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De Novo Design, Synthesis and Evaluation of a Non-Steroidal Diphenylnaphthyl Propylene Ligand for the Estrogen Receptor

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Abstract—There is still a strong need for additional diversity and new chemical scaffolds to allow for the exploration of improved tissue selectivity and finding better selective estrogen receptor modulators (SERMs). Using a de novo design technology a diphenylnaphthyl propylene scaffold, exemplified by (*E*)-**9b**, with ER antagonist activity has been generated. It was prepared by alkylating 1-[4-methoxyphenyl)-2-(4-(2-chloroethoxy)phenyl]-1-propanone under metal halogen exchange conditions with 1-iodo-6-methoxy-naphthalene. Following dehydration and cleavage of the methoxy groups, (*E*)-**9b** was formed by displacement of the chloro group with pyrrolidine. (*E*)-**9b** binding to ER generated calculated K_i values of 3.7 nM for hER_α and 72 nM for hER_β. The antagonism of (*E*)-**9b** was demonstrated in cell transfection assays using the ERE from the vitA2 promotor and the natural ER-responsive pS2 promotor. With increasing concentrations of (*E*)-**9b**, the E_2-dependent response was efficiently inhibited demonstrating that (*E*)-**9b** could function as an anti-estrogen in these assays. Interestingly, ER_α activity was inhibited even below basal levels suggesting that ligand-independent activity of ER_α was also inhibited. Computational docking studies suggest that the placement of the hydroxyl group on the naphthalene group may not be optimal and we are currently exploring additional analogues.

Introduction

Estrogen Receptors (ER) are members of a superfamily of ligand-activated transcription factors, which includes progesterone, androgen, glucocorticoid and mineralocorticoid receptors, as well as receptors for thyroid hormone, retinoids and vitamin D.[1] Additionally, a large group of receptors known as 'orphan receptors', for which no ligand or function has been described, also belong to this superfamily.

Stimulation of ER by endogenous estrogens plays an important role in both male and female physiology. Estrogens are involved in the regulation of cholesterol and lipid levels, the skeletal system, the central nervous system, and reproductive functions.[2,3] However, estradiol (E_2) stimulation is also implicated in the development of breast cancer.[4] Consequently, many ER ligands are being developed with the aim of preventing E_2-mediated tumour growth. Tamoxifen, which was originally developed as an ER antagonist, is currently the hormonal treatment of choice for both pre- and post-menopausal women with breast carcinoma. It is now known that tamoxifen and other selective estrogen receptor modulators (SERMs) display a broad range of agonist and antagonist activity dependent on the tissue and species being evaluated, and that several factors are thought to contribute to this phenomenon.[5–8] A new ER subtype was discovered and designated ER_β.[9] Experiments based on the tissue distribution and pharmacology of ER_α and ER_β suggest that the tissue selectivity of certain estrogens may be related, at least in part, to their different effects at the ER_α and ER_β subtypes.[7,10,11] Other factors that may contribute to selectivity include the tissue-specific presence of co-repressors and co-activators, and the tissue/species presence of different DNA-response elements.[8,12]

Since the tissue selective effects of tamoxifen have been discovered other SERMs with improved selectivity profiles have been or are being developed. As exemplified in Figure 1 these include the benzothiophenes[7,13,14] (raloxifene and related analogues), triphenylethylene

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analogues[7,15,16] (including tamoxifen), along with the indole analogue (TSE-424).[17] These have estrogenic activity on skeletal and cardiovascular systems and are being developed as alternatives to E_2 replacement therapy. On the other hand, ligands that have pure antiestrogenic activity on breast tissues are being developed for the treatment of breast cancer. These include faslodex, and EM-800 (although recently it has been suggested that EM-800 may possess more SERM like activity than pure antiestrogenic activity[18,19]), which are currently in clinical development.

Although these compounds display improved selectivity compared with tamoxifen, optimal tissue selectivity has not yet been demonstrated. As such there is a strong need for additional diversity and new chemical scaffolds to allow for exploration of improved tissue selectivity. Here we describe a diphenylnaphthyl propylene ER ligand that arose from our ligand based de novo design technology along with its ER binding and functional antagonism in cellular assays.

Results

Chemistry

De novo design. Our ligand based de novo design approach is based on the proprietary Evolutionary Molecular Design™ (EMD) computational technology.[20] Briefly, EMD utilizes structural information and biological activity of known pharmacologically active and inactive compounds as input structures (in this case, E_2 along with other steroidal estrogens) to generate new structures that share and/or improve the biological activity of the original compounds (see ref 21 for additional details).

Compounds generated in the de novo design process were grouped into six structural classes or scaffolds. The first group contained structures that were based on E_2, not surprisingly, since E_2 and E_2-like compounds were used as input for the design process. The second class consisted of the triphenylethylene skeleton and the third class a series of flavanoid structures. The remaining three scaffolds were novel and not known in the E_2 literature. We have already reported on one of these novel classes that was based on a phenanthrene scaffold.[21] This second diphenylnaphthyl propylene class, which in addition to having potential as an ER antagonist ligand, also serves to further demonstrate the utility and value of our drug design technology.

