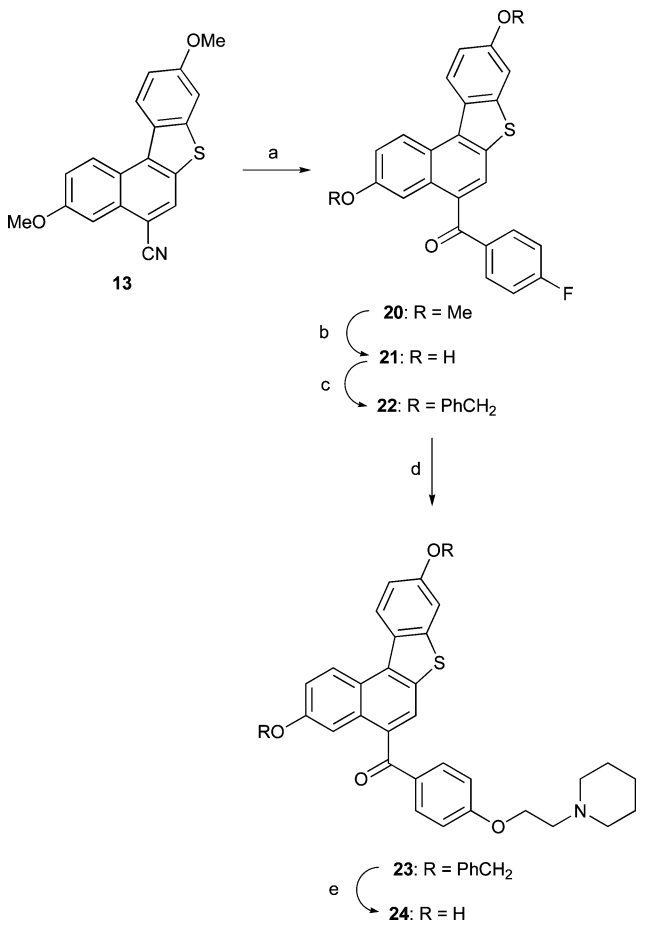

**Fig. 3** Aryl benzisoxazoles as ER β-ligands.

Docking studies for **4** (shown below) suggested that this compound could present ERβ selectivity. Scheme 3 shows the route used to prepare naphthothiophene **4**, beginning with commercially available 5-bromothiophene-2-carbaldehyde. Suzuki coupling led to **15**, and a three-step sequence, analogue to the one used for the synthesis of compounds **1–3** (Knoevenagel type condensation, photochemical cyclization and deprotection), provided compound **4**.

Our approach to obtaining compounds with potential SERM activity relied on the introduction of an aromatic nucleus with a leaving group at the *para* position, appropriate for a nucleophilic aromatic substitution ($S_NAr$), as depicted in Scheme 4. This strategy has been used before for the synthesis of raloxifene.[20] Thus, compound **20** was synthesized in 71% yield by reacting **13** with 4-fluorophenylmagnesium bromide, under standard conditions. At this stage, a change from methoxy to benzyloxy protective group became necessary, in order to avoid chain rupture in the last deprotection step. Treatment of **20** with $BBr_3$ to give **21**, followed by reaction of **21** with benzyl bromide in the presence of $K_2CO_3$,




**Scheme 3** Synthesis of ligand **4**. *Reagents and conditions*: (a) $K_2CO_3$ 2 M, $PdCl_2(PPh_3)_2$, 51%; (b) (3-methoxyphenyl)acetonitrile, NaOEt–EtOH, 88%; c) $I_2$, *hv*, EtOH, 57%; (d) $BBr_3$, DCM, 52%.




**Scheme 4** Synthesis of **24**. *Reagents and conditions*: (a) 4-fluorophenylmagnesium bromide, THF reflux for 24 h, 71%; (b) $BBr_3$, DCM, 95%; (c) benzyl bromide, $K_2CO_3$, EtOH, 33%; (d) 2-(piperidin-1-yl)ethanol–NaH, DMF, 57%; (e) $H_2$, Pd/C (10%).

gave **22**, which was reacted with 2-dimethylamino-1-ethoxide to bring about the substitution of the fluoro atom by the nucleophile, affording **23** (Scheme 4). Finally, deprotection of **23** by catalytic hydrogenation gave the desired compound **24**. This methodology will allow high yield access to a broad series of SERMs based in our new scaffolds (**1–4**), and a SAR study for this type of systems.

## Binding affinities and agonist/antagonist characterization towards ERα and ERβ

The ER binding affinity of compounds **1–4** was determined in a scintillation proximity assay (SPA) using human estrogen receptor expressed in *E. coli* (hERα-LBD or hERβ-LBD), while their agonist/antagonist character was determined using a commercially available EnBio estrogen receptor (α and β)/coactivator ligand assay. Since this assay contains a coactivator peptide (SRC1) which joins the reaction followed by receptor–ligand binding, both agonists and antagonists for ERα and ERβ can be detected.

Table 1 shows a summary of the results obtained in both assays. The most interesting result corresponds to benzonaphthofuran **2**, which presented reasonably good affinity for ERβ ($IC_{50}$ = 0.2066 μM), and behaved as an ERβ selective agonist in the ER/coactivator ligand binding assay ($EC_{50}$ = 0.1 μM). Interestingly, this compound acts through ERα as a weak antagonist with $IC_{50}$ = 9.9 μM. Thus, compound **2** appears to be more potent as an ERβ agonist than as an ERα antagonist, which may be explained by its preferential binding affinity toward ERβ. Although this ligand shows modest affinities for both receptors, such a ERβ-agonist/ERα-antagonist behaviour represents one of the most wanted profiles sought in SERMs research.[21] While β-agonism has been demonstrated to lead to chemopreventive and some other beneficial estrogenic effects, the α-antagonism may avoid undesirable estrogen effects on breast and uterus mediated by ERα-subtype stimulation.

The affinity toward both receptors decreased in benzonaphthothiophene **3**, where the furan ring present in **2** is substituted by a thiophene. Compound **3** clearly showed a reduced ERβ agonism ($EC_{50}$ = 26 μM) relative to that displayed by **2** in the scintillation proximity assay. Compound **1** was designed in order to evaluate the effect that the removal of the benzofuran hydroxyl group had on the binding affinity. The complete lack of activity observed for **1**, both in the scintillation proximity assay and in the ER/coactivator ligand binding assay, suggests that the benzofuran hydroxyl group in **2** makes the major contribution to ligand binding affinity. Thus, it may be involved in the hydrogen bonding network between ERβ residues Glu305 and Arg346 (ERα residues Glu353 and Arg394), and a highly ordered water molecule, mimicking the hydroxyl group present in the A-ring of estradiol. Finally, naphthothiophene **4**, where the oxygen–oxygen distance was increased in order to favour the affinity toward ERβ receptor, did not show the ERβ selectivity predicted by the molecular modelling studies, but it showed a 3-fold selective affinity toward ERα, and a weak antagonistic activity toward the same receptor.

## *In vitro* antiproliferative activity

Recently, it has been shown that *in vitro* pancreatic cell proliferation is highly estrogen sensitive.[15] ERs are frequently expressed in them, and ERβ expression usually outweighs ERα expression. Therefore, antiproliferative activity of compounds showing the highest affinity for ERβ (**2** and **3**) were tested against two

**Table 1** Agonist/antagonist characterization and binding affinity for compounds **1–4**

| | Coactivator ligand assay (μM) | | | | Scintillation proximity assay (μM) | |
|---|---|---|---|---|---|---|
| | ERα | | ERβ | | | |
| Comp. | Agon.[a] $EC_{50}$[c] | Antag.[b] $IC_{50}$[c] | Agon.[a] $EC_{50}$[c] | Antag.[b] $IC_{50}$[c] | ERα $IC_{50}$ | ERβ $IC_{50}$ |
| **1** | Weak | Weak | Weak | Weak | Weak | Weak |
| **2** | — | 9.9 | 0.1 | — | 0.7206 | 0.2066 |
| **3** | — | 6.5 | 26.1 | — | 1.0283 | 0.2935 |
| **4** | — | 12.5 | — | — | 0.5443 | 1.5606 |

[a] Agon.: agonist. [b] Antag.: antagonist. [c] The effective concentration ($EC_{50}$) and inhibitory concentration ($IC_{50}$) for each compound were calculated from the concentration of sample at which the absorbance was diminished to 50%. Estradiol and bisphenol A were used as controls.

pancreatic cancer cell lines, L36PL and Panc-1. Compounds were tested at concentrations ranging from 0.1 to 10 μM and the results are summarized in Fig. 4 and compared with the activity of gemcitabine. Interestingly, compounds **2** and **3** exhibited a cell growth inhibition in both cell lines, which was dose-dependent in the case of L36PL. They showed higher activity than gemcitabine, which was used as control. Therefore, compounds **2** and **3** have been selected to carry out *in vivo* assays and further studies on their mechanism of action.

