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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Effect of side-chain alteration on hormonal activity of nonsteroidal estrogen antagonists

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Abstract Incorporation of novel side-chains in 3,4-diaryl chromans, an established nucleus present in the nonsteroidal estrogen antagonist centchroman, has been carried out. The effect of variation in the nature of the chain on relative binding affinity (RBA) to estrogen receptor, estrogenicity, and antiestrogenicity has been studied. Presence of hydrazide residue on *cis*- and *trans*-3,4-diaryl chromans led to relatively higher RBA and estrogenicity as compared with corresponding acids and esters.

Keywords Centchroman · SERM · Estrogenicity · Antiestrogenicity · Enantiomers · Hydroxychroman

Introduction

The role of the side-chain in nonsteroidal compounds exhibiting estrogen antagonistic activity is well established in studies with ligand-bound nonsteroidal estrogen antagonist. It has been shown that the amino-residue-containing basic chain interacts with particular amino acid residue Asp351 present on estrogen receptor (Brzozowski *et al*., 1997). This interaction prevents the ligand binding pocket of the receptor from closing for initiation of estrogenic activity. Therefore, the nature of the chain, its dimension, and its orientation in space are crucial for determining its estrogen antagonistic activity.

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Recent interest in the development of nonsteroidal estrogen antagonists is due to the finding of their tissue-selective action, being agonists in some tissues such as bone, and antagonists in others such as uterine and breast tissues. Such compounds, termed selective estrogen receptor modulators (SERM), have the potential to be developed as drugs according to their selective activity profile (Kauffman and Bryant, 1995; Ray and Sangita, 2004; Jordan, 2003).

However, antiestrogenic activity has resulted in molecules containing other side-chains such as α,β-unsaturated propionic acid in GW 5638 (Wijayaratne *et al*., 1999). In order to study the adaptability of the antiestrogen binding site, compounds with acid, ester, and hydrazide chains have been prepared and their effect on biological activity studied. Since it was essential to compare activity in order to reach a meaningful conclusion, it was necessary to associate these chains with a nucleus having established estrogenic and antiestrogenic activities. We therefore substituted the pyrrolidinoethoxy chain of centchroman (Ray *et al*., 1976) (**i**), a well-studied molecule, with acid, ester, and hydrazide chains.