Synthesis. The synthetic route for the preparation of the diphenylnaphthyl ligand is given in Scheme 1. Alkylation of anisole with 2-(4-nitrophenyl)propanoic acid using polyphosphoric acid generated **4**. Catalytic

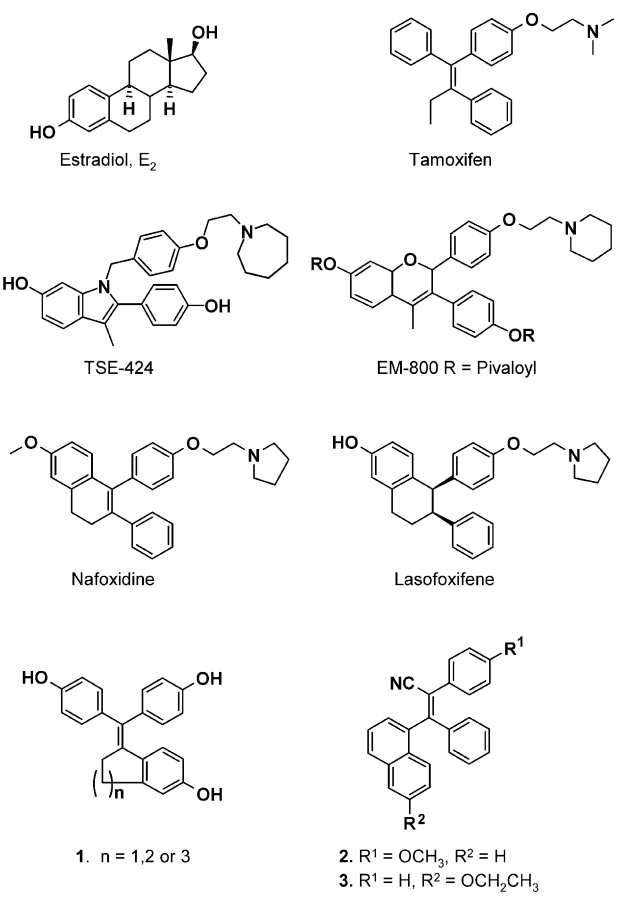
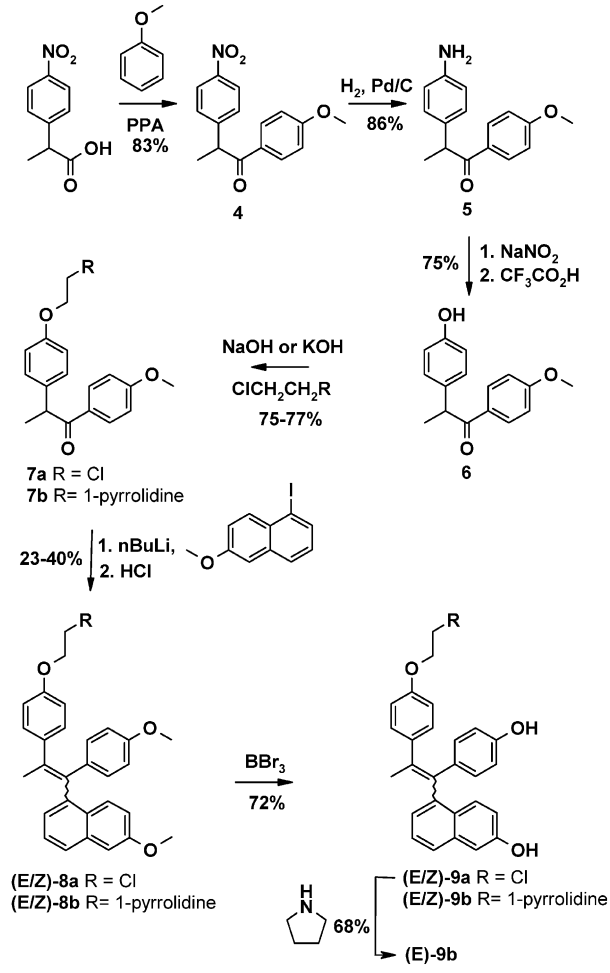


Figure 1.



Scheme 1.

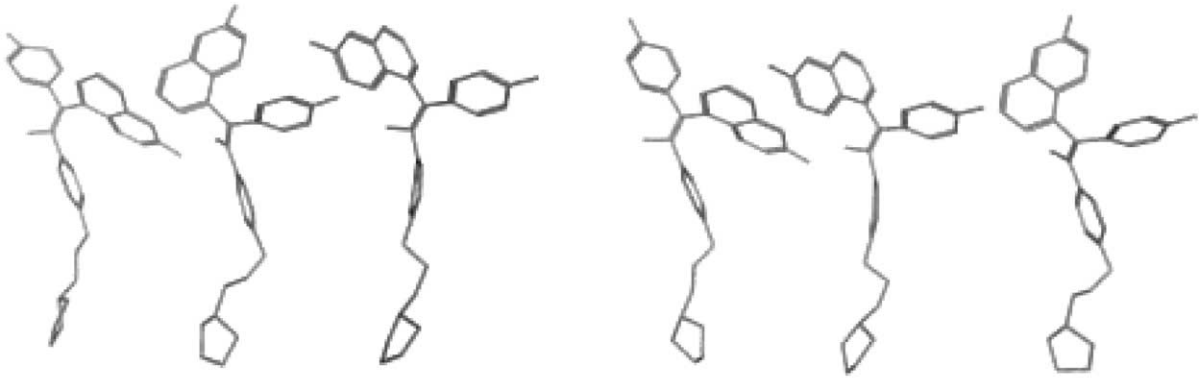


Figure 2. Best docked solutions starting from either the neutral or protonated forms of **9b**. The leftmost 3 structures show the best *Z* binding mode and the two best *E* binding modes of neutral **9b**. The rightmost three structures show the best *Z* binding mode and the two best *E* binding modes of the protonated form **9b**$^+$. All modes have the same absolute coordinates and are facing the same way.

