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Structure-Based Drug Design: Synthesis, Crystal Structure, Biological Evaluation and Docking Studies of Mono- and Bis-benzo[*b*]oxepines as Non-steroidal Estrogens☆

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Abstract—Mono- and bis-benzo[b]oxepine derivatives have been rationally synthesized to meet the molecular requirement for interaction with estrogen receptor. Bis-benzo[*b*]oxepines (**7** and **9**) and mono-benzo[b]oxepine (**10**) acquire geometry with phenolic groups disposed in a fashion to stimulate estrogen receptor. Structure-based investigation, in vivo activity and docking studies have been described and correlated to demonstrate a practical approach for suitable ligand design.
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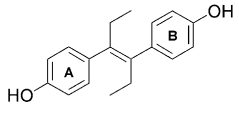
Introduction

The central role of endogenous estrogen in the development and maintenance of the female reproductive organs and other sexual characteristics has been long recognized. More recently their involvement in estrogen deficient syndrome such as osteoporosis,[1] Alzheimer and cardiovascular diseases in females as well as males has also been established.[2–5] Endogenous estrogens are also known to play an important role in lipid, cholesterol metabolism, the skeletal as well as, the central



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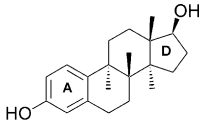
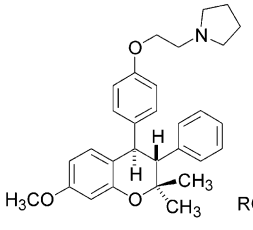


II

nervous systems and reproductive functions.[6,7] Various classes of molecules are being developed as alternatives to estradiol-replacement therapy on skeletal and cardiovascular systems, including raloxifene,[8–10] tamoxifen[11,12] and indole analogues.[13]

The development of nonsteroidal estrogens without the usual drawback of endogenous estrogen for the treatment and prevention of diseases associated with estrogen deficiency, has therefore, attracted much attention.

Structural studies of first nonsteroidal oral contraceptive[14] centchroman (**III**) reveals the importance of the conformation–activity relationship.[15,16] It is for this reason; synthesis of novel estrogens and study of their structural features with



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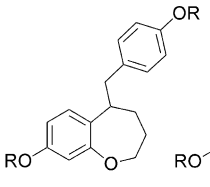

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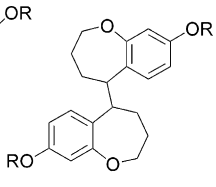

V

respect to their biological activity was undertaken. Although molecular requirement for a ligand to interact

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with estrogen receptor has been reasonably delineated,[17] but finer aspects which make them tissue selective is poorly understood. Since diethyl stilbestrol (DES) (**I**) and d-Estradiol (**II**) bind to estrogen receptor (ER) with high affinity, possess molecular similarity in having hydroxy groups projected in space in a particular orientation, it was conceived that mono-benzo[*b*]oxepine (**IV**) and bis-benzo[*b*]oxepine derivatives (**V**), which resemble with DES, would interact with ER and may provide estrogenic ligands of interest.

The accurate prediction of protein–ligand interaction geometries is essential for the success of virtual screening approaches in structure-based drug design. It requires docking tools that are able to generate suitable configuration and conformations of a ligand within a protein binding site and energetic measurements describing the quality of the interaction.[18–22] For this purpose an 'energy-driven' docking method was required that is solely based on the direct optimization of an energy criterion[23] and does not rely on other geometric or combinatorial rules for generating docked ligand orientations and conformations. Traditionally, Monte Carlo simulated annealing has been used as optimization algorithm with the AutoDock program.[24,25] In the most recent version available, a so-called Lamarckian genetic algorithm AutoDock 3.0.5 has been implemented as more efficient alternative.[26] Here, we describe a rational approach for the synthesis, structural correlation, biological evaluation and docking studies of novel benzo[*b*]oxepine ER-ligands (**IV** and **V**).