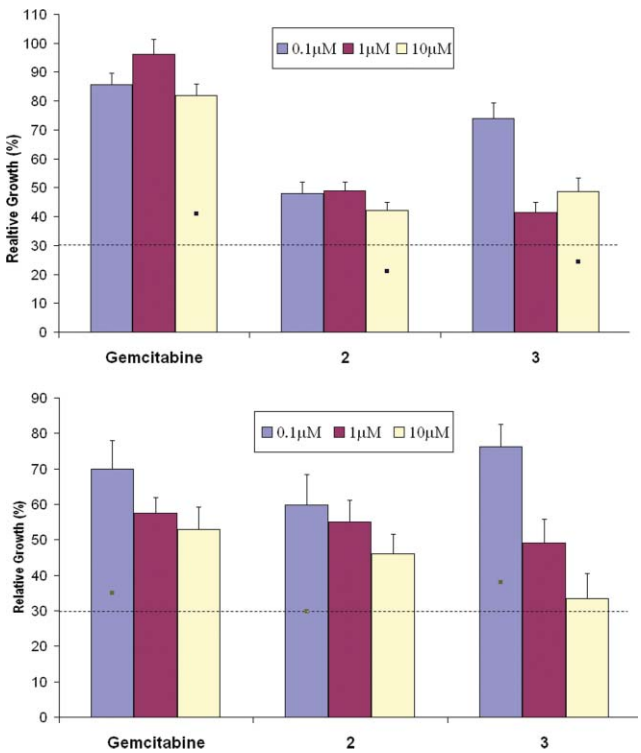



**Fig. 4** Relative growth inhibition of compounds **2** and **3** compared to gemcitabine against L36PL (above) and Panc-1 (below) pancreatic cancer cell lines at concentrations of 0.1, 1 and 10 μM.

## Molecular modelling

In order to analyze the binding mode of this family of compounds to the ER receptors, a docking study was undertaken for compounds **1–4**, employing the ligand binding domain (LBD) of both ERα and ERβ, and the automated docking program AutoDock on the basis of its wide reported use.[22] From the Protein Data Bank[23] we selected PDB codes 1A52, 1ERR (ERα LBD in complex with estradiol and the antagonist raloxifene, respectively), and 1L2J (complex between ERβ LBD and the antagonist THC [(*R*,*R*)-5,11-*cis*-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol]). We first validated AutoDock as an appropriate predictive tool by testing its ability to predict the binding modes present in the crystal structures of 1A52, 1ERR and 1L2J, for estradiol, raloxifene and THC, respectively. Evaluation of the docked structures indicated that AutoDock was able to predict the crystallographic binding poses (data not shown), so we considered it as a valid tool to perform the docking studies.

## Estradiol

Predicted binding orientations for estradiol were studied carefully for comparison purposes. Docking results of estradiol in 1A52 provided different solutions within an energy range of −19.9 to −16.9 kcal $mol^{-1}$. AutoDock was able to reproduce the same key interactions observed in the crystallographic structure: the hydrogen bonds between OH-3 (A ring) and the Glu353-Arg394-HOH triad, and between OH-17 (D ring) and His524 (Fig. 5). With this H-bonding pattern, two poses were predicted: one with the β-face oriented toward Leu525 (like the crystallographic orientation), and a second one, with the β-face pointing toward Met421. We also carried out the docking of estradiol within the ERα LBD from 1ERR (antagonist-like conformation). AutoDock did not predict any solution. On the contrary, the docking of estradiol within the ERβ from 1L2J, gave rise to several poses inside the binding site. Two of them were equivalent to that predicted for estradiol in the ERα (binding energy of −21.5 to

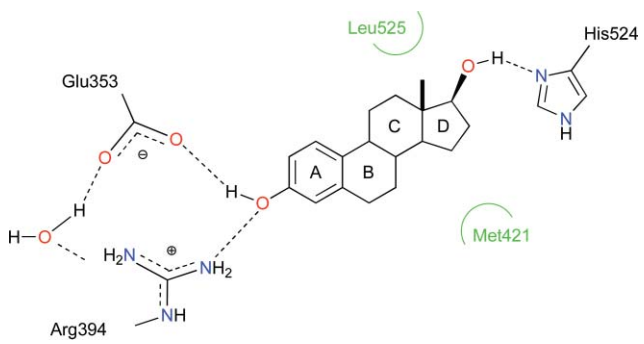



**Fig. 5** Schematic representation of the binding mode of estradiol inside the ERα LBD.

−18.0 kcal mol$^{-1}$), with the β-face oriented toward Leu476 (ERα Leu525), or with the β-face oriented toward Ile373 (ERα Met421).

**Compound 1.** For the three X-ray structures of ER considered, several solutions were identified showing a great heterogeneity on the different orientations obtained from the docking (1A52: −10.5 to −7.7 kcal mol$^{-1}$; 1ERR: unique solution, −9.1 kcal mol$^{-1}$; 1L2J: −13.0 to −10.1 kcal mol$^{-1}$). These orientations corresponded to several combinations of interactions, including the interaction of the OH group with ERα Glu353-Arg394-HOH triad (ERβ Glu305-Arg346-HOH); with ERα His524 (ERβ His475) or with ERα Met343 (ERβ Met295). The cyano group is involved in interactions with ERα His524 (ERβ His475) or with ERα Thr347 (ERβ Thr299), among others. None of the different binding orientations seemed to be preferred. These results show that the benzo[*b*]naphtho[1,2-*d*]furan-5-carbonitrile scaffold is capable of "horizontal and vertical flipping", as it may bind in two orientations that differ by 180° in each axis. The lack of one of the two hydroxyl groups, which are well known to be essential for ER binding, allows a high degree of mobility inside the binding site.

**Compound 2.** ERα: Predicted docking energies for ERα were within the range of −12.4 to −9.4 kcal mol$^{-1}$, and 39 out of 100 solutions were placed inside the binding site, with five different orientations. One predicted orientation (blue in Fig. 6A ), contains only three docking solutions with energy of −12.4 kcal mol$^{-1}$ and is equivalent to that for estradiol, involving interactions between both hydroxyl groups, the Glu353-Arg394-HOH triad and His524. Ring D is placed close to Met421, in a similar way to one of the orientations obtained from the docking of estradiol.

The other non estradiol-like orientations were higher in energy (−11.0 to −9.4 kcal mol$^{-1}$) and involved interactions between the cyano group and Met421, or between the hydroxyl group at position 3 and Met421. Several poses corresponded to orientations perpendicular to the estradiol plane, involving interactions between the OH-9 group and Asp351 (helix-12) and Lys529 (helix-18). This result is in agreement with the weak antagonistic character detected for this compound, taking into account that the antiestrogenic character of tamoxifen has been explained by forcing helix-12 out of position through the interaction of the side chain with Asp351.[24]

ERβ: Remarkably, predicted docking energies for ERβ were within the narrow range of −12.4 to −12.0 kcal mol$^{-1}$, and 95 out of 100 solutions were placed inside the binding site, oriented in four different poses. Two predicted orientations contained 84 docking solutions (blue and white, Fig. 6B. and Fig. 7), and corresponded to orientations equivalent to that for estradiol, in which the hydroxyl groups interact with Glu305-Arg346-HOH triad and His475. For the most populated orientation (68 solutions out of 100), an additional interaction is found between the cyano group and Thr299. It has been reported that hydrogen bonding through Thr299 (ERα Thr347) rather than His475 (ERα His524) can be an alternative-binding mode within the ER binding site.[25] This may represent a third anchorage point inside the ER LBD, suggesting that the cyano group could be modulating the interaction between the rest of the ligand and the receptor. Additionally, ring D nicely occupies the cavity between Met336 (ERα Leu384) and Ile373 (ERα Met421), establishing favourable contacts, not observed for the docking in ERα. Interestingly, it has been proposed that enhanced selectivity for ERβ can be achieved by designing ligands able to bind differently to ERβ Ile373 than to ERα Met421. This interaction has been reported for some ligands derived from the 2-phenylnaphthalene scaffold.[13] Our docking studies show that **2** binds to ERβ in a more efficient way than to ERα, as a result of several subtle interactions that can account for the ERβ selectivity experimentally shown by this compound.