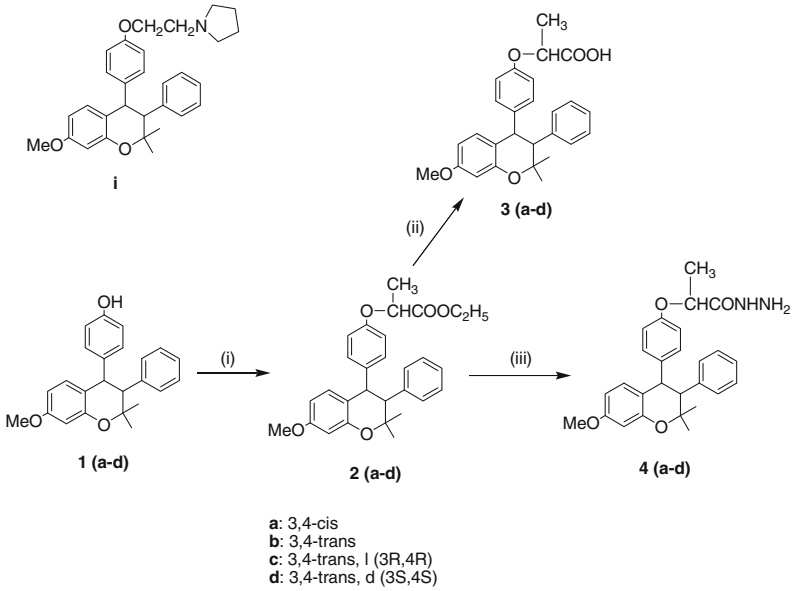
Chemistry

Synthesis of the novel compounds has been carried out by reacting the hydroxychroman derivatives with ethyl 2-bromopropionate in dry acetone in the presence of potassium carbonate to give the esters (**2a–d**). The hydroxy chroman derivatives used for the purpose are *cis*-2,2-dimethyl-3-phenyl-4-(*p*-hydroxyphenyl)-7-methoxy chroman (**1a**) *trans*-2,2-dimethyl-3-phenyl-4-(*p*-hydroxyphenyl)-7-methoxy chroman (**1b**), and its resolved *l*- (**1c**) and *d*-enantiomers (**1d**). Hydrolysis of the esters under alkaline condition gave the corresponding carboxylic acid (**3a–d**). Hydrazides of the compounds (**4a–d**) were prepared by treating the corresponding esters with hydrazine hydrate in alcohol as solvent. The reaction is shown in Scheme 1. Compounds thus prepared are given in Table 1.

Results and discussion

The biological activity of the compounds is shown in Table 1. The biological activity of different isomeric forms of centchroman has been reported earlier (Salman *et al*., 1986).

It was found that 3,4-*trans*-diaryl chromans were biologically manyfold more active than *cis*-chroman derivatives. In the *trans*-diaryl chroman the *l*-enantiomer (3*R*,4*R*) of centchroman showed sevenfold higher relative binding affinity (RBA) as compared with the *d*-form, and similar order of difference in their estrogenic and antiestrogenic activities (Salman *et al*., 1986). However, its 100-fold lower RBA and low antiestrogenicity would suggest inferior binding of the hydrazide chain as compared with pyrrolidinoethoxy on the antiestrogen binding site of the receptor. It will be of interest to study the role of the hydrazide chain in tissue-selective action.



Scheme 1 Reagents: (i) Ethyl 2-bromopropionate, anh. K_2CO_3 and dry acetone, (ii) NaOH and alcohol, (iii) Hydrazine hydrate and absolute alcohol

Estrogenic activity of *l-trans* hydrazide (**4c**) was similar to that of *l*-centchroman (**1c**). In the less active *d*-hydrazide (**4d**), the estrogenic activity was low and comparable to that of the corresponding acid (**3d**).

The nature of the chain had no definite effect on the antiestrogenicity of the molecule. A study of the three-dimensional (3D) molecular geometry of the *dl-trans* hydrazide (**4b**) and its superimposition on to the *dl-trans* centchroman (**1b**) on a silicon graphics Indy R 4000 workstation employing Molecular Simulations software (Insight II® 95 Molecular modeling system; Molecular Simulations and Discover® 95/3.0.0 Forcefield Simulations; Molecular Simulations) was carried out. Structures were built in the builder module of the Insight II software. The 3D structures were later optimized for their geometry using the consistent valence forcefield (CVFF) (Dauber-Osguthorpe *et al*., 1988) and energy minimization was performed using steepest-descent conjugate-gradient and Newton–Raphson algorithms in sequence followed by quasi-Newton–Raphson (va09a) implemented in discover module by using 0.001 kcal mol^{-1} energy gradient convergence and maximum number of iterations of 1,000. Energy-minimized structures were stored in MDL format.

The conformational resemblances of the molecules were assessed using the root-mean-square (RMS) fitting option of the Search–Compare forcefield (Search_compare 95.0 conformational Search and Molecular Comparison.; Molecular Simulations) module. One-to-one correspondences for atoms in the molecules to be superimposed were specified. The RMS fit between the superimposed molecules was noted.