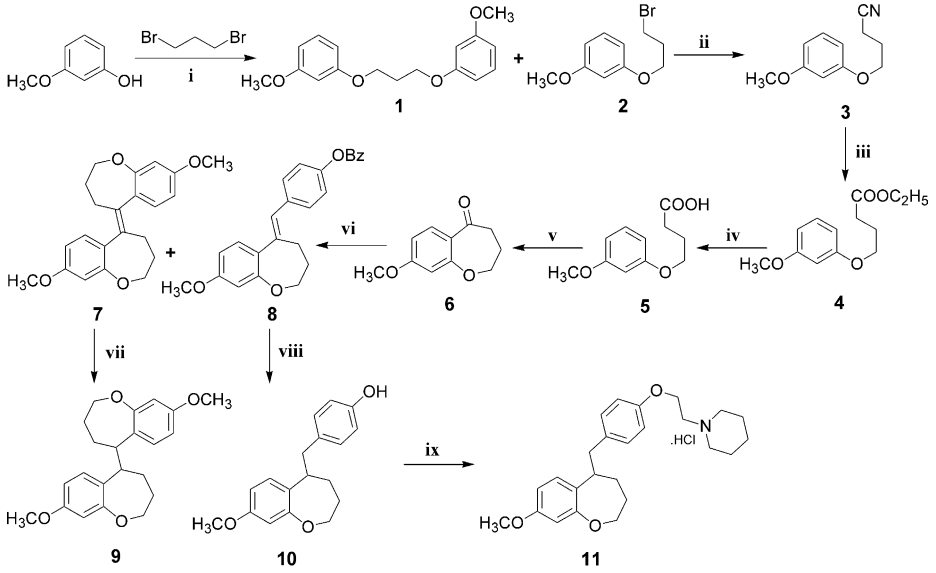
Chemistry

Preparations of the desired prototypes were carried out starting from *m*-methoxy phenol (Scheme 1). Its condensation with 1,3-dibromopropane in aqueous medium in the presence of base gave 1,3-bis-(3-methoxyphenoxy)propane (**1**) and 1-(3-bromopropoxy)-3-methoxybenzene (**2**) as minor and major products, respectively. Compound **2** was treated with NaCN in aqueous medium at elevated temperature to give the corresponding nitrile **3** which on hydrolysis followed by esterification in the presence of H_2SO_4 gave an ester **4** which on saponification led to yield corresponding carboxylic acid **5**. Cyclization of **5** in polyphosphoric acid under anhydrous condition gave 8-methoxy-3,4-dihydro-1-benzoxepin-5(2*H*)-one (**6**). Reductive coupling of **6** with 4-benzyloxybenzaldehyde in the presence of Zn/$TiCl_4$ under anhydrous condition yielded **7** and **8**. The mechanism of above reductive coupling reaction is probably that proposed by McMurray.[27] It involved an electron transfer and generation of an anion radical followed by dimerization. Coordination of the pinacolate to the surface of a small zero valent Ti particle and subsequent cleavage of the C–O bonds lead to the formation of the alkene. Self dimerization resulted in **7** whereas reaction of **6** with benzyloxy benzaldehyde unit produced **8**. Pd/C-catalyzed hydrogenation of **7** and **8** at 30 psi gave **9** and **10**, respectively. Condensation of **10** with 1-(2-chloroethyl)piperidine hydrochloride in acetone in the presence of K_2CO_3 under reflux gave the desired product which, on subsequent treatment with dry ethereal HCl, afforded **11**. All chiral compounds are in the racemate forms.

Result and Discussion

Ligand's structural studies

The stereochemistry at C5 double bond in **7** could be either syn or anti-conformation. The relative disposition of the rings would decide whether the compound elicits biological response and mimics estradiol (**II**). This would then require the rings to be anti-conformation with respect to each other so as to reach out to the D-ring region of estradiol. A NOE experiment indicates



Scheme 1. Reagents and conditions: (i) aq NaOH; (ii) NaCN/aq ethanol; (iii) abs. ethanol/conc H_2SO_4; (iv) aq NaOH; (v) PPA; (vi) 4-benzyloxybenzaldehyde/Zn/$TiCl_4$/THF; (viii) H_2-Pd/C; (ix) 1-(2-chlorohydrochloride/KOH, HCl.

for a possible E-conformation in compound **7**. The observed NOE H1–H3 = 2% and H3–H1 = 2% indicated for a possible E-geometry. The observed H2–H1 = 12% and H1–H2 = 12% indicated equal NOE for these neighbouring protons. Diffraction quality crystals were obtained only for compounds **7** and **9**. Accordingly, their crystallographic studies were undertaken to ascertain the conformation of the two benzo[*b*]oxepine moieties with respect to each other. The molecular structure and conformation of **7**, as assigned by single crystal X-ray analysis[28] is shown in Figure 1(a). There is half a molecule in one asymmetric unit. The benzo [*b*]oxepine moieties are anti- to each other. The aromatic ring is planar while the seven-membered oxepine ring is puckered and adopts a distorted chair conformation. The crystal packing [Fig. 1(b)] reveals that there are two intermolecular C–H...O interactions. The O1 atom in the oxepine ring forms bifurcated hydrogen bonds with H7 and H11A of the other molecule. This is an interesting observation in light of the documented fact that the 3-hydroxy of estradiol forms three hydrogen bonding interactions at the receptor site.[17] From the packing diagram [Fig. 1(b)], it is clear that methyl carbon C11 and aromatic C7 carbon atom may acts as a hydrogen bond donor. The H7...O1 distance of 2.6 Å is smaller compared to that of the H11A, indicates about more acidic nature of H7 than the methyl proton H11A. This has some implications. It is possible that the H7 atom also takes part in H-bonding (weak) interactions along with the stronger O–H... interactions at the receptor binding site.

The importance of these interactions towards the stabilization of the protein structures[29] has been recently highlighted. The idea behind the synthesis and study of the crystal structure of **9**[28] [Fig. 2(a)] was to investigate the effect of reduction of C5–C5B double bond on the molecular conformation/interaction and biological activity. The resulted single bond provide a certain degree of flexibility to the molecule, which might lead to a better binding at the receptor active site. Interestingly the intermolecular interaction pattern for **9** is quite different but the overall conformations of the molecules (**7** and **9**) are quite similar.

