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Resolution, Molecular Structure and Biological Activities of the D- and L-Enantiomers of Potent Anti-implantation Agent, DL-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran†

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Abstract—Compound **1** (DL-2-[4-(2-piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran, CDRI 85/287) a potent anti-estrogen and anti-implantation agent has been successfully resolved into its pure D- and L-enantiomers. Biological studies showed L-enantiomer to be the active form, exhibiting a fivefold higher receptor affinity for the rat uterine cytosolic estrogen receptor, 100% contraceptive efficacy at 1.3 mg/kg dose in single day schedule and 89% inhibition of estradiol induced increase of uterine weight at its contraceptive dose. The absolute stereochemistry determined by X-ray crystallographic analysis showed that the L-enantiomer has **2R** configuration at its asymmetric centre.

Introduction

Molecules with triarylethylene or ethane framework exemplified by tamoxifen, nafoxidene, clomiphene, centchroman, etc. (Chart 1) have long been identified as an important group of compounds associated with anti-estrogenic and anti-fertility activities.[1–3] In triarylethylenes (TAEs), the ethylene moiety carrying the three aryl substituents is either acyclic or constitutes a part of a carbocyclic or heterocyclic system and the molecules in general are geometrically labile. The biological profile of TAEs is characterised by mixed agonist–antagonist action, with antagonist activity depending crucially on certain favourable circumstances of molecular geometry.[4–7] Further studies in this area led to the development of another class of compounds, the triarylpropenones, both cyclic/acyclic and were reported to have better anti-fertility and anti-estrogenic activities than triarylethylenes.[8–10] One such compound, raloxifene (LY139481.HCl), is in advanced clinical trails for prevention and treatment of osteoporosis.[11] Molecular modelling studies with raloxifene indicated that the orientation of the basic amine containing side chain in these type of molecules is an important discriminating factor for the maintenance of tissue selectivity.[12]

Over the years our efforts in the design and development of post-coital contraceptives/anti-implantation agents have resulted in the discovery of 2,3-diaryl-2*H*-1-benzopyrans (DABPs) as a promising new class of non-steroidal molecules possessing significant anti-estrogenic and post-coital contraceptive activities. A series of such compounds with different substituent groups were synthesised and most of the compounds showed 100% post-coital contraceptive activity at very low doses.[13–18] Further detailed biological studies revealed that these molecules exhibit tissue selective effects acting as agonists on bone and cardiovascular systems while antagonising the effects of estrogen on uterine and breast tissues and, therefore, have been termed as selective estrogen receptor modulators.[12] Since these pharmacologically active molecules possess an asymmetric centre, there is a possibility that the individual enantiomers may exert different biological activities functioning synergistically in some tissues and antagonistically in others. In this communication we

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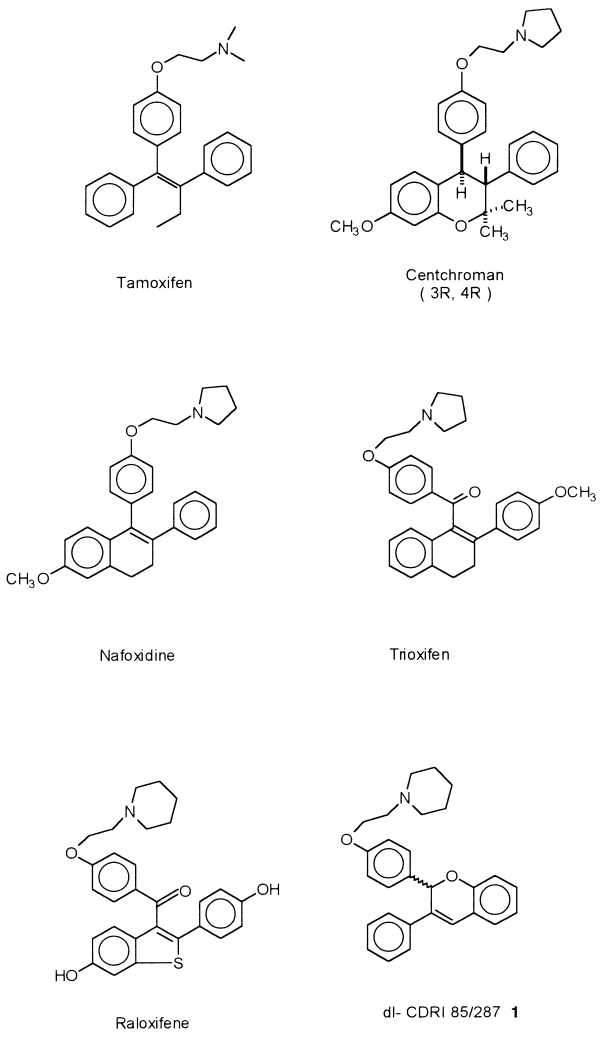
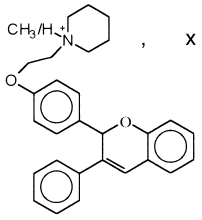