hydrogenation of the nitro group using Pd/C gave **5**. Diazotization of the aniline group of **5** followed by acid treatment gave the phenol **6** via a tetrafluoroborate salt intermediate. Alkylation of **6** with 1-(2-chloroethyl)pyrrolidine gave **7b**. Following halogen metal exchange using *n*-butyl lithium, 1-iodo-6-methoxynaphthalene,[22] was reacted with **7b** to form an alcohol that upon treatment with hydrochloric acid gave a mixture of (*E*/*Z*)-**8b**. Cleavage of the two methoxy groups of (*E*/*Z*)-**8b** using boron tribromide at −78 °C gave a mixture of (*E*/*Z*)-**9b**. As it proved difficult to separate the required *E* isomer form the *Z* isomer, (*E*)-**9b** was prepared via an alternative route. Alkylation of **6** with 1,2-dicloroethane gave **7a**, which was converted to (*E*/*Z*)-**8a** as described for (*E*/*Z*)-**8b** again to give a mixture of *E* and *Z* isomers. After demethylation to give (*E*/*Z*)-**9a** the *E* and *Z* isomers were separated and following nucleophilic replacement of the chlorine with pyrrolidine gave (*E*)-**9b**. However, for the initial binding and cellular based biological studies **9b** was determined to be 9/1 mixture of the *E*/*Z* isomers by NMR. The designations *Z* and *E* forms of **9b** relate to the position of the 6-hydroxynaphthyl group on C atom 1 with respect to the position of the 4-[2-(1-pyrrolidinyl)ethoxy]phenyl group on C atom 2 of the double bond. The *E* and *Z* forms are distinguished from themselves by analysis of the methyl group by ^{1}H NMR.

Computational chemistry. In order to assess its ability to bind to ER_α, **9b** was computationally docked into ER_α starting from either (*Z*)-**9b** or (*E*)-**9b**. Also, as it was unknown whether the basic pyrrolidine containing side chain interacts as the protonated form, both protonated and unprotonated (*Z*)-**9b** or (*E*)-**9b** were docked separately.

For the neutral and protonated forms of **9b**, three low-energy binding modes were identified, one having the *Z* (*cis*) and two having the *E* (*trans*) configuration. The docked solutions for the neutral case (leftmost structures) and for the protonated forms (rightmost structures) of **9b** are shown in Figure 2 with all modes having the same absolute coordinates and facing the same way.

The calculated binding energies given in Table 1 of these docked solutions are defined as follows: $\Delta E_{bind,H,lig}$ is the binding energy with the rigid receptor after relaxing the position of the ligand and neighbouring hydrogens via limited MM optimization, whereas $\Delta E_{bind,flex}$ is the binding energy after partial relaxation of the binding site.

As was the case for raloxifene, the protonated binding modes are generally lower in energy than the neutral binding modes. For both forms, the three binding modes occupy the pocket differently, with the naphthalene ring on either the left or the right hand side. Similarly, the oxygen atoms of the hydroxyl groups may also end up at different locations in the pocket. In this case, one guide to judge the appropriateness of the position of these groups is from the calculated binding energy. The inappropriate position of these groups will give a high calculated binding energy.

It is possible to get a sense of the position of the hydroxyl groups by comparing the binding modes with that of raloxifene. Shown in Figure 3 are the three binding modes of neutral **9b**, the *cis* mode in red, the

Table 1. Calculated binding energies of the lowest energy binding modes and double bond angles of both neutral and the protonated forms of **9b** and raloxifene, as a result of docking with ER_α

Compound binding mode	$\Delta E_{bind,H,lig}$ (kcal/mol)	$\Delta E_{bind,flex}$ (kcal/mol)	Double bond angle
(*Z*)-**9b**	42.9	7.6	5.5
(*E*)-**9b** mode 1	48.8	28.4	151.9
(*E*)-**9b** mode 2	60.4	14.7	165.4
(*Z*)-**9b**$^+$	50.4	10.2	5.6
(*E*)-**9b**$^+$ mode 1	34.0	−2.8	167.5
(*E*)-**9b**$^+$ mode 2	39.2	7.3	152.4
Raloxifene	−35.1	−43.7	–
Raloxifene$^+$	−66.8	−88.4	–

$\Delta E_{bind,H,lig}$ is the binding energy with the rigid receptor after relaxing the position of the ligand and neighbouring hydrogens via limited MM optimization, whereas $\Delta E_{bind,flex}$ is the binding energy after partial relaxation of the binding site.