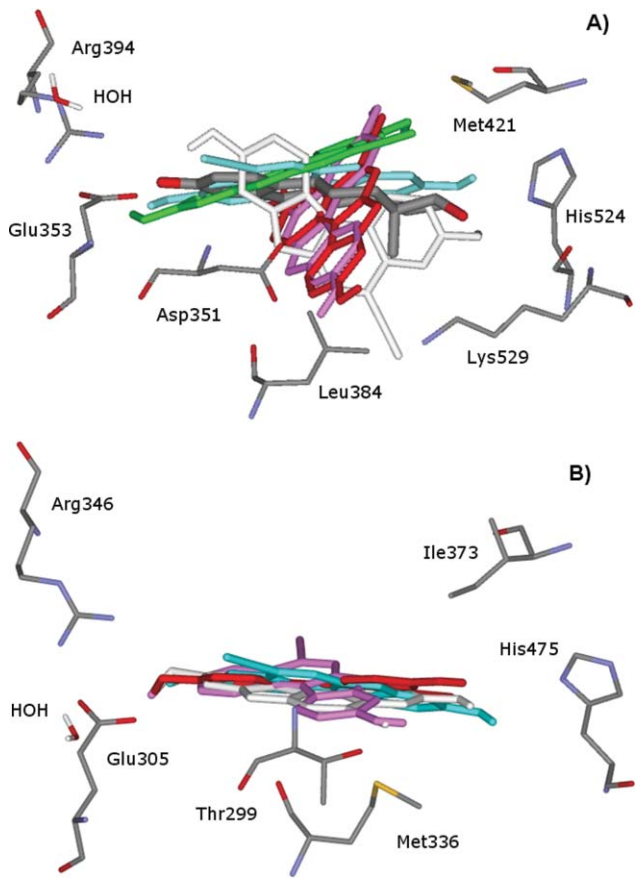



**Fig. 6** Superimposition of the docking orientations obtained for compound **2** in A) ERα LBD; B) ERβ LBD. Estradiol is shown in CPK colours as reference.

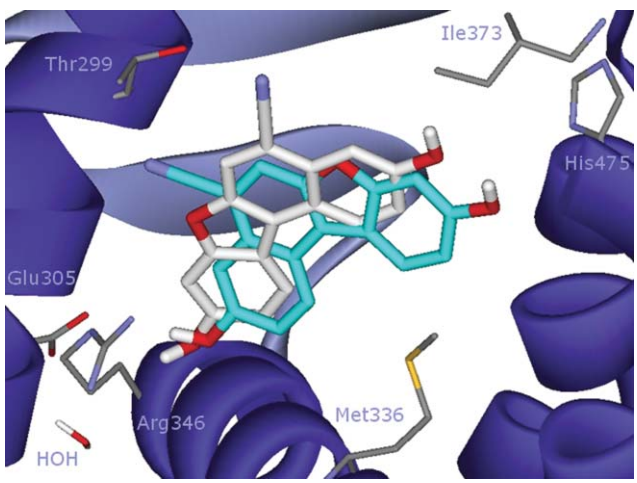



**Fig. 7** Compound **2** docked poses into the binding site of ERβ. Key residues of the site are denoted by sticks.

**Compound 3.** Energy range for solutions inside ERα LBD was −12.3 to −11.1 kcal mol$^{-1}$ (27 solutions out of 100), and for ERβ LBD was −12.3 to −11.9 kcal mol$^{-1}$ (37 solutions out of 100). Docking calculations did not predict any orientation involving similar interactions to those for estradiol, *i.e.* interactions in which both hydroxyl groups are involved in contacts with ERα Glu353-Arg394-HOH triad, and with ERα His524, simultaneously (Fig. 8). Only in ERβ, a pattern of interactions resembling the estradiol-like pattern could be identified (in 17 out of 37 docking solutions): interactions between OH-3 and His475, and between OH-9 and Arg346 (but not Glu305). With this pair of contacts, two "flipped" orientations were identified: with the cyano group oriented toward Leu394, and with the cyano group oriented toward Lys315. Nevertheless, the lack of an appropriate set of interactions could justify the weak ERβ agonistic behavior in comparison to **2**.

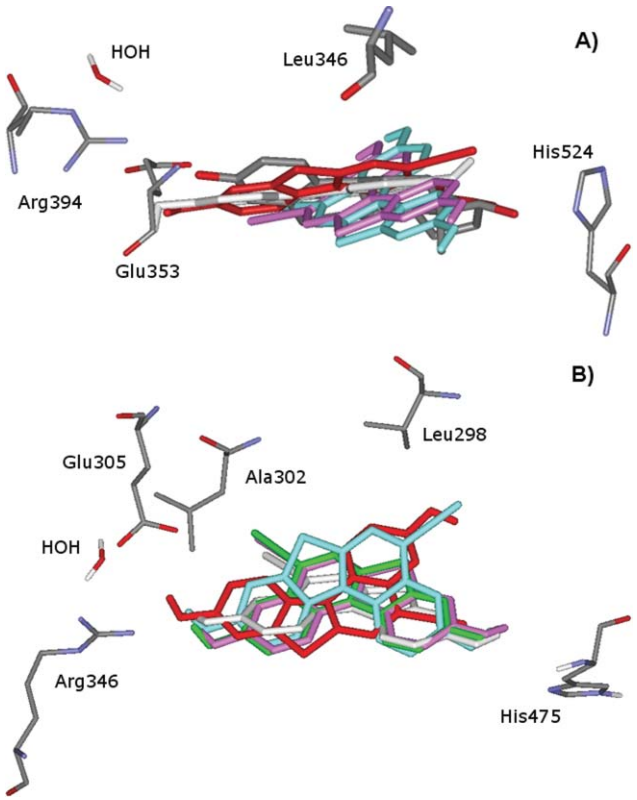



**Fig. 8** Superimposition of the docking orientations obtained for compound **3** in A) ERα LBD; B) ERβ LBD. Estradiol is shown in CPK colours as reference.

We also observed a repulsive interaction between the sulfur atom and the carbonyl group of ERα Leu346, due to its proximity (red in Fig. 8A.). However, in ERβ, the sulfur atom is close to the Cα of Ala302 (3.68 Å), and the equivalent carbonyl group (Leu298) is far away, so there is no repulsive interaction (see Fig. 8B.). In spite of the slight energy difference between ERα/ERβ docking solutions, this observation could explain the weak ERβ selectivity shown by **3**.

**Compound 4.** Docking studies inside the ERα LBD predicted only four solutions inside the binding site. Two of them were grouped into the same cluster (−10.9 kcal mol$^{-1}$), and corresponded to an estradiol-like orientation, with OH-7 pointing toward His524, and OH-4′ toward Glu353-Arg394-HOH triad. The sulfur atom is bumping on Leu387 side chain. Docked solutions within the ERβ LBD pointed to a potential ERβ selectivity. The energy range was −11.5 to −9.5 kcal mol$^{-1}$ (51 solutions out of 100), and four possible orientations could be identified. One of these orientations (16 solutions) corresponded to that for estradiol: the hydroxyl group at position 7 is oriented toward the Glu305-Arg346-HOH triad, and OH-4′ is oriented toward His475. The cyano group is placed into the cavity delimited by Leu343 and Leu380. An alternative orientation, and the most populated one (32 solutions), involves interactions between the OH-7 group and the sulfur atom of Met340, the OH-4′ group and the NH of the backbone of His475, and the stacking interaction between the phenyl ring and the imidazole of His475. Unexpectedly, naphthothiophene **4** only showed a very weak antagonistic activity on ERα, in disagreement with the docking prediction. In order to rationalize this result further computational work, such as molecular dynamic simulations, becomes necessary.

## Experimental

### General methods

Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. $^{1}$H and $^{13}$C NMR were recorded on a Bruker 300-AC instrument. Chemical shifts ($\delta$) are expressed in parts per million; coupling constants ($J$) are in Hertz. Mass spectra were run on a Bruker Esquire 3000 spectrometer. Elemental analyses (C, H, N) were performed on a LECO CHNS-932 equipment at the Microanalyses Service of the University Complutense of Madrid. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. Unless stated otherwise, starting materials used were high-grade commercial products. The photolyses were carried out in a quartz immersion well apparatus with a Pyrex filter and a 400 W medium-pressure Hg arc lamp.