Chart 1. Structures of some potent anti-estrogens.



Compound no.	Isomer	X
1	DL	—
2	D	di-*p*-toluoyl-D-tartrate
3	D	—
4	L	di-*p*-toluoyl-L-tartrate
5	L	—
6	D	I
7	L	I

Figure 1.

now report the resolution, absolute molecular structure and pharmacological characteristics of the D and L enantiomers of DL compound **1**.

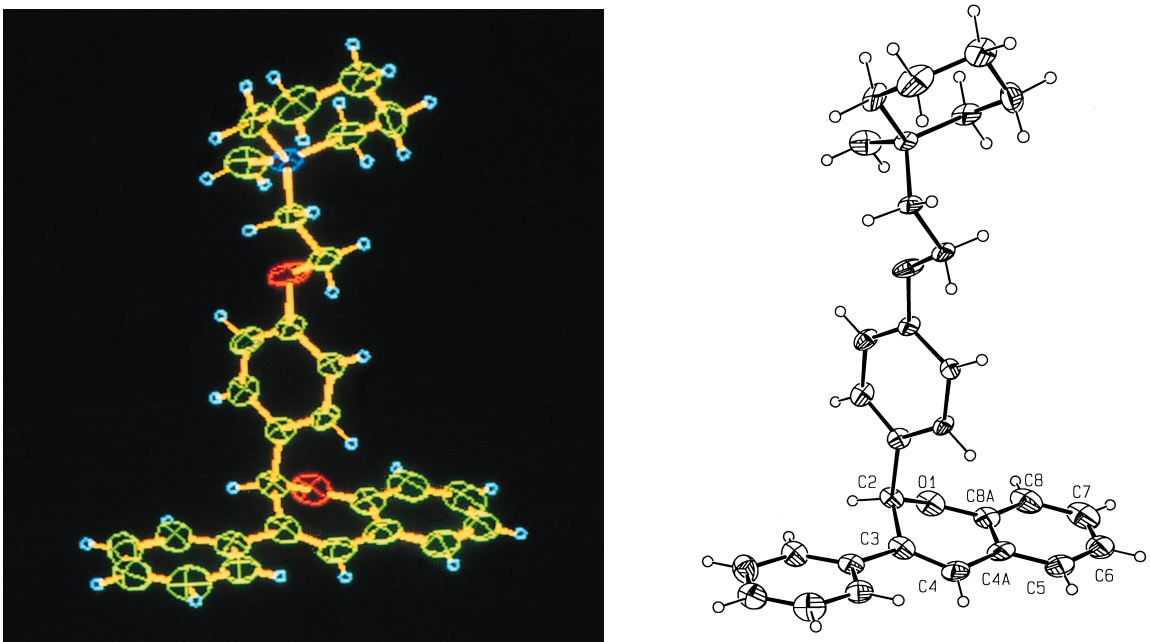
Chemistry

The DL compound **1** was synthesised as reported in the literature.[13] Resolution into its pure D- and L-enantiomers **3** and **5** was effected by formation of diastereoisomeric salts **2** and **4** (Fig. 1) with optically active D- and L-di-*p*-toluoyl tartaric acids. Repeated fractional crystallisation of salts was carried out to get the pure diastereoisomers of constant rotation. Alkaline hydrolysis of the tartrate salts liberated the free bases and their percent enantiomeric purity was checked by HPLC using a chiral stationary phase column. For X-ray crystallographic studies, all attempts to get suitable crystals of either the D- or L-enantiomer as free base were unsuccessful. A literature report on centchroman[3] mentioned the formation of crystalline *N*-methyl iodide derivatives of single enantiomers suitable for X-ray analysis. Following the similar approach, both the D- and L-enantiomers on refluxing with methyl iodide formed crystalline *N*-methyl iodide salts **6** and **7**. Subjecting them to slow crystallisation in alcohol gave plate like crystals of L-enantiomer **7** after several days which were found suitable for X-ray analysis.

Molecular structure and conformation of the L-enantiomer *N*-methyl iodide salt (7)

The conformational features of L-enantiomer *N*-methyl iodide salt **7** are shown in the ORTEP[19] diagram (Fig. 2(a)). The absolute structure confirms that the chiral centre (C2) of the molecule has **R** configuration. The C2 substituted ether side chain is twisted out of the plane of the chromene system (axial orientation) and the pendant phenyl substituent at C3 is almost planar with the chromene ring system. The O1 and C2 atoms of the pyran ring have a sofa-like