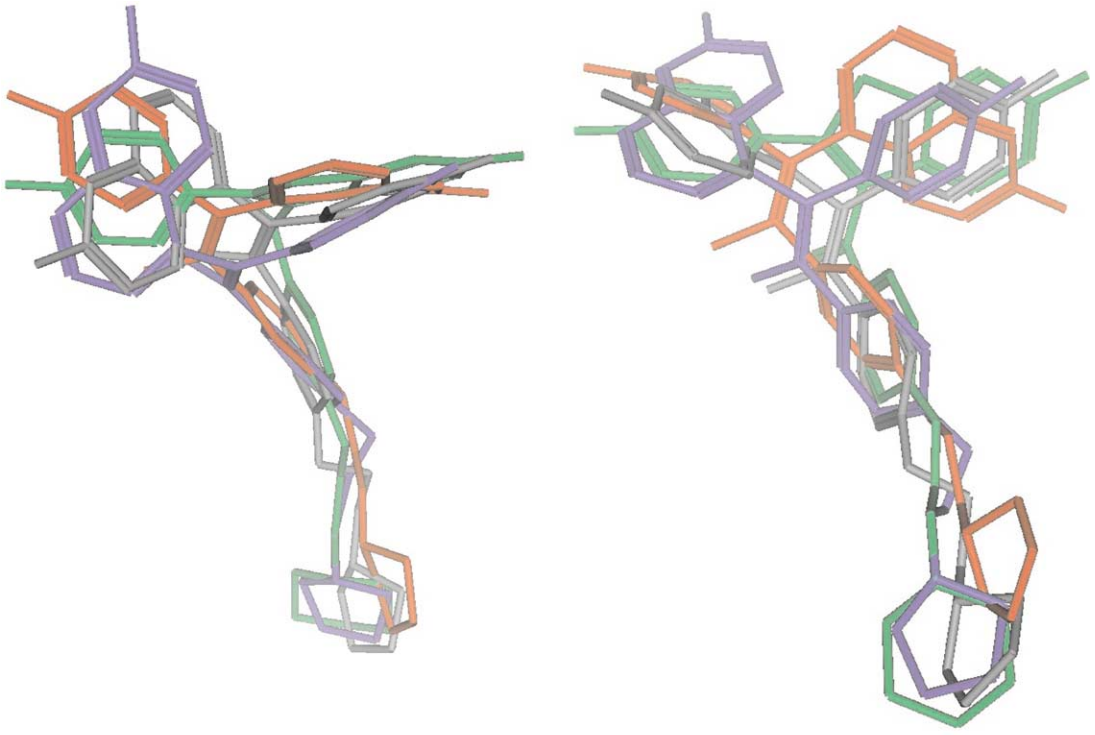


Figure 3. The best *Z* binding mode (red), *E* binding mode 1 (blue) and *E* binding mode 2 (grey) of neutral **9b** overlaid with raloxifene (green). Two different views are shown.

trans mode 1 in blue and the *trans* mode 2 in grey overlaid with raloxifene in green as found when co-crystallized with ER_α. The image is shown from two separate views. In all cases the overlay with raloxifene appears to be adequate although clearly not optimal. Empirically this overlay suggests that by altering the substitution pattern of the hydroxyl groups additional binding activity may be attained (see Discussion).

Biology

Receptor binding assay. In order to determine the relative affinity of (*E*)-**9b** (90% *E*: 10% *Z*) for each receptor, we tested whether (*E*)-**9b** could displace E_2 from ER_α and ER_β using a direct binding assay. As shown in Figure 4, (*E*)-**9b** was able to displace the bound E_2 with a calculated K_i of 3.7 nM for hER_α and 72 nM for hER_β. These results indicate that this compound is a high affinity ligand for ER with a preference for the ER_α isotype.

Cell transfection ER antagonism assays. The estrogen response element (ERE) from the vitellogenin A2 (vitA2) promoter is well known to bind to and mediate the transcriptional activity of ER_α.[23] We therefore used a reporter plasmid containing three synthetic copies of the consensus sequence from the vitA2ERE preceeding a luciferase reporter gene (ERE_3bLuc) to study the effects of (*E*)-**9b** on ER_α-mediated *trans*-activation. As indicated by Figure 5a, when transfected into the hepatocyte cell line, HepG2, human ER_α generated a robust hormonal response in the presence of 10 nM E_2 with more than a 4-fold induction over basal levels of transcription. In the presence of increasing concentrations of (*E*)-**9b**, the E_2-dependent response was efficiently abrogated indicating that this molecule could function as an anti-estrogen in this assay. Interestingly, inhibition of ER_α activity was reduced even below basal levels suggesting that ligand-independent activity of ER_α was also inhibited. Similar results were obtained in HeLa cells (data not shown).

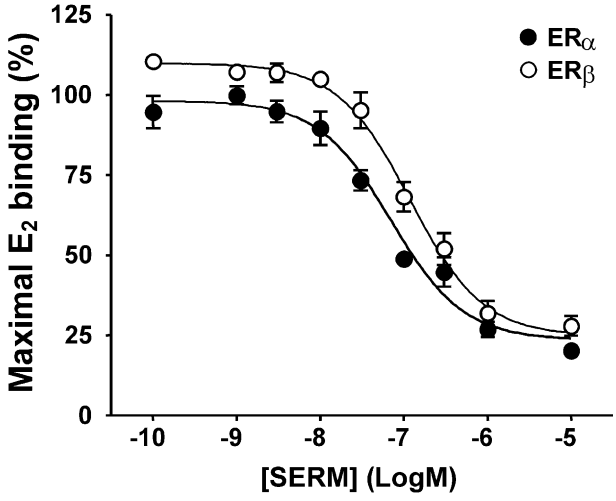


Figure 4. (*E*)-**9b** effectively competes for E_2 binding on both ER_α and ER_β. Receptors produced in vitro were incubated overnight with 0.5 nM [2,4,6,7-^{3}H]E_2 in the presence of an increasing concentration of (*E*)-**9b** as indicated. Unbound steroid was removed with dextran-coated charcoal and the amount of bound E_2 to the receptor was measured by liquid scintillation counting. E_2 binding in the absence of competitor was set to 100%. Results represent the mean ± SEM of three separate experiments conducted in duplicate.