Interaction pattern from crystal packing of **9** [Fig. 2(b)] reveals that there is a intermolecular C–H...O interaction (O10...H11–C11) involving oxygen atom of methoxy moiety leading to diad structure. It supports the possibility for the formation of better complex of **9** through H-bonding with estrogen receptor. It is evident from the biological data that **7** as well as **9** elicit slight estrogenicity (Table 1).[30] A flexible alignment of **9** with estradiol were performed using advance program MOE-Flex Align,[31] clearly indicates the similarity in their structures as far as disposing off the methoxy groups of **9** in place of hydroxy groups at the 17-positions of the estradiol is concerned. Best scored superposition (Fig. 3) shows matching the aromatic ring A of estradiol (II) with the corresponding phenyl ring of **9** and the oxygen atom of the methoxy group matched with that of the hydroxyl group for estradiol. The hydroxyl group at

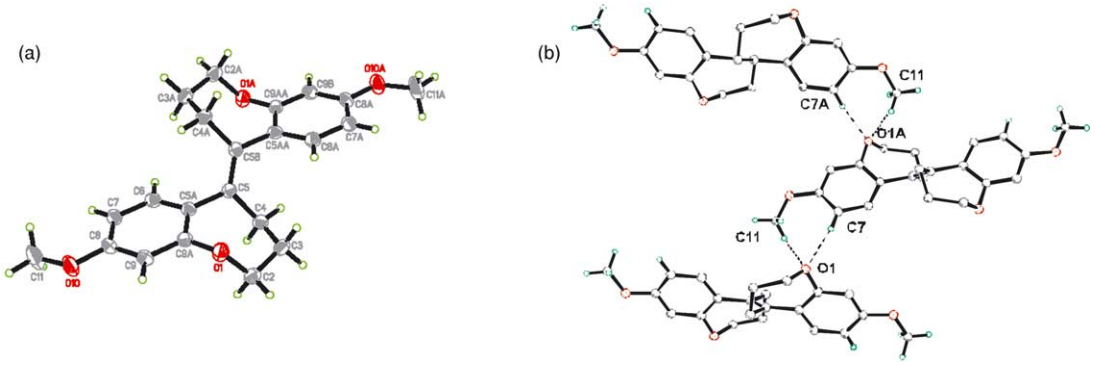


Figure 1. (a) ORTEP diagram showing the crystal structure of **7** with atomic numbering scheme; (b) partial crystal packing diagram of **7** showing weak H-bonding (dotted line) involving O1 atom.

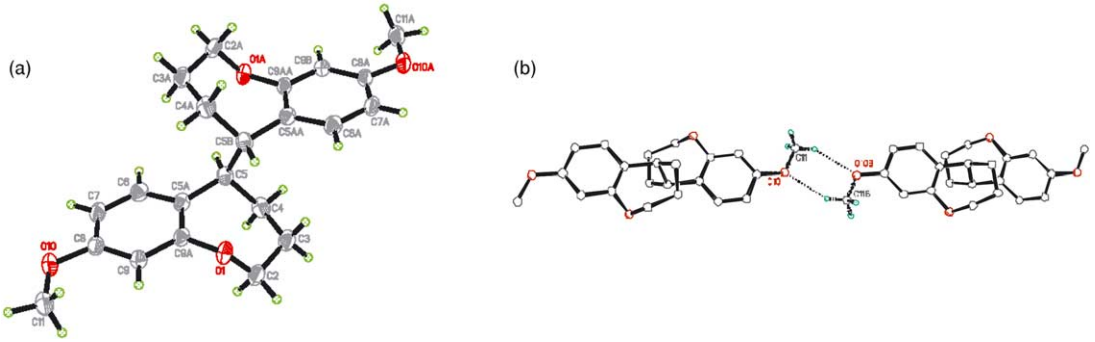


Figure 2. (a) **ORTEP** diagram showing the crystal structure of **9** with atomic numbering scheme; (b) partial crystal-packing diagram showing the dimerization of molecules (**9**) through C–H...O bond involving O10 (Phenoxy) atom.

Table 1. Biological activity data (in vivo)[30]

Compd	Daily dose (mg/kg)[a]	Uterine wt (mg)[b]		Vaginal cornification (%)
		Absolute	10 g body weight	
Ethynyl-estradiol	0.02	89.6±13.5	21.3±2.3	100
7	10	55.0±2.7	16.5±0.8	30–40
9	10	62.0±5.2	19.8±2.0	30
Vehicle	10	16.8±0.8	5.2±0.3	Nil
11	10	58.7±3.2	12.1±0.5	Nil
Vehicle	10	15.5±0.7	3.6±0.2	Nil
10	10	101.7±4.3	25.6±1.4	80–90
Vehicle	10	17.0±0.81	6.8±0.4	Nil

[a]Once daily for 3 days, po; six animals per group.
[b]Mean±SEM.