puckering. The piperidine ring of the ether side chain is in a chair conformation. In the present molecule the pendant aryl twist is 86.5° for the C2-ring and 9.6° for the C3-ring with respect to the chromene best plane. The C2-ring twist is comparable with the C4-ring twist in centchroman (98.7). However, the C3-ring twist is very different from the C3-ring in centchroman (71.5). The difference can be attributed to the potential for arene double bond conjugation in diarylbenzopyrans which tends to enforce ring double bond planarity. The pendant aryl groups at C2 and C3 are in the β-axial and planar orientation, a structural feature consistent with the proposed non-planar topology model for improved oral bioavailability.[20] A comparison of the present molecule with other anti-estrogens indicate different conformations of the side chain ether, which is synclinal in the present molecule (71.3°), centchroman (68.5°) and tamoxifen (78.7°) and *anti* in nafoxidine (173.8°). The different conformations are most likely a result of the crystal packing force

(a)



(b)

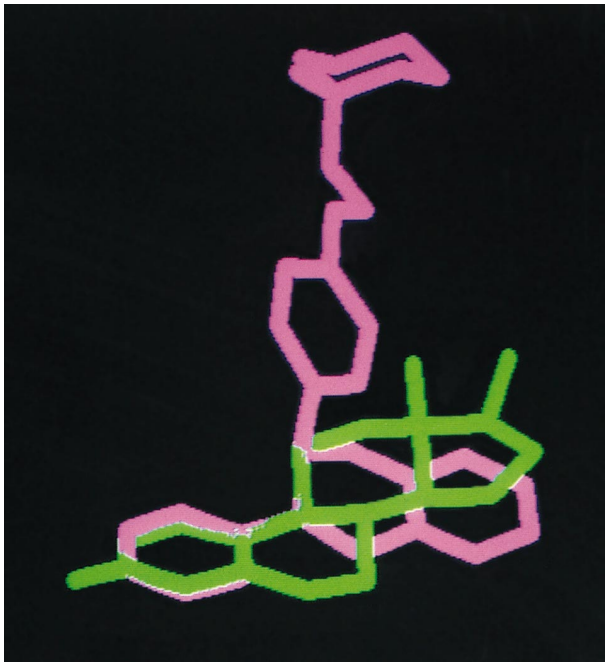


Figure 2. (a) The ORTEP diagram in colour and black and white showing the relaxed molecular structure of L-enantiomer *N*-methyl iodide salt **7**. Only non-H atoms of chromene ring have been numbered for clarity. (b) Overlay of the relaxed stereostructure of **7** and 17β-estradiol.

stabilisation rather than alterations in the core ring structure.[21]

Results

Receptor binding affinity

The resolved D- and L-enantiomers tested as their tartrate salts **2** and **4** showed a marked difference in their affinity for the rat uterine cytosol receptor (Table 1). The binding affinity of the active L-enantiomer **4** is about fivefold higher than that of the inactive D-enantiomer **2** and is also about three times more active than the DL-mixture **1**. On the other hand, the two enantiomers showed almost the same affinity for the antiestrogenic binding sites (AEBS), the racemic mixture however exhibited a slightly higher AEBS affinity as compared to its enantiomers.