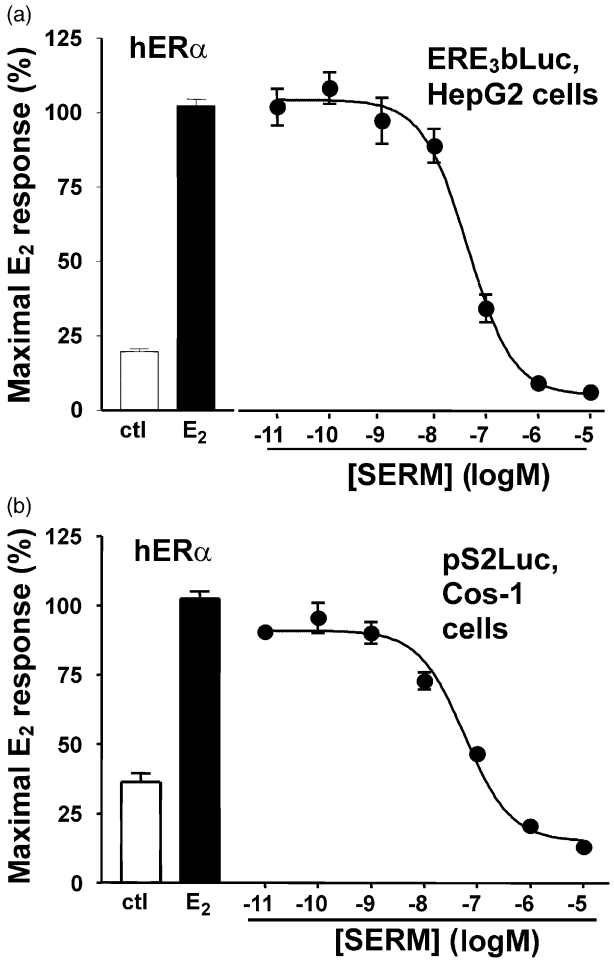


Figure 5. (a) (*E*)-**9b** blocks the E_2-dependent activity of ER_α. HepG2 cells were transfected with 1 μg ERE_3bLuc and 0.1 μg hER_α expression plasmid, treated for 16 h with 10 nM E_2 in the presence of an increasing concentration of (*E*)-**9b** as indicated, and assayed for luciferase activity. The white bar indicates the basal level of ER_α activity in the absence of the ligand and the maximun response in the presence of E_2 alone is depicted as the black bar. Results represent the mean ± SEM of three separate experiments conducted in duplicate. (b) As in Figure 5a except that Cos-1 cells were transfected with the natural E_2-responsive promoter, pS2.

Very few ER-responsive genes actually contain the perfect consensus ERE sequence in their promoters. Therefore, we wished to determine if (*E*)-**9b** could also function as an anti-estrogen on natural promoters. To this end, transient transfection studies were repeated in Cos-1 cells in the presence of a luciferase reporter gene under the control of the well characterised ER-reponsive pS2 promoter.[24] Again, the E_2-dependent response of ER_α was inhibited by (*E*)-**9b** (Fig. 5b).

Discussion

Utilizing a ligand based de novo design approach and steroidal estrogens as input ligands, the non-steroidal diphenylnaphthyl propylene, (*E*)-**9b**, ligand was identified with predicted high affinity for ER_α. As determined from Figure 4 the low nM binding of 3.7 nM with human recombinant ER_α for (*E*)-**9b** (90% E: 10% Z) corroborates the prediction. This de novo designed diphenylnaphthyl propylene scaffold ligand, (*E*)-**9b**, when taken together with the biological results of our previously described phenanthrene scaffold[21] provides another example in the validation of our de novo design technology. In addition, although not part of the design input criteria, (*E*)-**9b** showed a preference for ER_α over ER_β.

Although ER binding is required, to be of value as a potential SERM the antagonism characteristic of a compound must be examined. Preliminary in vitro studies were undertaken to evaluate the antagonistic character of (*E*)-**9b**. As shown in Figure 5a using the ERE from the vitA2 promotor and Figure 5b using the natural promotor pS2, increasing concentrations of (*E*)-**9b** efficiently inhibited the E_2-dependent response. Taken together, these results demonstrate that (*E*)-**9b** could function as an efficient anti-estrogen in different cellular and promoter contexts. We also observed that ER_β-mediated transcriptional activity could be inhibited by (*E*)-**9b** in these assays (data not shown). As predicted by the direct binding assay (Fig. 4), ER_β was inhibited with an efficacy of approximately 20-fold less than ER_α.

As already indicated (*E*-**9b**) as tested was a 9/1 mixture of the *E*/*Z* isomers. It is possible that the *Z* isomer is responsible for the biological activity or that one isomer functions as an agonist whereas the other functions as an antagonist. Although we see substantial antagonistic activity further work is required to isolate the *E*/*Z* isomers and determine their biological effects separately.

Returning to the basic scaffold of (*E*)-**9b** there is evidence to indicate it is not an unreasonable adaptation as an ER ligand. Previously it has been demonstrated that small lipophilic substituents such as vinyl, ethyl or fluoroethyl are well tolerated at the 11-β position of E_2 as shown by 11-β-vinyl estradiol (see Fig. 6), suggesting that there is steric/hydrophobic tolerance in this region.[25] Moreover, 7-estratetraene-3,17-β-diol has been shown to be nearly as effective as E_2 in terms of binding to ER.[26] Therefore the *trans* naphtholic and propylene moieties of (*E*)-**9b** are in the correct orientations allowing the 4-[(2-pyrrolidin-1-yl)ethoxy]phenyl side chain to be in the right position to potentially confer antagonism of ER.

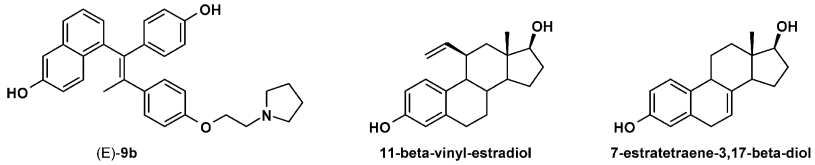


Figure 6.