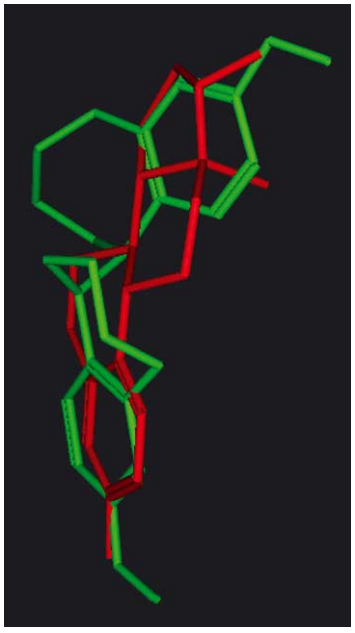


Figure 3. A flexible overlay of the molecule **9** (green) and estradiol (red) showing significant molecular alignment.[31]

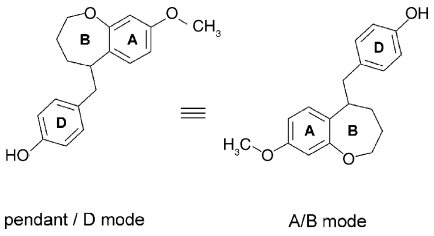


Figure 4. Chemical structure of compound **10**.

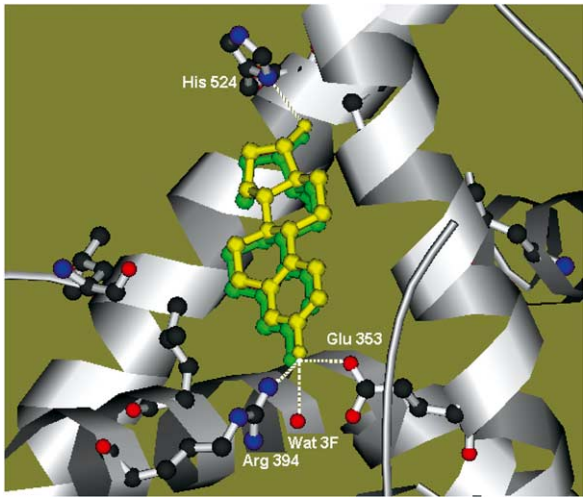


Figure 5. Superposition of the docked estradiol molecule (green) with crystallographic estradiol molecule (yellow) complexed in the binding cavity of ER. The H-bonding are shown by broken white lines.

3-position was considered as the most essential feature (Pharmacophore) as far as the binding with the ER is concerned. The flexible superposition (Fig. 3) of **9** clearly shows that a part of the puckered oxepine ring projects into the 7α-region of estradiol whereas a part of the ring (from the other half of the molecule) occupies the 11β-position of estradiol. Both these positions are known to be tolerant to steric bulk.[8] The moderate estrogenicity elicited by these compounds might then be rationalized in light of the above observations. The molecular structure of **7** and **9** thus explains the possible mimicking of the binding requirements of estradiol through hydrogen bonding at the receptor active site.

In light of the observation regarding the exceptionally high estrogenic profile of **10**, the free phenolic group might mimic the A ring of estradiol so as to dispose the hydroxyl group in the same space as that of the 3-OH group of estradiol. The 3-OH of estradiol contributed more significantly (3-fold) as compared to the 17β-OH towards the binding stability with the ER.[32] The high estrogenecity elicited by **10** might be a consequence of the higher stability of the formed complex between ER and **10** resulting from D-mode of binding (Fig. 4). This was further well established by molecular modeling studies. Regarding the estrogenic profile of **11**, absence of vaginal cornification but gain in weight of uterus may be attributed to non-specific mobilization of water or tissue-fluid in the uterus. It is postulated in the case of **11**, where both hydroxyl groups protected, the amino alkoxy side chain may not allow for adopt a suitable conformation for mimicking the estradiol and that may be the reason towards its poor estrogenecity. The presence of a free hydroxy group in compound **10** in place of the alkyl amino alkoxy chain as in **11**, would explain its relatively higher estrogenic activity.

Table 2. Docking results

Compd	Cluster rank	RMSD from estradiol (II)	Final intermolecular energy (kcal/mol)	Final docked energy (kcal/mol)
II (ref)	I	0.69	−10.71	−10.71
7	I	2.79	−9.98	−9.34
9	I	1.66	−10.98	10.08
10	I	1.85	−10.45	−10.44
11	I	4.71	−12.91	−7.77