### 6-Methoxy-1-benzofuran 14

To a solution of 6-hydroxy-1-benzofuran-3(2*H*)-one (5 g, 33 mmol) in dry acetone (20 cm$^3$) at room temperature were added $K_2CO_3$ (5.9 g, 43 mmol) and $Me_2SO_4$ (2.49 g, 20 mmol). After stirring for 2 h, the mixture was concentrated *in vacuo*, and diluted with water. The aqueous layer was extracted with DCM, and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give 6-methoxy-1-benzofuran-3(2*H*)-one (3.5 g, 65%) as a yellow solid, mp 102–103 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 2220; $\delta_H$(300 MHz, $CDCl_3$) 3.85 (3 H, s, OMe), 4.60 (2 H, s, $CH_2$), 6.51 (1 H, d, *J* 1.8, ArH), 6.62 (1 H, dd, *J* 8.6 and 1.8, ArH) and 7.52 (1 H, d, *J* 9.2, ArH); $\delta_C$(75.4 MHz, $CDCl_3$) 55.80, 75.44, 96.18, 112.10, 114.20, 124.94, 168.09, 176.43 and 197.49.

To a solution of 6-methoxy-1-benzofuran-3(2*H*)-one (0.5 g, 3.05 mmol) in MeOH (10 cm$^3$) was added $NaBH_4$ (0.17 g, 4.6 mmol) in four successive portions at room temperature. After stirring for 4 h, the crude reaction mixture was quenched by the addition of acetone and treated with an aqueous 3 N HCl solution for 1 h. The acetone and methanol were evaporated and

the aqueous solution was extracted with EtOAc. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to give **14** (0.305 g, 70%) as an oil; $\nu_{max}$ (KBr)/cm$^{-1}$ 1610 and 2810; $\delta_H$(300 MHz, $CDCl_3$) 3.87 (3 H, s, OMe), 6.73 (1 H, s, ArH), 6.93 (1 H, d, *J* 8.5, ArH), 7.09 (1 H, s, ArH), 7.49 (1 H, d, *J* 8.5, ArH) and 7.57 (1 H, s, ArH); $\delta_C$(75.4 MHz, $CDCl_3$) 55.56, 95.76, 106.26, 111.83, 120.54, 121.10, 143.95, 155.84 and 157.88.

### 6-Methoxy-1-benzofuran-2-carbaldehyde 6

To a solution of **14** (2.26 g, 15 mmol) in dry THF (20 cm$^3$), was added dropwise *n*-BuLi (11.25 cm$^3$, 1.6 M in hexane) at −78 °C under argon. The mixture was stirred for 1 h and then DMF (2.19 g, 30 mmol) was added. After stirring for 4 h at room temperature, the mixture was quenched with an aqueous saturated $NH_4Cl$ solution (15 cm$^3$) and stirred at 0 °C for 0.5 h. The aqueous phase was extracted with DCM (3 × 20 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated to dryness. The residue was purified by flash column chromatography using hexane–EtOAc (9 : 1) as eluent to give **6** (1.6 g, 61%) as brownish solid, mp 78–80 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 1655; $\delta_H$(300 MHz, $CDCl_3$) 3.69 (3 H, s, OMe), 6.79 (2 H, m, ArH), 7.35 (1 H, s, ArH), 7.42 (1 H, d, *J* 8.5, ArH) and 9.58 (1 H, s, CHO); $\delta_C$(75.4 MHz, $CDCl_3$) 55.20, 94.89, 114.32, 118.71, 119.50, 123.57, 151.95, 157.43, 161.29 and 178.28. EIMS (*m*/*z*) 199 [M + Na]$^+$.

### 6-Methoxy-1-benzothiophene-2-carbaldehyde 7

The procedure described above was used for the synthesis of **7**. From 6-methoxy-1-benzothiophene (3.824 g, 423 mmol), *n*-BuLi (14.4 cm$^3$, 1.6 M in hexane) and DMF (3.36 g, 46 mmol), **7** was obtained (3.69 g, 83%) as a white solid, mp 98–99 °C (from EtOH) (Found: C, 62.71; H, 4.35; S, 16.76. $C_{10}H_8OS$ requires C, 62.48; H, 4.19; N, 16.68%); $\nu_{max}$ (KBr)/cm$^{-1}$ 1652; $\delta_H$(300 MHz, $CDCl_3$) 3.86 (3 H, s, OMe), 7.02 (1 H, dd, *J* 8.8 and 2.2, ArH), 7.25 (1 H, d, *J* 2.2, ArH), 7.76 (1 H, d, *J* 8.8, ArH), 7.87 (1 H, s, ArH) and 9.97 (1 H, s, CHO); $\delta_H$(300 MHz, $CDCl_3$) 55.30, 104.23, 116.06, 126.82, 132.20, 134.57, 140.58, 144.68, 160.04 and 183.91; EIMS (*m*/*z*) 193 [M + H]$^+$.

### 5-(4-Methoxyphenyl)thiophene-2-carbaldehyde 15

To a solution of 5-bromothiophene-2-carbaldehyde (3.0 g, 16 mmol), 4-methoxyphenylboronic acid (3.65 g, 24 mmol) and bis(triphenylphosphine)palladium(II) dichloride (5% mol, 0.8 mmol) in THF (50 cm$^3$) was added a 2 M solution of $K_2CO_3$ (25 mL, 50 mmol) and the mixture was stirred at 40 °C for 3.5 h. Diethyl ether (30 cm$^3$) was added and the solution was washed with an aqueous 0.5 N NaOH solution (2 × 20 cm$^3$), water and brine, and then dried ($MgSO_4$), filtered and evaporated to dryness. The residue was purified by flash column chromatography using hexane–EtOAc (9 : 1) as eluent to give **15** (1.78 g, 51%) as an orange solid, mp 121–122 °C (Found: C, 65.97; H, 4.82; S, 14.52. $C_2H_{10}O_2S$ requires C, 66.03; H, 4.62; S, 14.69%); $\nu_{max}$ (KBr)/cm$^{-1}$ 1650; $\delta_H$(300 MHz, $CDCl_3$) 3.86 (3 H, s, OMe), 6.95 (2 H, AA′XX′, ArH), 7.30 (2 H, d, *J* 3.8, thiophene-H), 7.62 (2 H, AA′XX′, ArH), 7.71 (1 H, d, *J* 3.8 Hz, thiophene-H) and 9.86 (1 H, s, CHO); $\delta_C$(75.4 MHz, $CDCl_3$) 55.3, 114.4, 122.9, 125.6, 127.7, 137.7, 141.3, 154.4, 160.6 and 182.6; EIMS (*m*/*z*) 219 [M + H]$^+$.

### 3-(1-Benzofuran-2-yl)-2-(3-methoxyphenyl)prop-2-enenitrile 8

To a solution of 3-methoxyphenylacetonitrile (3.02 g, 20 mmol) and 1-benzofuran-2-carbaldehyde (3 g, 20 mmol) in absolute EtOH (50 cm$^3$) was added NaOEt (0.68 g, 10 mmol) and the mixture was stirred at room temperature for 5 h. The precipitate formed was collected by filtration to give **8** (3.78 g, 62%) as a solid, mp 88–90 °C (Found: C, 78.49; H, 4.81; N, 5.14. $C_{18}H_{13}NO_2$ requires C, 78.53; H, 4.76; N, 5.09%); $\nu_{max}$ (KBr)/cm$^{-1}$ 2220; $\delta_H$(300 MHz, $CDCl_3$) 3.87 (3 H, s, OMe), 6.95 (1 H, m, ArH), 7.21 (1 H, t, ArH), 7.27–7.50 (6 H, m, ArH, furan-H and HC=C), 7.56 (1H, d, *J* 8.2, ArH) and 7.65 (1 H, d, *J* 7.7, ArH); $\delta_C$(75.4 MHz, $CDCl_3$) 55.4, 110.7, 111.3, 111.6, 115.2, 117.4, 118.2, 122.0, 123.6, 126.8, 128.1, 128.3, 130.2, 134.8, 151.2, 155.2 and 160.1. EIMS (*m*/*z*) 276 [M + H]$^+$.