Table 1. Relative binding affinity and post-coital contraceptive activity data of compounds **1**, **2** and **4**

Compound	Dose (mg/kg; no. of animals)[a]	Treatment (day of administration)[b]	% Inhibition ($m.e.d._{100}$)[c]	RBA (% of estradiol)	
				ER	AEBS
17β-Estradiol	— (6)			100	< 0.001
Control	—				
DL, **1**	2.5[d]	day 1	100	0.34	276.50
D, **2**	1.3	day 1	Inactive	0.18	234.97
	2.5	day 1	60		
L, **4**	1.3[d]	day 1	100	1.0	250.96
Tamoxifen				2.86	100.00

[a] Six animals were used.
[b] Single day schedule, i.e. only on day 1 of pregnancy.
[c] $m.e.d._{100}$, minimal effective dose for 100% inhibition of pregnancy.
[d] Contraceptive dose.

Post-coital contraceptive activity

The importance of chirality in biological systems is reflected in the results of post-coital contraceptive efficacy of the two enantiomers (Table 1). The L-enantiomer **4** was found to be the active isomer showing 100% inhibition of implantation at 1.3 mg/kg dose (m.e.d.) in single day schedule. This is almost half the dose required for the racemic mixture (m.e.d. = 2.5 mg/kg, single day schedule). However, the D-enantiomer **2** was found to be inactive at 1.3 mg/kg and when tested up to 2.5 mg/kg showed only 60% efficacy.

Uterotrophic activity

The results of individual enantiomers evaluated for uterotrophic activity (Table 2) in immature rats showed an increase in the uterine wet weight of 108% for the L-enantiomer **4** at its contraceptive dose, whereas it was 71% increase for the D-enantiomer **2** at 1.5 mg/kg dose. However, the DL-compound **1** showed an increase of 96% at its contraceptive dose (2.5 mg/kg). None of the compounds induced vaginal cornification at any of the doses tested. As there was no stimulation of other uterine parameters, this uterine weight increase may therefore be attributed to water retention. With the administration of estradiol at 0.1 or 1.0 μg per rat, a three- to fivefold increase in the uterine weight was evident and the vaginal smear showed cornified epithelial cells.

Table 2. Uterotrophic activity data of compounds **1**, **2** and **4** in immature rats

Compounds	Dose, μg ×3 days	Uterine wet weight. Mean ± SE ($n = 6$)[a]	% Increase	Status of vaginal opening
OV control	—	1.70 ± 0.39	—	—
DL, **1**	100	29.6 ± 2.44	74	—
	500	26.3 ± 1.40	54	—
	1300	26.0 ± 1.86	53	—
	2500[b]	33.3 ± 2.17	96	—
D, **2**	100	27.6 ± 0.61	49	—
	500	30.6 ± 0.49	65	—
	1300	35.0 ± 1.12	89	—
	1500	31.8 ± 0.90	71	—
	2500	29.6 ± 0.95	60	—
L, **4**	100	30.6 ± 1.20	61	—
	500	33.2 ± 1.48	74	—
	1300	39.6 ± 2.44	108	—
	1500	4.1 ± 1.22	132	—

[a] Six animals were used.
[b] Contraceptive dose.

Antiuterotrophic activity

Results of the D- and L-enantiomers for the antiuterotrophic activity (Table 3) showed significantly higher potency for the L-enantiomer **4**. At its contraceptive dose the L-enantiomer showed 89% inhibition of estradiol (0.1 μg) induced uterotrophic effect, whereas with the D-enantiomer **2** inhibition was found to be 80% at 1.3 mg/kg and the DL-mixture showed inhibition of 77% at its contraceptive dose. Complete inhibition of vaginal cornification was achieved at 1.0 mg/kg for the DL-mixture and 1.3 mg/kg for both D- and L-enantiomers. When the dose of estradiol was raised to 1.0 μg, the percent inhibition noted at the respective contraceptive doses was 74% for L-enantiomer, 48% for D-enantiomer (at 1.3 mg/kg) and 62% for DL-mixture. Complete inhibition of vaginal cornification was evident at 1.5 mg/kg for the DL compound, while the two enantiomers showed only partial inhibition.