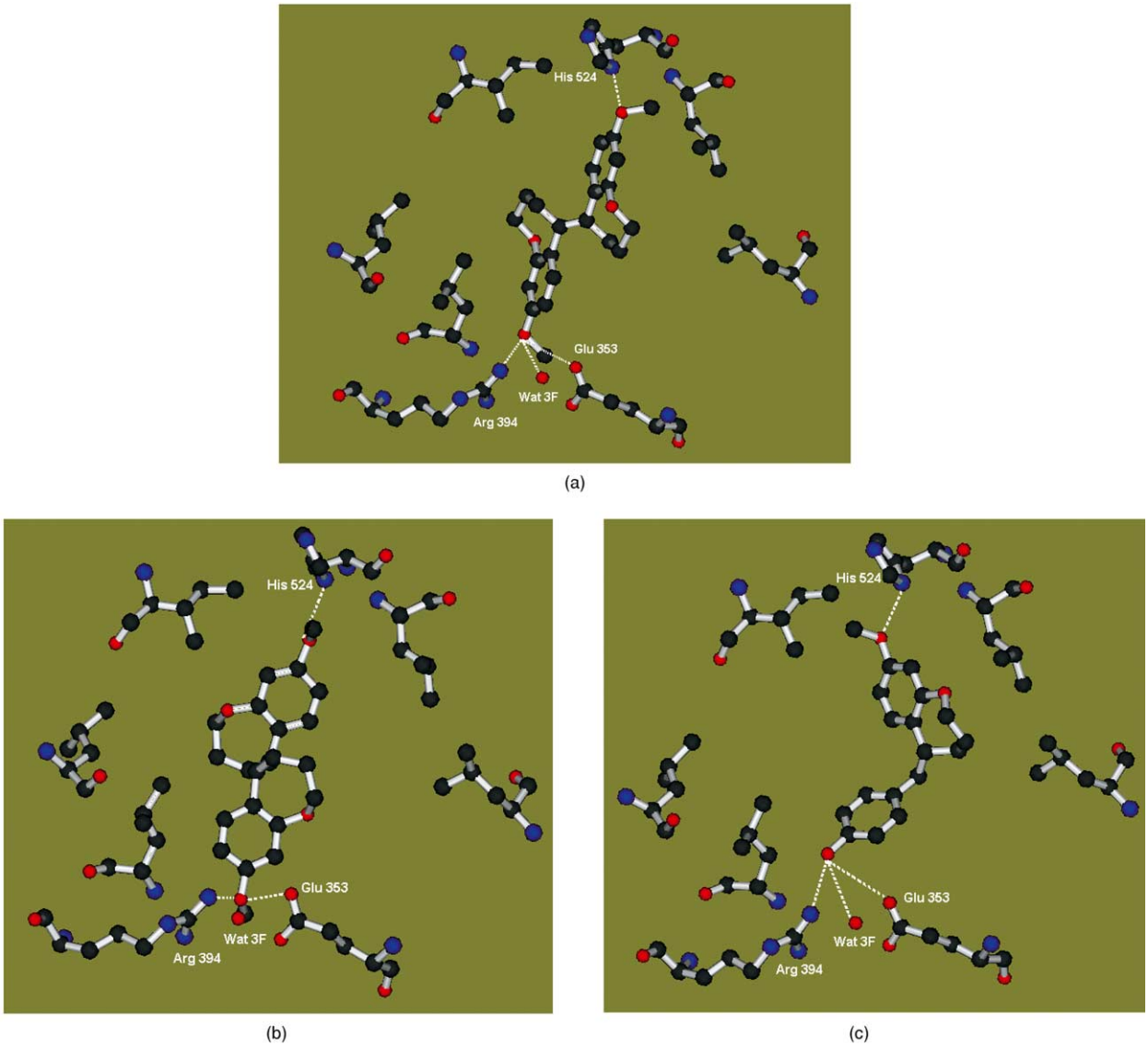


Figure 6. (a) Docked compound **7** in the binding cavity of ER showing interaction with neighbouring residues through H-bonding (broken white lines); (b) docking of the compound **9** in the binding cavity of ER showing the interaction with neighbouring residues through H-bonding (broken white lines); (c) docking of the compound **10** in the binding cavity of ER showing the interaction with neighbouring residues through H-bonding (broken white lines).

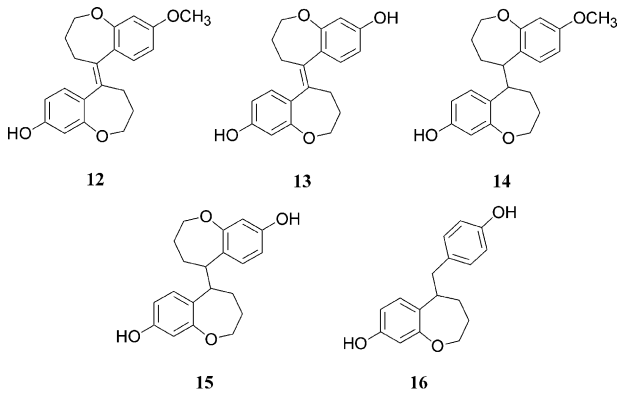
Docking studies

The docking values for these compounds were calculated as described in the experimental section and are summarized in Table 2. From the docking experiment and in vivo activity, it is evident that docking scores indeed correlated to in vivo activity of the compounds and X-ray structural studies of ligands.

The AutoDock predicted conformation of **II** (estradiol, green) is shown in Figure 5 with the X-ray crystallographic obtained conformation[17] (yellow) superposition. The root mean square deviation (RMSD) between these two conformations is ~0.69 Å, indicating that the parameter set for the AutoDock simulation is reasonable to reproduce the X-ray structure. The AutoDock method and the parameter set could be extended

Table 3. Docking results

Compd	Cluster rank	RMSD from estradiol (II)	Final intermolecular energy (kcal/mol)	Final docked energy (kcal/mol)
II (ref)	I	0.69	−10.71	−10.71
12	I	2.51	−11.90	−11.67
13	I	2.18	−11.14	−11.14
14	I	1.85	−10.88	−9.72
15	I	1.96	−12.02	−11.63
16	I	2.28	−10.42	−10.63



Scheme 2.

to search the binding conformations for other ligands accordingly. Table 2 lists the calculated energies and docked results from the compounds **7**, **9**, **10** and **11** with comparison of docked estradiol.