### 3-(6-Methoxy-1-benzofuran-2-yl)-2-(3-methoxyphenyl)prop-2-enenitrile 9

A solution of 3-methoxyphenylacetonitrile (1.25 g, 8.5 mmol), compound **6** (1.5 g, 8.5 mmol) and NaOEt (0,068 g, 1.0 mmol) in absolute EtOH (30 cm$^3$) was refluxed for 1 h and cooled to room temperature to produce a precipitate of yellow crystals. The solid was removed by filtration and an additional amount of NaOMe (0.21 g, 3.1 mmol) was added to the filtrate. The mixture was refluxed for 2 h and cooled to room temperature to precipitate more crystals. The two crops were washed with a small amount of EtOH and dried to give **9** (2.1 g, 80%) as a yellow solid, mp 99–101 °C (from hexane) (Found: C, 75.24; H, 4.97; N, 4.86. $C_{19}H_{15}NO_3$ requires C, 74.74; H, 4.95; N, 4.59%); $\nu_{max}$ (KBr)/cm$^{-1}$ 2200; $\delta_H$ (300 MHz; $CDCl_3$) 3.89 (3 H, s, OMe), 3.90 (3 H, s, OMe), 6.91–6.96 (m, 2 H, ArH), 7.08 (1 H, d, *J* 1.8, ArH), 7.20 (1 H, t, ArH), 7.27–7.30 (1 H, m, ArH), 7.37, (1 H, t, *J* 7.9, ArH), 7.39 and 7.43 (2 H, 2 s, ArH and CH=C) and 7.51 (1 H, d, *J* 9.2, ArH). $\delta_C$ (75.4 MHz; $CDCl_3$) 55.42, 55.74, 95.29, 108.58, 111.18, 111.99, 113.86, 114.83, 117.78, 118.12, 121.47, 122.28, 128.16, 130.13, 135.15, 150.52, 156.72, 160.08 and 160.11; EIMS (*m*/*z*) 328 [M + Na]$^+$.

### 3-(6-Methoxy-1-benzothiophen-2-yl)-2-(3-methoxyphenyl)prop-2-enenitrile 10

The procedure described above was used for the synthesis of **10**. From 3-methoxyphenylacetonitrile (2.8 g, 19 mmol), **7** (3.7 g, 19 mmol) and NaOEt (0.32 g, 4.6 mmol), **10** (4.427 g, 73%) was obtained as a yellow solid, mp 149–150 °C (Found: C, 70.79; H, 4.76; N, 4.59; S, 9.90. $C_{19}H_{15}NO_2S$ requires C, 71.00; H, 4.70; N, 4.36; S, 9.98%); $\nu_{max}$ (KBr)/cm$^{-1}$ 2220; $\delta_H$(300 MHz, $CDCl_3$) 3.86 (3 H, s, OMe), 3.88 (3 H, s, OMe), 6.91 (1 H, dd, *J* 8.2, and 2.2, ArH), 7.00 (1 H, dd, *J* 8.8 and 2.2, ArH), 7.15 (1 H, m, ArH), 7.22–7.37 (3 H, m, ArH) and 7.67–7.69 (3 H, m, ArH); $\delta_C$(75.4 MHz, $CDCl_3$) 55.35, 55.55, 104.17, 108.47, 111.18, 114.54, 115.69, 118.07, 118.12, 125.36, 130.05, 130.15, 132.47, 135.14, 135.24, 143.14, 159.06 and 159.97; EIMS (*m*/*z*) 344 [M + Na]$^+$.

### 2-(3-Methoxyphenyl)-3-[5-(4-methoxyphenyl)thiophen-2-yl]prop-2-enenitrile 16

To a solution of 3-methoxyphenylacetonitrile (0.5 g, 3.4 mmol) and 5-(4-methoxyphenyl)thiophene-2-carbaldehyde (0.74 g, 3.4 mmol)

in absolute EtOH (20 $cm^3$) was added NaOEt (0.09 g, 1.7 mmol) and the mixture was stirred at room temperature for 5 h. The precipitate formed was collected by filtration to give **16** (1.04 g, 88%) as an orange solid, mp 132–133 °C (Found: C, 72.50; H, 5.01; N, 4.11; S, 9.39. $C_{21}H_{17}NO_2S$ requires C, 72.60; H, 4.93; N, 4.03; S, 9.23%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220; $\delta_H$(300 MHz, $CDCl_3$) 3.85 (3 H, s, OMe), 3.87 (3 H, s, OMe), 6.88–6.91 (1 H, m, ArH), 6.94 (2 H, AA′XX′, ArH), 7.16 (1 H, t, *J* 2.2, ArH), 7.22–7.26 (2 H, m, ArH and thiophene-H), 7.34 (1 H, t, ArH), 7.55 (1 H, d, *J* 3.8, thiophene-H), 7.61 (1H, s, HC=C) and 7.62 (2 H, AA′XX′, ArH). $\delta_C$(75.4 MHz, $CDCl_3$) 55.3, 106.4, 111.0, 114.2, 114.4, 117.9, 118.3, 122.4, 126.0, 127.4, 130.0, 134.5, 134.5, 135.4, 136.0, 149.2, 160.0 and 160.0. EIMS (*m*/*z*) 348 $[M + H]^+$.

### 3-Methoxybenzo[*b*]naphtho[1,2-*d*]furan-5-carbonitrile 11

A solution of **8** (0.6 g, 1.96 mmol) and $I_2$ (0.5 g, 1.96 mmol) in absolute EtOH (350 mL) was irradiated for 4 h. The solid formed was isolated by filtration to give **11** (0.39 g, 65%) as a white solid, mp 202–204 °C (Found: C, 79.15; H, 4.20; N, 5.22. $C_{18}H_{11}NO_2$ requires C, 79.11; H, 4.06; N, 5.13%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220; $\delta_H$(300 MHz, $CDCl_3$) 4.04 (3 H, s, OMe), 7.46–7.74 (5 H, m, ArH), 8.15 (1 H, s, ArH), 8.39 (1 H, br d, *J* 8.8, ArH) and 8.58 (1 H, d, *J* 9.4, ArH); $\delta_C$(75.4 MHz, $CDCl_3$) 55.5, 104.7, 107.1, 112.3, 118.7, 118.7, 121.0, 122.6, 122.6, 123.4, 123.7, 125.3, 128.1, 131.0, 150.5, 156.9 and 158.2. EIMS (*m*/*z*) 273 $[M]^+$.

### 3,9-Dimethoxybenzo[*b*]naphtho[1,2-*d*]furan-5-carbonitrile 12

A solution of **9** (0.30 g, 0.98 mmol) and $I_2$ (0.25 g, 0.98 mmol) in absolute EtOH (350 $cm^3$) was irradiated for 4 h in a quartz immersion well apparatus with a Pyrex filter and a 400 W medium-pressure Hg arc lamp. The solid formed was isolated by filtration to give **12** (0.247 g, 82%) as a solid, mp 262–263 °C (Found: C, 74.89; H, 4.28; N, 4.83. $C_{19}H_{13}NO_3$ requires C, 75.24; H, 4.32; N, 4.62%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2200; $\delta_H$ (300 MHz, $CDCl_3$) 3.96 (3 H, s, OMe), 4.04 (3 H, s, OMe), 7.10 (1 H, dd, *J* 8.8 and 2.2, ArH), 7.20 (1 H, d, *J* 2.2, ArH), 7.46 (1 H, dd, *J* 8.8 and 2.7, ArH), 7.67 (1 H. d, *J* 2.7, ArH), 8.12 (1 H, s, ArH), 8.24 (1 H, d, *J* 8.8, ArH) and 8.52 (1 H, d, *J* 8.8, ArH); EIMS (*m*/*z*) 304 $[M + H^+]$.

### 3,9-Dimethoxybenzo[*b*]naphtho[1,2-*d*]thiophene-5-carbonitrile 13

A solution of **10** (0.3 g, 0.94 mmol) and $I_2$ (0.24 g, 0.94 mmol) in absolute EtOH (350 $cm^3$) was irradiated for 8 h. The solid formed was isolated by filtration to give **13** (0.2 g, 84%) as a white solid, mp 226–227 °C (Found: C, 71.14; H, 4.27; N, 4.81; S, 10.04. $C_{19}H_{13}NO_2S$ requires C, 71.45; H, 4.10; N, 4.39; S, 10.04%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2200; $\delta_H$ (300 MHz, $CDCl_3$) 3.98 (3 H, s, OMe), 4.05 (3 H, s, OMe), 7.23 (1 H, dd, *J* 9.2 and 2.4, ArH), 7.43–7.47 (2 H, m, ArH), 7.70 (1 H, d, *J* 2.4, ArH), 8.28 (1 H, s, ArH), 8.70 (1 H, d, *J* 9.2, ArH) and 8.90 (1 H, d, *J* 9.2, ArH); EIMS (*m*/*z*) 342 $[M + Na]^+$.