Figure 1 shows the molecular similarity between the two molecules discussed above with RMS fit value of 0.007118. This would explain the observed similar

Table 1 Biological activity data

Compound no.	RBA, % of estradiol	Dose (mg kg^{-1})	Estrogenicity (% increase in uterine weight)	Antiestrogenicity (% inhibition in ethynyl estradiol-induced uterine weight gain)
2a	Not detected	10	71.58	3.71
3a	Not detected	10	44.2	3.8
4a	0.011	10	110.22	1.07
2b	0.029	10	43.7	3.98
3b	Not detected	10	21.6	10.8
4b	0.052	10	101.91	12.14
2c	Not detected	3	122.2	40.64
3c	0.037	10	149	10
4c	0.37	10	155.02	25.37
2d	Not detected	10	0.19	14.33
3d	Not detected	10	6.88	16.09
4d	0.24	10	6.68	6.21
1b	5.42	3	126.83	60
1c	15.7	3	165	46.11
1d	2.10	3	109	50
1a	<0.001	10	33.6	

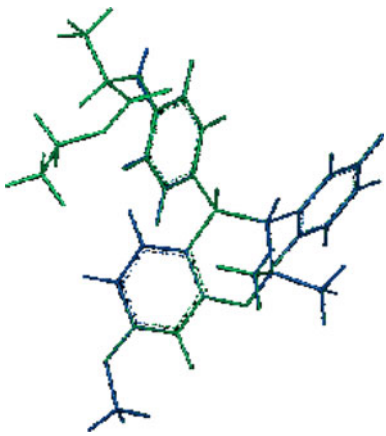


Fig. 1 Superposition of *dl-trans* centchroman with *dl-trans* hydrazide

order of estrogenicity and hence binding ability of the ligand to the estrogen receptor as seen with *dl*-centchroman.

Experimental section

All reactions were monitored by thin-layer chromatography (TLC) over silica-gel-coated TLC plates. Spots on TLC plates were visualized by warming plates sprayed

with $CeSO_4$ (1% in H_2SO_4) in an oven at 100°C or in iodine vapors. For column chromatography, silica gel (60–120 mesh) was used. Infrared (IR) spectra were recorded on Perkin-Elmer 881 and FTIR-8210 PC Shimadzu spectrophotometers and values are expressed in cm^{-1}, ^1H nuclear magnetic resonance (NMR) spectra were recorded on Advance DPX 200 FT Bruker Robotics spectrometer using TMS as an internal reference. Fast atom bombardment (FAB) mass spectra were recorded on Jeol SX 102/DA 6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Elemental analyses were carried out on Carlo-Erba-1108 instrument. Optical rotations were determined on Autopol III polarimeter using 1 dm cell at 28°C in methanol as the solvent with concentrations stated in units of g/100 ml.

Ethyl 2-(4-*cis*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (**2a**)

A mixture of 3,4-*cis*-2,2-dimethyl-3-phenyl-4-[4-hydroxy phenyl]-7-methoxychroman (1.5 g, 4.17 mmol), anhydrous potassium carbonate (6 g, 43.4 mmol), and ethyl 2-bromopropionate (8 ml, 43.4 mmol) in dry acetone (30 ml) was refluxed for 4 h. Potassium carbonate was filtered off and the solvent was distilled off. The residue was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and concentrated to give an oil.

Yield: 1.3 g (67.83%); IR (neat, cm^{-1}): 1446, 1504, 1582, 1615 (ArH), 1750 (ester), 1240 (OMe), 2983 (CH), 1374 (*gem*-dimethyl), 1662 (C=O); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.76 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.2 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 1.1 (t, 3H, CH_2CH_3) 4.6 (q, 1H, $CHCH_3$), 4.1 (q, 2H, CH_2CH_3), 1.5 (d, 3H, $CHCH_3$); MS *m/z*: 460. Anal.: ($C_{29}H_{32}O_5$), C and H.

2-[4-(*cis*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid (**3a**)

A mixture of ethyl 2-(4-*cis*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.75 g, 1.63 mmol), and sodium hydroxide (2.0 g, 50 mmol) in alcohol (20 ml) was refluxed for 1.5 h. The solvent was distilled off. The reaction mixture was acidified with 5% HCl solution and extracted with ethyl acetate, washed with water to pH 7, and dried over anhydrous sodium sulfate, then ethyl acetate was distilled off to give an oil, which was crystallized from methanol to give the desired product.