Figure 6(a–c) shows the 3-D docked model of compound **7**, **9** and **10** with ER. Figure 6 illustrates the probable binding conformational alignment of the synthesized compounds. Figure 6(a) indicates that there is a short contact between water (3FO) and C11 atom of **7** within an ER, as well as lack of good fit, represented by poor energy parameters through docking results. Figure 6(b) also indicates the same result as Figure 6(a) but better fit in compared to **7**. Interestingly this result is also supported by initial crystallographic discussion, where packing diagram showed the involvement of O10 in hydrogen bonding, only in the case of **9**. Hydrogen-bonding is one of the important characteristics of the ligand-receptor interaction. It was nicely found in the case of compound **10**, as shown in Figure 6(c), for which similar binding pattern and energy parameters were obtained as were found in original X-ray structure of ER–estradiol complex.

From the above results it could be speculated that conformation of the compound **10** [Fig. 6(c)] (the most potent ligand among the four compounds) is suitable for interaction with ER. Interestingly only compound **10** contains a phenolic group, which plays an important role in receptor binding. So we undertook modeling experiments taking hydroxy compounds (**12**–**16**) (Scheme 2) in place of methoxy. The result of virtual docking of free phenolic compounds, shown in Table 3, would suggest that the presence of mono-hydroxy group increases receptor binding significantly but no additional advantage is seen with dihydroxy compounds (**13**, **15** and **16**). Significantly lowered intermolecular energies (Table 3) of hydroxyl compounds **12**–**15** corresponds to **7** and **9** suggest partial or slow in vivo metabolism of **7** and **9** to its demethylated products. Thus moderate in-vivo estrogenicity shown by compound **7** and **9** justified.

Experimental

All reactions were monitored by thin-layer chromatography over precoated silica gel plates and visualized by irradiation or exposure to iodine vapours. The melting points were recorded on electrically heated block and are uncorrected. NMR spectra were recorded on Bruker Avance 200 and 300 MHz FT spectrometers using TMS as internal reference. Chemical shifts and coupling constants (J) were repported in δ (ppm) and in Hz, respectively. Mass spectra of the compounds were taken with Jeol-JMS-D-300 instrument. Microanalysis was carried out on Carlo Ebra model EA-11108 and Heraeus CHN rapid instrument. IR spectra of liquid samples were run neat, and solids as KBr pellets.

Preparation

1-(3-Bromopropoxy)-3-methoxybenzene (2). A 25% aqueous solution of NaOH (35 mL) was added to a mixture of 3-methoxyphenol (10 g, 0.081 mol), 1,3-dibromopropane (32 g, 0.158 mol) in boiling water (70 mL) and heating was continued with stirring for 10 h. The reaction was monitored through TLC and extracted with ether. The ethereal layer was dried over anhydrous $CaCl_2$ and concentrated to dryness. The crude product was chromatographed over silica gel (60–120 mesh) using 5% ethyl acetate–hexane mixture as an elutant. The oily product was identified as **2**.

Yield: 14.5 g (73.5%), oil, MS (EI): 245 (M^+), ^{1}H NMR ($CDCl_3$): δ 2.307 (m, 2H, CH_2), 3.598 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 4.087 (m, 2H, OCH_2), 6.493 (m, 3H, ArH), 7.181 (m, 1H, ArH).

1-Cyano-3-(3-mehtoxyphenoxy)propane (3). A mixture of **2** (13 g, 0.053 mol), NaCN (6 g, 0.122 mol), 95% Ethanol (100 mL) and water (30 mL) was heated under reflux. After 40 h the TLC monitored showed two major spots, the upper corresponding to starting material 1.

Further refluxing did not show any appreciable change in TLC. The reaction mixture was concentrated, extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulphate and removed the solvent. The crude reaction mixture was chromatographed over silica gel (60–120 mesh) using 20% benzene-hexane mixture as eluent. The pure compound was obtained as an oil.

Yield 5.4 g (53%), oil, MS (EI): 191 (M^+), ^{1}H NMR ($CDCl_3$): δ 2.127 (m, 2H, CH_2), 2.582 (t, 2H, CH_2), 3.790 (s, 3H, OCH_3), 4.054 (t, 2H, OCH_2), 6.477 (m, 3H, ArH), 7.186 (t, 1H, ArH).

Ethyl-4-(3-methoxyphenoxy)butyrate (4). A mixture of **3** (5 g, 0.026 mol), absolute ethanol (30 mL) and concd H_2SO_4 (4 mL) was heated under reflux for 25 h. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and dried over anhydrous sodium sulphate. The crude product was then chromatographed over silica gel using 3% EtOAc–hexane as the eluent.