### 7-Methoxy-2-(4-methoxyphenyl)naphtho[2,1-*b*]thiophene-5-carbonitrile 17

A solution of **16** (0.3 g, 0.867 mmol) and $I_2$ (0.2 g, 0.867 mmol) in absolute EtOH (350 $cm^3$) was irradiated for 15 h. The solid formed was isolated by filtration to give **17** (0.17 g, 57%) as an orange solid, mp 216–217 °C (Found: C, 72.91; H, 4.49; N, 4.24; S, 9.22. $C_{21}H_{15}NO_2S$ requires C, 73.02; H, 4.38; N, 4.06; S, 9.28%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220; $\delta_H$ (300 MHz, $CDCl_3$) 3.89 (3 H, s, OMe), 4.01 (3 H, s, OMe), 7.01 (2 H, AA′XX′, ArH), 7.34 (1 H, dd, *J* 8.8 and 1.7, ArH), 7.58 (1 H, d, *J* 1.7, ArH), 7.72 (2 H, AA′XX′, ArH), 7.98 (1 H, s, thiophene-H), 8.21 (1 H, s, ArH) and 8.26 (1 H, d, *J* 8.8, ArH); $\delta_C$ (75.4 MHz; $CDCl_3$) 55.4, 55.5, 104.7, 114.5, 116.1, 118.6, 119.7, 123.4, 125.7, 126.1, 127.9, 127.9, 128.1, 131.2, 132.4, 140.8, 149.5, 158.8 and 160.4; EIMS (*m*/*z*) 346 $[M + H]^+$.

### 3-Hydroxybenzo[*b*]naphtho[1,2-*d*]furan-5-carbonitrile 1

To a solution of **11** (0.20 g, 0.73 mmol) in dry DCM (20 $cm^3$), was added $BBr_3$ (14 $cm^3$, 1 M in DCM, 14 mmol) at 0 °C. The mixture was stirred in a sealed tube at 70 °C for 48 h. After cooling to room temperature, the crude reaction mixture was quenched carefully with ice, water and 1 N HCl. The aqueous layer was extracted with AcOEt (3 × 50 $cm^3$) and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated to dryness to give **1** (0.11 g, 99%) as a solid, which was recrystallized from *n*-butanol, mp > 300 °C (Found: C, 78.22; H, 3.76; N, 5.51. $C_{17}H_9NO_2$ requires C, 78.76; H, 3.50; N, 5.40%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220; $\delta_H$ (300 MHz, DMSO) 7.46 (1 H, dd, *J* 8.5 and 2.4, ArH), 7.53–7.60 (2 H, m, ArH), 7.67 (1 H, t, ArH), 7.88 (1 H, d, *J* 7.9, ArH), 8.64 (1 H, s, ArH), 8.67 (1 H, d, *J* 7.3, ArH) and 8.79 (1 H, d, *J* 8.5, ArH); $\delta_C$ (75.4 MHz; DMSO) 105.9, 107.4, 112.3, 118.0, 119.6, 119.7, 121.0, 121.7, 122.2, 122.8, 123.2, 124.1, 126.4, 128.6, 131.0, 149.8, 156.3 and 156.7; EIMS (*m*/*z*) 260 $[M + H]^+$.

### 3,9-Dihydroxybenzo[*b*]naphtho[1,2-*d*]furan-5-carbonitrile 2

The procedure described above was used for the synthesis of **2**. From **12** (0.16 g, 0.53 mmol), and $BBr_3$ (10.5 $cm^3$, 1M in DCM, 10.5 mmol), and after work up, the residue was purified by column chromatography using AcOEt : MeOH (20 : 1) as eluent to afford **2** (0.12 g, 86%) as a solid, mp > 300 °C (Found: C, 73.68; H, 3.45; N, 5.28. $C_{17}H_9NO_3$ requires C, 74.18; H, 3.30; N, 5.09%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220; $\delta_H$(300 MHz, DMSO) 7.01 (1 H, dd, *J* 8.5 and 2.4, ArH), 7.16 (1 H, d, *J* 2.4, ArH), 7.41 (1 H, dd, *J* 9.2 and 2.4 Hz, ArH), 7.56 (1 H, d, *J* 2.5, ArH), 8.43 (1 H, d, *J* 8.5 Hz, ArH), 8.52 (1 H, s, ArH), 8.68 (1 H, d, *J* 9.2 ArH) and 10.38 (2 H, br s, 2 OH); $\delta_C$(75.4 MHz, DMSO) 98.29, 103.40, 107.21, 113.55, 114.79, 118.44, 119.25, 120.70, 121.19, 123.14, 123.88, 126.55, 131.34, 149.40, 156.63, 158.34 and 159.04; EIMS (*m*/*z*) 274 $[M - H]^+$.

### 3,9-Dihydroxybenzo[*b*]naphtho[1,2-*d*]thiophene-5-carbonitrile 3

The procedure described above was used for the synthesis of **3**. From **13** (0.13 g, 0.41 mmol) and $BBr_3$ (6.12 $cm^3$, 1 M in DCM, 6.12 mmol), and after work-up, the residue was purified by flash column chromatography using AcOEt : hexane (7 : 3) as eluent to give **3** (0.071 g, 60%) as a solid, mp > 300 °C (Found: C, 69.08; H, 3.52; N, 4.71; S, 10.48. $C_{17}H_9NO_2S{\cdot}1/3H_2O$ requires C, 68.67; H, 3.28; N, 4.71; S, 10.78%); $\nu_{max}$ (KBr)/$cm^{-1}$ 2220, 3250 and 3400; $\delta_H$(300 MHz, DMSO) 7.12 (1 H, dd, *J* 9.2 and 2.4, ArH), 7.40 (1 H, dd, *J* 9.2 and 2.5, ArH), 7.49 (1 H, d, *J* 2.4, ArH), 7.53 (1 H, d, *J* 2.5, ArH), 8.70 (1 H, s, ArH), 8.80 (1 H, d, *J* 9.2, ArH), 8.99 (1 H, d, *J* 9.2, ArH), 10.30 (1 H, s, OH) and

10.43 (1 H, s, OH); $\delta_C$(75.4 MHz; DMSO) 104.49, 107.73, 108.49, 115.56, 118.37, 120.25, 122.85, 126.25, 127.19, 128.99, 129.22, 131.65, 132.10, 133.26, 143.29, 156.62 and 157.26; EIMS (*m*/*z*) 290 $[M - H]^+$.

### 7-Hydroxy-2-(4-hydroxyphenyl)naphtho[2,1-*b*]thiophene-5-carbonitrile 4

The procedure described above was used for the synthesis of **4**. From **17** (0.145 g, 0.42 mmol) and $BBr_3$ (8.43 $cm^3$, 1 M in DCM, 18.43 mmol), **4** (0.069 g, 52%) was obtained as a solid, mp > 300 °C; $\nu_{max}$ (KBr)/$cm^{-1}$ 1690 and 3400; $\delta_H$(300 MHz, acetone-$d_6$) 6.90 (2 H, AA′XX′, ArH), 7.30 (1 H, dd, *J* 9.2 and 2.4, ArH), 7.45 (1 H, d, *J* 2.4, ArH), 7.74 (2 H, AA′XX′, ArH), 8.51 (1 H, s, thiophene-H), 8.54 (1 H, d, *J* 9.2, ArH) and 8.63 (1 H, s, ArH), 10.00 (1 H, s OH) and 10.35 (1 H, s OH); $\delta_C$ (75.4 MHz; acetone-$d_6$) 102.9, 106.5, 115.0, 115.3, 115.4, 117.2, 118.32, 121.9, 124.0, 125.7, 127.0, 127.2, 127.3, 130.6, 130.7, 140.4, 149.1, 156.2 and 157.8; EIMS (*m*/*z*) 316 $[M - H]^+$.