Yield: 0.6 g (85.19 %); m.p.: 150°C; IR (KBr, cm^{-1}): 1459, 1506, 1599 (ArH), 1725 (C=O), 1228 (OMe), 2933 (CH), 1360 (*gem*-dimethyl), 3410 (OH); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.76 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.1 (d, 1H, dibenzylic H, J = 12 Hz), 6.28–7.26 (m, 12H, ArH), 4.3 (q, 1H, $CHCH_3$), 1.5 (d, 3H, $CHCH_3$); MS *m/z*: 432. Anal.: ($C_{27}H_{28}O_5$), Cald: C and H.

2-[4-(*cis*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid hydrazide (**4a**)

A mixture of ethyl 2-(4-*cis*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.15 g, 0.33 mmol) and hydrazine hydrate (2 ml, 64.2 mmol) in alcohol (15 ml) was refluxed for 7 h. The solvent was distilled off to give an oil.

Yield: 0.136 g (93.51 %); m.p.: 60°C; IR (KBr, cm^{-1}): 1449, 1504, 1617, (ArH), 1677 (C=O), 1225 (OMe), 2929 (CH), 1375 (*gem*-dimethyl), 3329, 3439 (NH), 3695 (NH_2); ^1H NMR (δ, $CDCl_3$): 1.25 (s, 6H, *gem*-dimethyl), 3.79 (s, 3H, MeO), 2.9 (d, 1H, monobenzylic H, J = 12 Hz), 4.3 (d, 1H, dibenzylic H, J = 12 Hz), 6.35–7.51 (m, 12H, ArH), 1.5 (d, 3H, $CHCH_3$) 4.6 (q, 1H, $CHCH_3$), 7.26 (s, 1H, NH); MS *m/z*: 446. Anal.: ($C_{27}H_{30}O_4N_2$), C, H, and N.

Ethyl 2-(4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (**2b**)

A mixture of 3,4-*trans*-2,2-dimethyl-3-phenyl-4-(4-hydroxy-phenyl)-7-methoxychroman (0.2 g, 0.56 mmol), anhydrous potassium carbonate (0.8 g, 5.7 mmol), and ethyl 2-bromopropionate (0.39 ml, 3.0 mmol) in dry acetone (10 ml) was refluxed for 4 h. Potassium carbonate was filtered off and the solvent was distilled off. The residue was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and concentrated to give oil, which was crystallized from methanol.

Yield: 0.180 g, (70.44 %); m.p.: 120°C; IR (KBr, cm^{-1}): 1450, 1506, 1585, 1612 (ArH), 1745 (ester), 1207 (OMe), 2985 (CH), 1377 (*gem*-dimethyl); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.76 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.5 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 1.1 (t, 3H, CH_2CH_3), 4.1 (q, 2H, CH_2CH_3), 1.5 (d, 3H, $CHCH_3$), 4.5 (q, 1H, $CHCH_3$); MS *m/z*: 460. Anal.: ($C_{29}H_{32}O_5$), C, H, and N.

2-[4-(*trans*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid (**3b**)

A mixture of ethyl 2-(4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.5 g, 1.09 mmol) and sodium hydroxide (1.0 g, 25 mmol) in alcohol (20 ml) was refluxed for 1.5 h. The solvent was distilled off. The reaction mixture was acidified with 5% HCl solution and extracted with ethyl acetate, washed with water to pH 7, and dried over anhydrous sodium sulfate, then ethyl acetate was distilled off to give an oil, which was crystallized from methanol to give the desired product.