Yield 4.67 g (75%), oil, MS (EI): 238 (M^+), ^{1}H NMR ($CDCl_3$): δ 1.255 (t, 3H, CH_3), 2.097 (m, 2H, CH_2), 2.507 (t, 2H, CH_2), 3.777 (s, 3H, OCH_3), 3.983 (t, 2H, OCH_2), 4.130 (q, 2H, CH_2), 6.480 (m, 3H, ArH), 7.163 (t, 1H, ArH).

4-(3-Mehtoxyphenoxy)butyric acid (5). A mixture of **4** (4 g, 0.168 mol) and a solution of 20% KOH was heated under reflux for 2 h. The reaction mixture was concentrated and poured into water. It was acidified with dil. HCl and extacted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulphate. Concentration of the organic layer yielded **4** which was recrystallized from hexane.

Yield 3.3 g (93.48%), solid, mp: 70 °C, MS (EI): 210 (M^+), ^{1}H NMR ($CDCl_3$): δ 2.012 (m, 2H, CH_2), 2.497 (t, 2H, CH_2), 3.782 (s, 3H, OCH_3), 3.88 (t, 2H, OCH_2), 6.461 (m, 3H, ArH), 7.083 (m, 1H, ArH).

8-Methoxy-3,4-dihydro-1-benzoxepin-5-(2*H*)-one (6). A mixture of polyphosphoric acid (PPA) (35 g) and **5** (3g, 0.014 mol) was taken in a dried R. B. flask fitted with a guard tube. The mixture was heated for 3 h on a steam bath. The colour of the mixture turned red. The reaction mixture was poured into water (70 mL) and left overnight. It was extracted with ethyl acetate, washed with $NaHCO_3$ solution and the remaining insoluble gummy residue was dissolved in dry methanol. The combined ethyl acetate and methanol extracts were concentrated to yield the crude product, which was purified from column chromatography using 5% EtOAc–hexane as the eluent.

Yield 1.35 g (49.5%), MS (EI): 192 (M^+), ^{1}H NMR ($CDCl_3$): δ 2.192 (m, 2H, CH_2), 2.867 (t, 2H, CH_2), 3.833 (s, 3H, OCH_3), 4.241 (t, 2H, OCH_2), 6.55–6.68 (m, 3H, ArH), 7.765 (d, 1H, ArH).

5,5′-Bi[8-methoxy-2,3,4,5-tetrahydro-1-benzoxepin]ylidene (6) and 4-{[(8-methoxy-3,4-dihydro-1-benzoxepin-5(2*H*)-ylidene]methyl}phenol (7). To a mixture of Zn (3.6 g) and dry THF (70 mL) at 40–45 °C, $TiCl_4$ (3.2 mL) was added with and refluxed under anhydrous condition for 2 h. To this a mixture of **6** (1.0 g, 5 mmol) and *p*-benzyloxybenzaldehyde (1.65 g, 8 mmol), was added slowly, refluxed for 5 h, filtered and the filtrate poured into water. It was acidified with HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude extract was flash chromatographed over silica gel to obtain **7** and **8**. Compound **7** and **8** were recrystallized from EtOAc–hexane mixture.

Yield **7**: 0.35 g (19%), mp: 132 °C, MS (EI): 352 (M^+), ^{1}H NMR ($CDCl_3$): δ 1.64–1.75 (m, 4H, CH_2), 1.78–1.82 (m, 4H, CH_2), 3.74 (s, 6H, OCH_3), 4.22–4.34 (t, 4H, OCH_2), 6.21–6.65 (m, 4H, ArH), 7.09–7.13 (d, 2H, ArH). Anal. calcd for $C_{22}H_{24}O_4$: C, 74.98; H, 6.86; O, 18.16. Found: C, 74.74; H, 6.96; O, 18.30.

Yield **8**: 0.25 g (13%), Oil, MS (EI): 372 (M^+), Anal. calcd for $C_{25}H_{24}O_3$: C, 80.62; H, 6.49; O, 12.89. Found: C, 80.32; H, 6.89.

5,5′-Bis[8-methoxy-2,3,4,5-tetrahydro-1-benzoxepin] (9). Hydrogenation of **7** (200 mg, 0.568 mmol) was carried out over Pd/C at 30 psi pressure for 7 h in methanol. The mixture was filtered and the filtrate was concentrated to yield a semi-solid residue. Which was crystallized from ethyl acetate to yield **9**.

Yield: 90 mg (45%), mp: 186–188 °C, MS (EI): 354 (M^+), ^{1}H NMR ($CDCl_3$): δ 1.48 (m, 4H, CH_2), 1.55 (m, 4H, CH_2), 2.22–2.24 (m, 4H, CH_2), 3.79 (s, 6H, OCH_3), 4.38–4.44 (t, 4H, OCH_2), 6.55–6.66 (m, 4H, ArH), 7.15 (d, 2H, ArH). Anal. calcd for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39; O, 18.06. Found: C, 74.46; H, 7.67.

4-[(8-Methoxy-2,3,4,5-tetrahydro-1-benzoxepin-5-yl)methyl]phenol (10). Hydrogenation of **8** (200 mg, 0.537 mmol) was carried out under similar condition as in case of **7**. Recrystallization form ethyl acetate gave the crude product as a solid.