### (3,9-Dimethoxybenzo[*b*]naphtho[1,2-*d*]thien-5-yl)(4-fluorophenyl)methanone 20

To a solution of **13** (0.23 g, 0.72 mmol) in THF (10 $cm^3$) was added a solution of 4-fluorophenylmagnesium bromide (14.5 ml, 14.5 mmol, 1.0 M in THF) at RT, and the mixture was refluxed for 24 h. After cooling, HCl 3 N (20 mL) was added to the crude, and a red solid, which was characterized as the imine of **20**, precipitated. The solid was isolated by filtration, and after adding HCl 3 N (125 $cm^3$), the suspension was refluxed for 48 h. The new yellow precipitate formed was extracted with DCM (3 × 20 $cm^3$) and the organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to give **20** (0.21 g, 71%), mp 179–180 °C; $\nu_{max}$ (KBr)/$cm^{-1}$ 1640; $\delta_H$ (300 MHz, $CDCl_3$) 3.87 (3 H, s, $OCH_3$), 3.96 (3 H, s, $OCH_3$), 7.16–7.23 (3 H, m, ArH), 7.40 (1 H, dd, *J* 9.3 and 2.5, ArH), 7.45 (1 H, d, *J* 2.5, ArH), 7.71 (1 H, d, *J* 2.9, ArH), 7.93–7.98 (3 H, m, ArH), 8.70 (1 H, d, *J* 9.3, ArH) and 8.92 (1 H, d, *J* 9.8, ArH); $\delta_C$ (75.4 MHz; $CDCl_3$) 55.11, 55.43, 105.65, 106.03, 114.31, 115,45, 115.74, 118.96, 124.33, 124.70, 125.49, 125.81, 129.37, 131.04, 131.97, 132.41, 134.93, 142.88, 157.22, 158.20, 163.99, 167.38 and 196.11.

### (3,9-Dibenzyloxybenzo[*b*]naphtho[1,2-*d*]thien-5-yl)(4-fluorophenyl)methanone 22

The procedure described above for **1**–**4** was used for the synthesis of **21**. From **20** (0.13 g, 0.31 mmol), and $BBr_3$ (6.2 $cm^3$, 1 M in DCM, 6.2 mmol), and after work up, a solid was obtained. Then, HCl 3 N (125 mL) was added and the mixture was refluxed for 48 h. After cooling, the mixture was extracted with AcOEt (3 × 50 $cm^3$) and the combined organic extracts were washed with $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated to dryness to afford **21** (0.114 g, 95%) as a brown solid, which was used in the next step without further purification; $\delta_H$ (300 MHz, acetone-$d_6$) 7.22 (1 H, d, *J* 8.6, ArH), 7.32 (2 H, m, ArH), 7.42 (1 H, d, *J* 9.2 Hz, ArH), 7.54 (1 H, s, ArH), 7.58 (1 H, s, ArH), 7.98 (2 H, m, ArH), 8.05 (1 H, s, ArH), 8.83 (1 H, d, *J* 8.6 Hz, ArH) and 9.05 (1 H, d, *J* 9.2 Hz, ArH).

A solution of **21** (0.12 g, 0.31 mmol), $K_2CO_3$ (0.43 g, 3.1 mmol) and benzyl bromide (0.4 ml, 3.1 mmol) in EtOH (3 $cm^3$) was refluxed for 24 h. The reaction was then diluted with AcOEt, washed with water and brine, dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash column chromatography using hexane : AcOEt (9 : 1) as eluent to give **22** (0.06 g, 33%) as a yellow oil; $\delta_H$ (300 MHz, $CDCl_3$) 5.11 (2 H, s, $CH_2$), 5.20 (2 H, s, $CH_2$), 7.14–7.53 (15 H, m, ArH), 7.80 (1 H, s, ArH), 7.94 (3 H, m, ArH), 8.70 (1 H, d, *J* 9.2, ArH) and 8.92 (1 H, d, *J* 9.3 ArH).

### (3,9-Dibenzyloxybenzo[*b*]naphtho[1,2]thien-5-yl)(4-[2-(piperidin-1-yl)ethoxy]phenyl)methanone 23

To a solution of 2-(piperidin-1-yl)ethanol (0.26 g, 2 mmol) in DMF (5 ml) was added NaH (0.05 g, 2 mmol) and the mixture was stirred at RT for 30 minutes. Then **22** (0.06 g, 0.1 mmol) was added and the stirring was continued for 6 h. To the solution was added water, and the aqueous layer was extracted with EtOAc, washed with brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography using AcOEt as eluent to give **23** (0.04 g, 57%) as a yellow oil; $\delta_H$ (300 MHz, $CDCl_3$) 1.47 (2 H, m, $CH_2$-piperidine), 1.64 (4 H, m, $2CH_2$-piperidine), 2.52 (4 H, m, $2CH_2$-piperidine), 2.82 (2 H, t, *J* 5.9, $CH_2N$), 4.20 (2 H, t, *J* 5.9, $CH_2O$), 5.08 (2 H, s, $CH_2Ph$), 5.20 (2H, s, $CH_2Ph$), 6.97 (2 H, d, *J* 8.8, ArH), 7.37–7.52 (13 H, m, ArH), 7.75 (1 H, d, *J* 2.4, ArH), 7.90 (1 H, d, *J* 8.8, ArH), 7.94 (1 H, s, ArH), 8.71 (1H, d, *J* 9.3, ArH), 8.92 (1H, d, *J* 9.8, ArH); $\delta_C$ (75.4 MHz; $CDCl_3$) 24.08, 25.86, 55.03, 57.65, 66.27, 69.89, 70.29, 107.03, 107.45, 114.28, 114.87, 119.39, 123.31, 124.77, 125.68, 125.85, 127.52, 127.72, 127.97, 128.14, 128.50, 128.65, 128.74, 129.87, 131.07, 131.35, 132.77, 133.34, 133.83, 136.44, 136.49, 142.65, 156.30, 157.32, 163.06 and 196.49; EIMS (*m*/*z*) 678 $[M + H]^+$.

### (3,9-Dihydroxybenzo[*b*]naphtho[1,2]thien-5-yl)(4-[2-(piperidin-1-yl)ethoxy]phenyl)methanone 24

To a solution of **23** (40 mg, 0.06 mmol) in EtOH (4 $cm^3$) was added Pd/C 10% (30 mg) and the mixture was introduced into a Parr apparatus under an initial pressure of 20 psi. After 2 h the catalyst was removed and the solvent evaporated. The residue was purified by flash column chromatography using AcOEt : MeOH (9 : 1) as eluent to give **24** (13 mg, 46%) as a yellow solid; $\nu_{max}$ (KBr)/$cm^{-1}$ 1640, 3300–3600; $\delta_H$ (300 MHz, $CD_3OD$) 1.65 (2 H, m, $CH_2$-piperidine), 1.81 (4 H, m, $2CH_2$-piperidine), 2.84 (4 H, m, $2CH_2$-piperidine), 3.07 (2 H, t, *J* 4.8, $CH_2N$), 4.34 (2 H, t, *J* 4.8, $CH_2O$), 7.13 (2 H, d, *J* 7.9, ArH), 7.28 (1 H, d, ArH), 7.46–7.53 (3 H, m, ArH), 7.94–7.96 (3 H, m, ArH), 8.84 (1 H, d, *J* 9.2, ArH), 9.07 (1H, d, *J* 9.2, ArH); EIMS (*m*/*z*) 498 $[M + H]^+$.

### Computational methods

Geometries of compounds **1**, **2**, **3**, **4**, estradiol, raloxifene, and THC were first optimized using the *ab initio* quantum chemistry program Gaussian 98[23] and the B3LYP/3-21G* basis set. Partial atomic charges were then obtained using the RESP[24] methodology with the 6-31G* basis set. Different conformers of the ligands were docked using the Lamarckian genetic algorithm (LGA) implemented in AutoDock, by randomly changing the torsion angles and overall orientation of the molecule. A volume for exploration was defined in the shape of a three-dimensional grid (80 × 80 × 90 $Å^3$ for 1A52; 60 × 70 × 95 $Å^3$ for 1ERR; 60 × 90 × 60 $Å^3$ for 1L2J) with a spacing of 0.375 Å that enclosed

the binding site, and included the residues that are known to be crucial for activity. Missing atoms inside the grids were added and crystallographic water close to Glu-Arg was kept for the docking calculations. At each grid point, the receptor's atomic affinity potentials for carbon, aromatic carbon, oxygen, nitrogen, sulfur, and hydrogen atoms were precalculated for rapid intra- and intermolecular energy evaluation of the docking solutions for each ligand. The original Lennard–Jones and hydrogen-bonding potentials provided by the program were used. The parameters for the docking using the LGA were identical for all docking jobs. After docking, the 100 solutions were clustered in groups with root mean square deviations less than 1.0 Å. The clusters were ranked by the lowest energy representative of each cluster.