Discussion

The absolute stereochemistry at the asymmetric centre of the active L-enantiomer as determined by X-ray analysis (ORTEP, Fig. 2(a)) has been found to be **2R**. It was earlier hypothesised[13,14] in a 3-D model for estrogen receptor that the active enantiomer would bind to the receptor in a fashion such that the tertiary aminoalkoxy phenyl residue would occupy a region corresponding to the 11β-position of the estradiol. This would be possible only if the pendant aryl ring at 3-position overlays on to the aromatic A ring of the E_2 as shown in the overlay picture of the relaxed stereostructure of **7** and 17β-estradiol (Fig. 2(b)). A recent report[22] on the crystal structure of the ligand binding domain (LBD) of estrogen receptor (ER) in complex with 17β-estradiol and raloxifene has supported this hypothesis. Raloxifene,

Table 3. Antiuterotrophic activity data of compounds **1**, **2** and **4** in immature rats

Compound	Dose (μg)	Against 0.1 μg of E_2		Against 1.0 μg of E_2	
		Uterine wet weight. Mean ± SE ($n = 6$)[a]	% Inhibition	Uterine wet weight. Mean ± SE ($n = 6$)[a]	% Inhibition
	OV control	18.2 ± 1.20		16.3 ± 0.66	
	E_2	57.6 ± 2.66		80.5 ± 1.62	
DL, **1**	E_2 + 100	52.2 ± 2.90	29	48.4 ± 4.0	40
	E_2 + 500	44.0 ± 0.73	35	49.6 ± 1.25	48
	E_2 + 1300	33.2 ± 1.87	62	32.5 ± 1.81	59
	E_2 + 2500[b]	27.0 ± 1.69	77	30.3 ± 1.30	62
D, **2**	OV control	18.5 ± 0.61		18.5 ± 0.61	
	E_2	67.0 ± 0.81		85.8 ± 0.74	
	E_2 + 100	38.5 ± 1.08	52	66.3 ± 0.95	32
	E_2 + 500	35.6 ± 0.95	65	60.5 ± 2.44	38
	E_2 + 1300	28.0 ± 0.73	80	53.3 ± 1.70	48
	E_2 + 1500	27.0 ± 0.66	82	42.6 ± 2.29	64
L, **4**	OV control	17.8 ± 0.83		19.0 ± 0.40	
	E_2	80.0 ± 4.00		90.3 ± 2.44	
	E_2 + 100	39.6 ± 3.07	50	71.6 ± 2.44	29
	E_2 + 500	38.0 ± 2.87	68	56.6 ± 2.44	44
	E_2 + 1300[b]	24.6 ± 1.42	89	37.6 ± 2.33	74
	E_2 + 1500	27.3 ± 0.98	85	44.3 ± 1.42	64

[a] Six animals were used.
[b] Contraceptive dose.

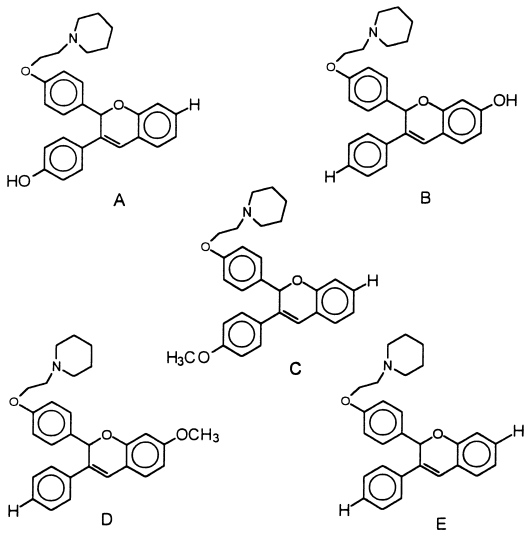
one of the most potent selective estrogen receptor modulators, is shown to bind at the same site as E_2 within the LBD with the hydroxyl group of the benzothiophene moiety mimicking the A ring phenolic hydroxyl of E_2. The 3-benzoyl group with its basic aminoalkoxy residue is oriented along the 11β-axis of estradiol. However, there exists the possibility that the free rotation of the benzoyl group may align it along the 11α-axis. A 7α-orientation of the 3-benzoyl residue was also proposed for an analogue of raloxifene LY 117018.[23] Nevertheless, the X-ray crystal picture of raloxifene–ER ligand binding complex clearly depicts it in the binding cavity along the 11β-axis. Since diarylbenzopyrans share structural features with raloxifene[12] in very similar positioning of the basic aminoalkoxy residue and the stilbene core, the active L-enantiomer would occupy position similar to that of raloxifene in the binding cavity of LBD of the hormone receptor.