As given in Figure 1, there are examples of anti-estrogenic and estrogenic ligands for ER containing naphthalene or hydrogenated naphthalene moieties. Dihydro- and tetrahydronaphthalene compounds occur when the proximal phenyl ring in tamoxifen is fused to the ethyl group as in nafoxidine and lasofoxifene.[27] Also, tetrahydro naphthalenes where the distal phenyl ring of tamoxifen ring has been fused to the ethyl group such as in **1** have recently been described.[28] Moreover, naphthalene (and also tetrahydronaphthalene) acrylamides such as in **2** and **3** have been shown to have estrogenic activity.[29] However, this diphenylnaphthyl propylene is, to our knowledge, the first example of a ligand for ER that extends the aromatic bulk with requisite hydroxyl functionality to bind to ER but with the appropriate basic side chain to provide ER binding and antagonistic activity.

We have also carried out extensive computational docking studies to evaluate **9b** as a ligand for ER. Since it was unknown as to the protonation state of the pyrrolidine when bound to ER_α both forms of **9b** were evaluated. As shown in Figure 2, for both forms of **9b**, three low-energy binding modes have been observed, one having the *Z* (*cis*) and two having the *E* (*trans*) configuration. Clearly, protonation of **9b** had no effect on the possible binding modes, only on their relative energies as shown in Table 1. There are, however, a number of observations that arise from these docking solutions. First, there appears to be a tendency for the angle about the double bond of **9b** to be distorted somewhat in the forcefield and letting the double bond flex during the docking process seems to lead to lower energies. The double bond angle is distorted a maximum of 5.6° for the *cis* mode to a maximum of 28.1° for the *trans* mode. Probably in reality these ligands induce a conformational change in the receptor to maintain the geometry of the double bond. Secondly, the lowest energy solution is (*Z*)-**9b** without protonation, whereas it is (*E*)-**9b**$^+$ with protonation. Thirdly, the two-(*E*) binding modes change their relative order of energy on protonation. Finally all of the docked solutions are predicted to bind much less than raloxifene.

A possible explanation as to why the binding energy of **9b** is predicted to be less than raloxifene can be derived from Figure 3. All three binding mode solutions for neutral **9b** from the flexible docking studies are overlaid with raloxifene and although the overlay may be adequate, not all the hydroxyl groups overlay very well with the hydroxyl groups of raloxifene. Hence, even though (*E*)-**9b** exhibited good binding at ER_α of 3.7 nM the overlaid binding modes in Figure 3 suggest that altering the substitution pattern could possibly enhance the activity of the **9b** scaffold. For example, if the hydroxyl group on the naphthyl moiety was moved from the 6 -to the 4- or 5-position, the resulting binding modes may overlay better with raloxifene. We are currently exploring this and have preliminary evidence that suggests this is indeed the case and will be reporting on the results in the future.

Conclusion

The de novo designed non-steroidal diphenylnaphthyl propylene, (*E*)-**9b**, ligand was predicted to have high affinity for ER_α and experimentally gave a K_i binding value of 3.7 nM with human recombinant ER_α that corroborates the prediction. (*E*)-**9b** also displayed antagonist activity in transfection assays. However, agonistic studies are still required to evaluate the full potential of (*E*)-**9b** as a SERM. In addition, computational docking studies suggest that the placement of the hydroxyl group on the naphthalene moiety may not be optimal and we are currently exploring additional analogues. Nonetheless based strictly on a de novo method we are reporting a second scaffold, exemplified by (*E*)-**9b**, with ER antagonistic activity that further demonstrates the value of our de novo design technology.

Experimental

1-(4-Methoxyphenyl)-2-(4-nitrophenyl)-1-propanone (4). 2-(4-Nitrophenyl)propanoic acid (11.1 g, 57 mmol) in anisole (5.8 g, 62 mmol) were mixed with polyphosphoric acid (8.0 g) and heated to 80 °C for 3 h under Ar. The reaction was cooled to room temperature, water (600 mL) was added and the mixture extracted three times with ethyl acetate (100 mL) to yield 13.5 g (83%) of 4. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.58 (3H, d, CH_3), 3.85 (3H, s, OCH_3), 4.80 (1H, q, CH), 6.90 (2H, d, ArH), 7.49 (2H, d, ArH), 7.94 (2H, d, ArH), 8.17 (2H, d, ArH).

1-(4-Methoxyphenyl)-2-(4-aminophenyl)-1-propanone (5). Compound **4** (10.1 g, 35.4 mmol) was dissolved in dioxane (200 mL) and acetic acid (5.0 mL) and 10% Pd/C (0.45 g) were added. The mixture was flushed with Ar and shaken under H_2 (30 psi) at room temperature. The reaction was extracted three times with ethyl acetate (350 mL) and the crude product chromatographed on silica gel with 7% CH_3OH/CH_2Cl_2 to yield 7.8 g (86%) of 5. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.48 (3H, d, CH_3), 3.79 (3H, s, OCH_3), 4.53 (1H, q, CH), 6.61–7.93 (8H, ArH).

1-(4-Methoxyphenyl)-2-(4-hydroxyphenyl)-1-propanone (6). Compound **5** (45 g, 0.176 mol) was added to concentrated HCl (200 mL) and water (150 mL) and cooled to 0 °C in an ice/salt bath. Sodium nitrite (13.4 g, 0.195 mol) was added and the reaction stirred for 30 min. Cold sodium tetrafluoroborate (29.0 g, 0.265 mol) in water (80 mL) was added and the mixture stirred for 2 h in the ice/salt bath. The solid was collected, washed with Et_2O and dried under vacuum overnight. Trifluoroacetic acid (300 mL) was cooled to 0–5 °C and K_2CO_3 (12.5 g, 0.090 mol) was added followed by the solid from above under Ar. The reaction was refluxed for 24 h. The reaction was treated with water (1 L) and stirred for 2 h. The crude product was separated from both the aqueous layer and precipitate and chromatographed on silica gel using 7% CH_3OH/CH_2Cl_2 to yield

33.7 g (74.8%) of 6. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 1.34 (3H, d, CH_3), 3.83 (3H, s, OCH_3), 4.76 (1H, q, CH), 6.67–7.96 (8H, ArH), 9.31 (1H, s, OH).