### $IC_{50}$ assessment on hERα-LBD and hERβ-LBD with scintillation proximity assay (SPA)

All dilutions except the compound dilution series were made in pH 8 assay buffer (1 mM EDTA, 18 mM $K_2HPO_4$, 2 mM $KH_2PO_4$, 20 mM $Na_2MoO_4$, 1mM TCEP). Extract of human estrogen receptor expressed in *E. coli* (hERα-LBD or hERβ-LBD) was thawed on ice from −70 °C and mixed with streptavidin coated SPA beads from Perkin Elmer (RPNQ00007). The amount of receptor used will give an assay signal of approximately 300 ccpm. Compounds were stored in 10 mM DMSO stock solutions at −30 °C and thawed to room temperature prior to use. The compounds were diluted in DMSO to 12 concentrations and 0.018 $cm^3$ of each dilution was added in duplicates to a 384 well assay plate with clear bottom (Corning 3706). The final assay concentration of tracer was 1.2 ± 0.08 nM and the compound concentrations ranged from 37 pM to 157 μM in a total volume of 0.088 $cm^3$. The plates were incubated on a shaker overnight at room temperature, centrifuged (2000 rpm, 5 min) and measured with top and bottom detectors on a 12 detector Trilux Microbeta from Perkin Elmer. A four parameter logistic fit (4PL) was used to analyze the data with XLfit software from IDBS (Guildford, UK) in Microsoft Excel.

### Agonist/antagonist characterization towards ERα and ERβ

The estrogen receptor α and β competitive binding assays were conducted using an estrogen receptor (α and β)/coactivator ligand assay kit (EnBioTec Laboratories, Japan), in accordance with protocol. In brief, the mixture of recombinant human estrogen receptor α or β and samples (compounds **1** to **4**, estradiol or bisfenol A) were added to the wells of a plate coated with biotinylated coactivator peptide, and they were allowed to react at room temperature for 1 h. After washing the wells with wash solution, the anti-ER-HRP solution was added to each well. The absorbance at 450 nm was measured with a microplate reader (Power Wave XS, Biotek) after incubation with the chromogen. For antagonist assay the mixture added to the wells of the plate coated with biotinylated coactivator peptide contained the samples together with a known concentration of estradiol and the recombinant human estrogen receptor α or β. The effective concentration ($EC_{50}$) and inhibitory concentration ($IC_{50}$) for each compound were calculated from the concentration of sample at which the absorbance was diminished to 50%. Estradiol and bisphenol A were used as controls.

### *In vitro* cytotoxicity assays

Two pancreas tumoural cell lines, Panc-1 and L36PL were used in this study. *In vitro* drug sensibility was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye conversion assay. Briefly, cells were trypsinized, seeded at $5 \times 10^3$ per well in 96-well plate, and allowed to grow for 24 h before treatment with exponential increasing concentrations of drugs (Gemcitabine, compounds **2** and **3**) in the presence 0.5% fetal bovine serum. After a 96 h period of treatment, 0.02 $cm^3$ of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (5 mg $cm^{-3}$ in PBS; Sigma) were added to each well and the plates were then incubated for 3 h at 37 °C. The medium was then replaced with 0.1 $cm^3$ DMSO per well. Plates were shaken and the absorbance was measured at 570 nm using a multiwell plate reader (Model 550, Bio-Rad, Inc., Hercules, CA). Each experiment was done in triplicate for each drug concentration and carried out at least thrice.

## Conclusion

A set of ER ligands based on novel tetracyclic scaffolds have been synthesized through an efficient photochemical approach. The affinity toward both ERα and ERβ receptors has been evaluated, and they have been characterized as agonists or antagonists. The most interesting result has been found for compound **2**, which behaves as an ERβ agonist ($EC_{50} = 0.1$ μM) and an ERα antagonist ($IC_{50} = 9.9$ μM), and presents 3.5-fold higher affinity toward ERβ. This result has been rationalized, based on molecular modelling studies, by an additional interaction between the cyano group present in **2** and ERβ Thr299, which is not present in the complex formed by **2** with ERα. Further optimization of this scaffold as ERβ-selective agonist is now underway.

All synthesized compounds are candidates to be transformed into ER antagonists by introduction of an appropriate basic side chain through the cyano group present in the molecule. In fact, a basic side chain has been introduced in compound **3** *via* a five step synthetic pathway, demonstrating that these systems are scaffolds suitable for the construction of new potential SERMs.

Compounds **2** and **3** shown an interesting antitumour profile towards two pancreatic cancer cell lines and have been selected to carry out *in vivo* assays. Interestingly, compound **2** behaves as a ERβ-agonist/ERα-antagonist.

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## References

1 J. A. Katzenellenbogen, R. Muthyala and B. S. Katzenellenbogen, *Pure Appl. Chem.*, 2003, **75**, 2397–2403.
2 J. A. Gustafsson, *J. Endocrinol.*, 1999, **163**, 379–383.
3 V. C. Jordan, *J. Med. Chem.*, 2003, **46**, 883–908.
4 V. C. Jordan, *J. Med. Chem.*, 2003, **46**, 1081–1111.

5 M. J. Meegan and D. G. Lloyd, *Curr. Med. Chem.*, 2003, **10**, 181–210.
6 V. C. Jordan, *Nature*, 2003, **2**, 205–213.
7 A. Cranney and J. D. Adachi, *Drug Saf.*, 2005, **28**, 721–730.
8 T. B. Bevers, *Expert Opin. Pharmacother.*, 2006, **7**, 2301–2307.
9 E. A. Ariazi, J. L. Ariazi, F. Cordera and V. C. Jordan, *Curr. Top. Med. Chem.*, 2006, **6**, 195–216.
10 T. A. Grese, L. D. Pennington, J. P. Sluka, M. D. Adrian, H. W. Cole, T. R. Fuson, D. E. Magee, D. L. Phillips, E. R. Rowley, P. K. Shetler, L. L. Short, M. Venugopalan, N. N. Yang, M. Sato, A. L. Glasebrook and H. U. Bryant, *J. Med. Chem.*, 1998, **41**, 1272–1283.
11 B. R. Henke and D. Heyer, *Curr. Opin. Drug Discovery Dev.*, 2005, **8**, 437–448.
12 C. J. Yang, R. Edsall, H. A. Harris, X. C. Zhang, E. S. Manas and R. E. Mewshaw, *Bioorg. Med. Chem.*, 2004, **12**, 2553–2570.
13 R. E. Mewshaw, J. Edsall, J. Richard, C. Yang, E. S. Manas, Z. B. Xu, R. A. Henderson, J. C. Keith, Jr. and H. A. Harris, *J. Med. Chem.*, 2005, **48**, 3953–3979.
14 H. A. Harris, L. M. Albert, Y. Leathurby, M. S. Malamas, R. E. Mewshaw, C. P. Miller, Y. P. Kharode, J. Marzolf, B. S. Komm, R. C. Winneker, D. E. Frail, R. A. Henderson, Y. Zhu and J. C. Keith, *Endocrinology*, 2003, **144**, 4241–4249.
15 S. Konduri and R. E. Schwarz, *J. Surg. Res.*, 2007, **140**, 55–66.
16 G. Schumacher, M. Kataoka, J. A. Roth and T. Mukhopadhyay, *Clin. Cancer Res.*, 1999, **5**, 493–499.
17 C. Sumi, T. Brinckjohnsen and D. S. Longnecker, *Cancer Res.*, 1989, **49**, 6687–6692.
18 K. Takeuchi, T. J. Kohn, D. J. Sall, M. L. Denney, J. R. McCowan, G. F. Smith and D. S. Gifford-Moore, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 759–764.
19 M. S. Malamas, E. S. Manas, R. E. McDevitt, I. Gunawa, Z. B. Xu, M. D. Collini, C. P. Miller, T. Dinh, R. A. Henderson, J. C. Keith, Jr. and H. A. Harris, *J. Med. Chem.*, 2004, **47**, 5021–5040.
20 C. R. Schmid, J. P. Sluka and K. M. Duke, *Tetrahedron Lett.*, 1999, **40**, 675–678.
21 B. J. Deroo and K. S. Korach, *J. Clin. Invest.*, 2006, **116**, 561–570.
22 G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.*, 1998, **19**, 1639–1662.
23 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, *Nucleic Acids Res.*, 2000, **28**, 235–242.
24 A. J. Knox, M. J. Meegan and D. G. Lloyd, *Curr. Top. Med. Chem.*, 2006, **6**, 217–243.
25 P. M. Kekenes-Huskey, I. Muegge, M. v. Rauch, R. Gust and E.-W. Knapp, *Bioorg. Med. Chem.*, 2004, **12**, 6527–6537.

 