Recently Labrie et al.[24] have suggested a very similar compound, EM-800, a dextrorotatory enantiomer of 4-methyl dihydroxy analogue of the compound **1**, as the active enantiomer that binds to the ER receptor in an orientation in which the 7-hydroxyl group of the chromene takes the position of the 3-hydroxyl group of estradiol and the tertiary aminoalkoxy phenyl residue occupies a position corresponding to 7α- of E_2 as in ICI estrogen antagonists. With the presence of two hydroxyl groups in the stilbene core of dihydroxy benzopyrans there is a possibility of the molecule orienting in the manner having 2-phenyl substituent with the flexible basic aminoalkoxy residue occupying the 7α-like position in the binding cavity. However, that this orientation may not be the desired one is supported by the observations[25] that there exists a large difference in the volume of the unoccupied hydrophobic cavities opposite the 11β-face of the C ring (45 Å) and 7α-face of the B ring (17 Å) of E_2 in the ligand binding domain of estrogen receptor.

Furthermore, in the same report, structure–activity relationship studies with different steroidal and non-steroidal molecules have shown that the 11β-site has high steric tolerance and can accommodate even aromatic substituents while still retaining high affinity, whereas the 7α-site with a comparatively smaller volume has lower steric compatability and can accommodate only small groups. If these findings hold true then the molecule in orientation with tertiary aminoalkoxy residue in the 11β-axis of E_2 will be the more acceptable form. This argument is further supported by the RBA and biological activity results of diarylbenzopyran analogues having a hydroxyl group either at the 4′-position of the 3-phenyl ring or at the 7-position of the chromene moiety.[26,27] RBA results indicate only a small difference (the same findings of the minor difference in RBA have recently been reported for the monohydroxy tetracyclic raloxifene analogues)[28] in the binding affinity of the two monohydroxy compound (Table 4), but there is a very obvious dissimilarity in their biological potency. The compound A with a hydroxyl group in the 4′-position of 3-phenyl substituent is substantially more potent both as post-coital contraceptive and as estrogen antagonist. Similarly its methyl ether C is the most active compound of the series[18] whereas compound B with hydroxyl group in the 7-position is only moderately active, whereas its methyl ether D is inactive. The apparent difference in the affinity and activity could be due to the preferential hydroxylation of the pendant aromatic ring forcing that ring to approximate the aromatic ring of estradiol. The final evidence will however be provided by the X-ray crystal structure studies.

We have also separated the pure enantiomers of methyl ether compound C and preliminary biological results have shown the *laevo* form to be the active enantiomer. Detailed studies are in progress with other analogues also and will be reported in due course.

Table 4. Relative binding affinity of monohydroxy DABPs and their methyl ether analogues to rat uterine cytosol receptor



Compound	RBA ER	
A	6.6	Active
B	7.5	Moderately active
C	0.55	Most active
D	0.02	Inactive
E	0.34	Active

Experimental

General methods

The melting points were determined on Toshniwal melting point apparatus and are uncorrected. The ^{1}H NMR were recorded on Brucker DRX (300 MHz-FT NMR) spectrometer using tetramethylsilane as internal standard. The values are reported in the δ scale. Mass spectra (EI) were recorded on Jeol JMS D-300 instrument fitted with a direct inlet system. FAB-HRMS spectra of *N*-methyl iodide salt **7** was recorded on a Jeol SX-102 machine. Optical rotations were measured on Rudolph III automatic polarimeter. The percent purity (% ee) of the enantiomers was determined by HPLC on a chiral chiradex column using MeOH:H_2O:AcOH: tetramethyl ammonium hydroxide mixture as the mobile phase, pH 4.2.