1-(4-Methoxyphenyl)-2-(4-(2-chloroethoxy)phenyl)-1-propanone (7a). Compound **6** (4.0 g, 15.6 mmol) was dissolved in 1,2-dichloroethane (25 mL). Tetrabutylammonium hydrogen sulfate (0.24 g, 0.7 mmol) and 3M NaOH (20 mL) were added and the reaction was refluxed for 21 h. The mixture was then extracted with Et_2O (2×30 mL) and washed with 1 M HCl (30 mL), and H_2O (2×30 mL). The crude product was chromatographed on silica gel with hexane/ethyl acetate (5:1) to give 3.8 g (77%) **7a** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.52 (3H, d, CH_3), 3.80 (2H, m, $ClCH_2$), 3.85 (3H, s, OCH_3), 4.20 (2H, t, CH_2O), 4.63 (1H, q, CH), 6.88–7.97 (8H, ArH).

(*E*)-1-(4-Methoxyphenyl)-2-(4-(2-chloroethoxy)phenyl)-1-(6-methoxynaphthalen-1-yl)propene (*E*-8a). 1-Iodo-6-methoxy-naphthalene[22] (3.8 g, 11.9 mmol) and *n*-butyl lithium (7.8 mL, 12.5 mmol) were mixed in THF (50 mL) at −78 °C. To this mixture was added **7a** (3.38 g, 11.9 mmol) in THF (40 mL) was added and the mixture stirred at −78 °C for 2 h then at room temperature for 19 h. The reaction was quenched with saturated NH_4Cl (10 mL), washed with brine (60 mL) and water (2×60 mL). The crude product was chromatographed on silica gel with hexane/ethyl acetate (10:1) to give 1.28 g (23.4%) of (*E*)-**8a** as a white solid and 0.95 g (17.4%) of a mixture of (*E*/*Z*)-**8a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (*E*)-isomer 1.88 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 3.85 (2H, t, $ClCH_2$), 3.97 (3H, s, OCH_3), 4.25 (2H, t, OCH_2), 6.57–8.01 (14H, ArH). (*Z*)-isomer 2.35 (3H, s, CH_3), 3.71(2H, t, $ClCH_2$), 3.80 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.07 (2H, t, OCH_2), 6.52–7.91 (14H, ArH).

(*E*)-1-(4-Hydroxyphenyl)-2-(4-(2-chloroethoxy)phenyl)-1-(6-hydroxynaphthalen-1-yl)propene (*E*-9a). The *E*/*Z*-**8a** mixture (0.59 g, 1.3 mmol) was dissolved in CH_2Cl_2 (50 m) and cooled to −78 °C under Ar. BBr_3 in CH_2Cl_2 (1.0 M, 17.5 mL, 17.5 mmol) was slowly added to the solution at −78 °C under Ar. The reaction was stirred at room temperature for 15 h and treated with water (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2×30 mL), washed with 20% $NaHCO_3$ and water (30 mL). The crude product was chromatographed on silica gel with 3% MeOH/CH_2Cl_2) to give 0.07 g of (*E*)-**9a** and 0.27 g of a mixture of (*E*/*Z*)-**9a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (*E*)-isomer 1.88 (3H, s, CH_3), 3.85 (2H, t, $ClCH_2$), 4.25 (2H, t, OCH_2), 6.49–8.00 (14H, ArH). (*Z*)-isomer 2.34 (3H, s, CH_3), 3.72 (2H, t, $ClCH_2$), 4.06 (2H, t, OCH_2), 6.52–7.90 (14H, ArH).

(*E*)-1-(4-Hydroxyphenyl)-2-(4-(2-(1-pyrrolidinyl)ethoxy)-phenyl)-1-(6-hydroxynaphthalen-1-yl)propene (*E*-9b). (*E*)-**9a** (54 mg, 0.125 mmol) was dissolved in ethanol (2 mL) and mixed with of pyrrolidine (0.5 mL). The mixture was sealed and heated to 105 °C under stirring for 15 h. The solvents were removed and the residue chromatographed on silica gel with 7% MeOH/CH_2Cl_2) to give 40 mg (68%) of (*E*)-**9b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 1.76 (3H, s, CH_3), 1.86 (4H, m, $NCH_2\underline{CH_2}$), 2.78 (4H, m, $NC\underline{H_2}CH_2$), 3.02 (2H, m, $NC\underline{H_2}CH_2O$), 4.03 (2H, t, $O\underline{CH_2}$), 6.42–7.87 (14H, ArH), 9.19 (1H, br s, OH), 9.75 (1H, br s, OH).

An alternative method useful for the preparation of (*E*/*Z*)-**9b** is described below.