Yield: 130 mg (85%), mp: 97 °C, MS (EI): 284 (M^+), ^{1}H NMR ($CDCl_3$): δ 1.41 (m, 2H, CH_2), 1.62–1.72 (m, 2H, CH_2), 2.75 (m, 1H, CH_2), 2.83–2.95 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 4.15 (t, 2H, OCH_2), 6.35–6.79 (m, 7H, ArH).

1-(2-{4-[(8-Methoxy-2,3,4,5-tetrahydro-1-benzoxepin-5-yl)methyl]phenoxyl}ethyl)piperidine hydrochloride (11). A mixture of (**10**) (130 mg, 0.458 mmol), chloroethyl piperidine hydrochloride (120 mg), and a pellet of KOH was heated under reflux for 3 h. The reaction mixture was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was chromatographed over silica gel and the product was obtained as a viscous oil. The hydrochloride salt was prepared by treating with ethereal solution of HCl. Removal of the ether under vacuum yielded the product

11 as a solid. Which was recrystallized from absolute ethanol–ether mixture.

Yield 26 mg, MS (EI): 395 (M^+), ^{1}H NMR ($CDCl_3$): δ 1.27–1.35 s(m, 6H, CH_2), 1.47 (m, 2H, CH_2), 2.20–2.22 (m, 4H, CH_2), 2.641 (m, 4H, CH_2), 2.777 (m, 1H, CH), 2.847 (m, 2H, CH_2), 3.766 (s, 3H, OCH_3), 4.112 (m, 2H, OCH_2), 4.274 (t, 2H, OCH_2), 6.47–6.59 (m, 7H, ArH). Anal. calcd for $C_{25}H_{33}NO_3$. HCl: C, 69.51; H, 7.93; N, 3.24. Found: C, 69.12; H, 7.91; N, 2.83.

Computational method

Docking, molecular dynamics, energy minimization and molecular graphics work were performed on a Silicon Graphics Octane workstation. The molecular alignment was performed using MOE 2002.03 program. The genetic algorithm of AutoDock 3.0 has been employed for docking the benzo[*b*]oxipene class compounds into the active sites of estrogen receptor.

Docking studies/protocol

The crystal structure of the LBD of estrogen receptor in complex with the endogenous oestrogen, 17β-oestradiol (II)[17] was recovered from the Brookhaven Protein Data Bank (http;//http://www.rcsb.org/pdb/) (entry codes 1ERE). Ligand structures of **7** and **9** were obtained in pdb-format from its crystal structure. The ligand structure of **10** and **11** were generated in pdb-format from and energy minimized by 3DChem draw modeling software. The advanced docking program AutoDock 3.0 was used to perform the automated molecular docking for **7**–**16**. The whole docking operation could be stated as follows. First, the ligand molecules were checked for polar hydrogens and assigned for Gasteiger–Hückel[33] partial atomic charges. Flexible torsions were defined with the help of AutoTors. This allowed the conformational search of ligands during the process of docking. The PDBQ file was created for ligands. For macromolecules ER, polar hydrogens were added and Kollman-all-atom[34] atomic charges were taken and the atomic solvation parameters were also assigned using the ADDSOL utility of AutoDock 3.0.5 program. Second, the 3-D grid of 0.3 Å resolutions was centered on the active site using AutoGrid algorithm[26] to evaluate the interaction energies between the ligands and the ER-LBD. In this stage, the ER was embedded in the 3-D grid and a probe atom was placed at each grid point. The affinity and electrostatic potential grid were calculated for each type of atom in the ligands. Third, a series of the docking parameters were set on. Not only the atom types but also the generations and the number of runs for GA algorithm were edited and properly assigned. The number of generations, energy evaluations, and GA runs were set of 27,000, 250,000 and 20, respectively. Finally, the docked complexes of ligand-receptor were selected according to the criteria of interacting energy combined with geometrical matching quality. These complexes were used for comparative study and correlation between and activity and its structural conformations. On the basis of the traditional molecular force field model of interaction energy, a new score function at the level of binding free energy was derived and adopted in the version of AutoDock3.0.[25] The total binding free energy was empirically calibrated based on the above-stated terms and set of coefficient factors. The same rational was applied to the system of benzo[*b*]oxepine compounds and estrogen receptor in order to evaluate the binding properties more precisely than the traditional molecular mechanics method did. The total binding free energy and corresponding inhibitory constant between compound and ER were calculated according to the algorithm in the AutoDock 3.0 program.[25]

Biological activity method

For determination of estrogenic activity,[30] immature female rats ovariectomized 7 days earlier were treated orally with the test agent or the vehicle (Tween-80 in glass distilled water). 17α-Ethynylestradiol (EE; Sigma Chemical Co., USA) was dissolved in 2–3 drops of redistilled ethanol and diluted to the desired concentration with glass distilled water. All treatments were done by the oral route once daily for 3 consecutive days and at autopsy 24 h after the last treatment, uterine fresh weight was taken and premature opening of vagina and the extent of vaginal cornification, if any, were recorded.

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