DL-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran (1). This was synthesised following the process as reported in earlier communication.[13]

D-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran-di-*p*-toluoyl-D-tartrate (2). DL Compound **1** (411 mg, 1 mmol) and di-*p*-toluoyl-D-tartaric acid (386 mg, 1 mmol) were dissolved by gentle warming in commercial double distilled ethanol (25 mL) and the solution was allowed to stir for 4 h. Excess of ethanol was removed under vacuo and the viscous residue obtained was subjected to repeated crystallisation in ethanol to give white amorphous solid of pure D-2-[4-(2-piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran-di-*p*-toluoyl-D-tartrate **2**, yield 104 mg; mp 132°C; $[\alpha]_D^{20}$ +72.4 (C1 in EtOH). ^{1}H NMR ($CDCl_3$) δ 1.3–1.7 (m, 6H $(CH_2)_3$), 2.25 (s, 6H, $ArCH_3$), 2.3–2.4 (m, 4H, $(CH_2)_2$ N), 2.5–2.7 (t, 2H, N CH_2), 4.0 (t, 2H, OCH_2), 5.7 (s, 2H, CH of tartaric acid), 6.1 (s, 1H, OCH), 6.6–7.3 (m, 14H, ArH and olefinic H); MS *m*/*z* 411 (M^+−386, di-*p*-toluoyl-D-tartaric acid).

D-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran (3). The D-tartrate salt **2** (100 mg) was dissolved in minimum quantity of ethyl acetate and the solution stirred with 10% aq NaOH solution (2 mL) for 1/2 h. Thereafter the organic layer was separated, washed with water to neutral, dried over anhydrous Na_2SO_4 and concentrated. On cooling and addition of a little hexane colorless needle shaped crystals of pure D-enantiomer base **3** were formed; yield 50 mg; mp 69°C; $[\alpha]_D^{20}$ +34.3 (C1 in EtOH). ^{1}H NMR ($CDCl_3$) δ 1.4–1.6 (m, 6H $(CH_2)_3$), 2.4 (m, 4H, CH_2NCH_2), 2.6–2.7 (t, 2H, NCH_2), 4.0 (t, 2H, OCH_2), 6.1 (s, 1H, OCH), 6.8–7.4 (m, 14H, ArH and olefinic H); MS *m*/*z* 411 (M^+).

L-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran-di-*p*-toluoyl-L-tartrate (4). The L-enantiomer tartrate salt **4** was prepared similarly as reported for the D-enantiomer by treating again the DL-compound and di-*p*-toluoyl-L-tartaric acid in equimolar ratio; yield 120 mg; mp 126°C; $[\alpha]_D^{20}$ −72.2 (C1 in EtOH). ^{1}H NMR and mass spectra were identical with the D-enantiomer tartrate salt.

L-2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran (5). The pure L-enantiomer free base **5** was obtained as white solid on aqueous alkaline hydrolysis of the L-tartrate salt **4** as described for the D-enantiomer; yield 57 mg; mp 75°C; $[\alpha]_D^{20}$ −34.3 (C1 in EtOH). ^{1}H NMR and mass spectra were identical to that of D-enantiomer pure base.

***N*-Methyl iodide salt of L-2-[4-(2-piperidinoethoxy)-phenyl]-3-phenyl-2*H*-1-benzopyran (7).** A mixture of L-enantiomer **3** (100 mg, 0.24 mmol), methyl iodide (180 mg, 1.5 mmol), anhydrous K_2CO_3 (170 mg, 1.5 mmol) in dry acetone (5 mL) was heated under reflux for 6 h in an oil bath (60°C). After cooling, the solution was filtered and concentrated under vacuo. The residue obtained was dissolved in minimum of alcohol and allowed to stand at room temperature. Slow crystallisation after several days afforded plate like crystals of compound **7**, mp 228°C; ^{1}H NMR (CD_3OD) δ 1.5–1.7 (m, 6H $(CH_2)_3$), 3.14 (s, 3H, NCH_3), 3.29 (m, 4H, $N(CH_2)_2$), 3.66 (t, 2H, NCH_2), 4.26 (t, 2H, OCH_2), 6.1 (s, 1H, OCH), 6.7–7.3 (m, 14H, ArH and olefinic H). HRMS-FAB *m*/*z* 426 (M^+-127I); 427 (M^+ + 1) -127I.