1-(4-Methoxyphenyl)-2-(4-(2-pyrrolidin-1-yl-ethoxy)-phenyl)-1-propanone (7b). Compound **6** (7.2 g, 28 mmol) and K_2CO_3 (9.5 g, 69 mmol) were added to DMF (50 mL) and heated to 100 °C under Ar. 1-(2-Chloroethyl)pyrrolidine hydrochloride (5.7 g, 33 mmol) was added in portions over 10 min and the reaction maintained at 100 °C for 1.5 h. After cooling, the reaction was filtered and the DMF removed from the filtrate by evaporation. The residue was extracted with ethyl acetate (2×100 mL) and added to the oil concentrated from the filtrate, washed with brine and the solvent evaporated. The crude product was chromatographed on silica gel using 7% CH_3OH/CH_2Cl_2 to yield 7.4 g (75%) of **7b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 1.36 (3H, d, CH_3), 1.67 (4H, m $NCH_2\underline{CH_2}$), 2.51 (4H, m $NC\underline{H_2}CH_2$), 2.76 (2H, t, $NC\underline{H_2}CH_2O$), 3.81 (3H, s, $OC\underline{H_3}$), 4.00 (2H, t, $NCH_2C\underline{H_2}O$), 4.85 (1H, q, CH), 6.85–7.97 (8H, ArH).

(*E*/*Z*)-1-(4-Methoxyphenyl)-2-(4-(2-pyrrolidin-1-yl-ethoxy)phenyl)-1-(6-methoxynaphthalen-1-yl)propene (*E*/*Z*-8b). 1-Iodo-6-methoxy-naphthalene (4.0 g, 14.2 mmol) and *n*-butyl lithium (0.9 g, 14.1 mmol) were mixed in THF (20 mL) at −78 °C. To this was added **7b** (5.0 g, 14.1 mmol) dissolved in THF (25 mL) and the mixture stirred at −78 °C for 1.5 h then at room temperature for 5 days. The crude product was mixed with 30% HCl (20 mL) in ethanol (60 mL) and refluxed for 3 h to give 2.76 g (40%) of **8b** as a mixture of *E* and *Z* isomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (*E*)-Isomer 1.87 (3H, s, CH_3), 1.89 (4H, m, $NCH_2\underline{CH_2}$), 2.77 (4H, m, $NC\underline{H_2}CH_2$), 3.00 (2H, m, $NC\underline{H_2}CH_2O$), 3.77 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.18 (2H, t, OCH_2), 6.56–8.01 (14H, ArH). (*Z*)-isomer 1.87 (4H, m, $NCH_2\underline{CH_2}$), 2.34 (3H, s, CH_3), 2.72 (4H, m, $NC\underline{H_2}CH_2$), 2.91 (2H, m, $NC\underline{H_2}CH_2O$), 3.80 (3H, s, $OC\underline{H_3}$), 3.89 (3H, s, OCH_3), 4.02 (2H, t, OCH_2), 6.52–7.91 (14H, ArH).

(*E*/*Z*)-1-(4-Hydroxyphenyl)-2-(4-(2-pyrrolidin-1-yl-ethoxy)phenyl)-1-(6-hydroxynaphthalen-1-yl)propene (*E*/*Z*-9b). The mixture of (*E*/*Z*)-**8b** (2.5 g, 5.0 mmol) was dissolved in CH_2Cl_2 (200 mL) and cooled to −78 °C under Ar. BBr_3 (8 mL, 79.8 mmol) was diluted with CH_2Cl_2 and added slowly at −78 °C under Ar. The reaction was stirred at room temperature overnight and then treated with water (250 mL). The crude product was chromatographed on silica gel using 7% CH_3OH/CH_2Cl_2 to yield 2.0 g (72%) of **9b** as a mixture of *E* and *Z* isomers.

Computational docking

ER_α co-crystallyzed with the antagonist raloxifene, was taken from the PDB (1ERR) and after the removal of raloxifene, the *cis* and *trans* isomers of neutral and protonated **9b** were rigidly docked into the pocket using

our previously described docking procedure based on the multiple start Monte Carlo algorithm.[21] Due to the high number of conformations tested and optimized in the binding pocket, this method is capable of detecting highly diverse binding modes. In all cases at least 200 random attempts were utilized in docking each form of **9b**. After identifying all reasonable binding modes, these were then redocked (i.e., biasing the calculations using these solutions as first estimates). The possibility to engage in favourable hydrogen bonding interactions was assessed by our protocol of relaxing both the ligand and the receptor hydrogens in the immediate vicinity (3 Å) using 10 molecular mechanics steps.[21] The minimum energy geometry was determined using a stochastic conformational search[30] with Born continuum solvation[31] and the MMFF94 force field.[32] The search was terminated after 100 conformers were found.

Competitive binding

The human ER_α and ER_β proteins were in vitro transcribed-translated using the rabbit reticulocyte lysate (Promega, Madison, WI, USA) with pCMX-hER_α and pCMX-hER_β templates, respectively, as previously described.[33] K_is were calculated using Prism (Graphpad Software, Inc., San Diego, CA). The cDNAs encoding the full length ERs were generous gifts from Dr. V. Giguère, McGill University Health Center.

Cell culture, DNA transfection, and luciferase assay

For transient transfections, Cos-1 and HepG2 cells (ATCC, Manassas, VA, USA) were seeded in 12-well plates in phenol red-free DMEM (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) supplemented with 10% charcoal-treated FBS, 100 μg/mL penicillin, and 100 μg/mL streptomycin. At 50–75% confluence, cells were transfected with 1.0 μg luciferase reporter plasmid, 0.1 μg receptor expression plasmid, and 0.5 μg pCMX-β-galactosidase expression plasmid using the Polyfect reagent as described by the manufacturer (Qiagen Inc., Mississauga, ON, Canada). Cell treatment, lysis, and assays for luciferase and β-galactosidase were as described previously.[33] Values were expressed as arbitrary light units normalized to the β-galactosidase activity of each sample.

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