Compound **6** was prepared similarly following the above procedure.

X-ray crystal structure analysis of compound (7). A colourless, transparent, prismatic crystal was used on an Enraf–Nonius CAD4 diffractometer for intensity data

collection. The systematic absences supported the choice of space groups $Pca2_1$. Scattering factors and anamolous dispersions were taken from ref 29. The structure was solved by Patterson synthesis method using SHELXS 86.[30] Correct position for the iodide ion was located from the Patterson vector map followed by a partial structure determination, isotropic least-squares refinement and by successive difference. Fourier synthesis revealed the remaining part of the structure. The structure was refined anisotropically for the non-H atoms by full matrix least square methods on F^2 using SHELXL 93.[31] All H atoms were placed in geometrically idealised position and allowed to ride on their parent atoms, to which each was bonded for the final cycles of refinement. Fifteen reflections (most disagreeable; $\Delta(F^2)\sigma/ > 6.0$) were suppressed during the last cycles of refinement. The absolute configuration of the molecule was supported by refinement studies. The highest peaks in the final difference Fourier map were in the vicinity of iodide anions; the final map had no other significant features.

Crystal data. $C_{29}H_{32}INO_2$, FW = 553.46, $T = 293°$ (2) K, $\lambda = 1.5418$ Å, orthorhombic, space group = $Pca2_1$, a = 17.944 (2), b = 13.070 (1), c = 10.951 (1) Å, V = 2568.3 (4) Å^3, Z = 4, D = 1.431 mg/m^3, $\mu = 9.985\,mm^{-1}$, F (000) = 1128, θ range for data collection = 3.38–67.84°, index ranges $0 \leq h \leq 21$, $-15 \leq K \leq 0$, $0 \leq 1 \leq 13$; reflections collected 2672; independent reflections 2468 [R (int) = 0.0062]; refinement [(Δ/σ) max = 0.000]: full-matrix least squares on F^2; data/restraints/parameters 2452/1/298; Goodness-of-fit on F^2 0.866; final R indices $1 > 2\sigma(1)$: $R_1 = 0.0431$, $R_2 = 0.1105$; R indices (all data) $R_1 = 0.0581$, $R_2 = 0.1870$; absolute structure parameter 0.002 (19); largest difference, peak and hole 0.484 and −1.474e Å^{-3}.

Biological Methods

Competitive ER and AEBS binding affinity

The relative binding affinity of the compounds for the estrogen receptor and antiestrogenic binding sites was determined by methods as reported earlier.[13]

Anti implantation activity

Male and female rats of Sprague–Dawley strain were cocaged and sperm positive groups divided into different groups. The three compounds **1**, **2** and **4** were administered per oral in graded doses to different groups as aqueous gum acacia suspension on day 1 post-coitum. At the time of laparotomy of animals on day 11 post-coitum, number of implantations and corpora lutea was recorded. The results were considered positive when implantation sites were totally absent in both the uterine horns.

Uterotrophic activity

The estrogenic activity was evaluated in immature ovariectomised rats after a rest period of 1 week. Different groups of animals were orally administered the test material in graded doses for 3 consecutive days. Uterine weight and status of vaginal opening were noted at the time of autopsy, i.e. 24 h after the last treatment. The activity was assessed by uterine weight gain.

Antiuterotrophic activity

The estrogen antagonistic activity was assayed by inhibition of estrogen induced uterine weight gain and vaginal cornification using immature ovariectomised rats. 17β-Estradiol at 0.1 μg or 1.0 μg in olive oil was given by subcutaneous route along with graded doses of compounds for 3 consecutive days. Inhibition was expressed as percent inhibition of estradiol induced increase in uterine wet weight. Separate controls were maintained for all the groups. The DL mixture was taken up for comparison with the D and L enantiomers.

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