Yield: 0.3 g (63.89 %); m.p.:185°C; IR (KBr, cm^{-1}): 1417, 1512, 1593, 1614 (ArH), 1722 (C=O), 1217 (OMe), 3022 (CH), 1373 (*gem*-dimethyl), 3425 (OH); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.69 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.1 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 4.4 (q, 1H, $CHCH_3$), 1.51 (d, 3H, $CHCH_3$); MS *m/z*: 432. Anal.: ($C_{27}H_{28}O_5$), C and H.

2-[4-(*trans*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid hydrazide (**4b**)

A mixture of ethyl 2-(4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.15 g, 0.33 mmol) and hydrazine hydrate (2 ml, 64.2 mmol) in absolute alcohol (20 ml) was refluxed for 7 h. The solvent was distilled off to give an oil, which was crystallized from ethanol to give the desired product.

Yield: 0.1 g (68.76%); m.p.: 195°C; IR (KBr, cm^{-1}): 1417, 1505, 1617, (ArH), 1670 (C=O), 1217 (OMe), 2930 (CH), 1382 (*gem*-dimethyl), 3290 (NH), 3437 (NH_2); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.11 (d, 1H, monobenzylic H, J = 12 Hz), 4.29 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 1.51 (d, 3H, $CHCH_3$) 4.60–4.64 (q, 1H, $CHCH_3$), 7.26 (s, 1H, NH); MS *m/z*: 446. Anal.: ($C_{27}H_{30}O_4N_2$) C, H, and N.

Ethyl 2-(4-*l*(-)3,4 *trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (**2c**)

A mixture of *l*(-)3,4-*trans*-2,2-dimethyl-3-phenyl-4-[4-hydroxy phenyl]-7-methoxychroman (1.5 g, 4.17 mmol), anhydrous potassium carbonate (6 g, 43.4 mmol), and ethyl 2-bromopropionate (4.5 ml, 34.6 mmol) in dry acetone (50 ml) was refluxed for 4 h. Potassium carbonate was filtered off and the solvent was distilled off. The residue was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and concentrated to give an oil, which was crystallized from methanol.

Yield: 1.4 g (73.04 %); m.p.: 96°C; $[\alpha]_D^{20}$: (C = 1, MeOH): –193.52; IR (KBr, cm^{-1}): 1450, 1506, 1598, 1612 (ArH), 1745 (ester), 1207 (OMe), 2985 (CH), 1382 (*gem*-dimethyl); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.11 (d, 1H, monobenzylic H, J = 12 Hz), 4.5 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 1.1 (t, 3H, CH_2CH_3) 4.58 (q, 1H, $CHCH_3$), 4.11 (q, 2H, CH_2CH_3), 1.5 (d, 3H, $CHCH_3$). MS *m/z*: 460. Anal.: ($C_{29}H_{32}O_5$), C, H, and N.

2-[4-(*l*(-)-*trans*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid (**3c**)

A mixture of ethyl 2-(4-*l*(-)-3,4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.75 g, 1.63 mmol) and sodium hydroxide (2.0 g, 50 mmol) in alcohol (20 ml) was refluxed for 1.5 h. The solvent was distilled off. The reaction mixture was acidified with 5% HCl solution and extracted with ethyl acetate, washed with water to pH 7, and dried over anhydrous sodium sulfate, and ethyl acetate was distilled off to give an oil, which was crystallized from methanol to give the desired product.

Yield: 0.61 g (86.60%); m.p.: 153°C; $[\alpha]_D^{20}$: (C = 1, MeOH): –204.761; IR (KBr, cm^{-1}): 1447, 1506, 1585, 1609 (ArH), 1725 (C=O), 1231 (OMe), 2983 (CH), 1350 (*gem*-dimethyl), 3402 (OH); ^1H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz),

4.1 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 4.6 (q, 1H, $CHCH_3$), 1.5 (d, 3H, $CHCH_3$); MS *m/z*: 432. Anal.: ($C_{27}H_{28}O_5$), C and H.

2-[4-(*l*(-)-*trans*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid hydrazide (**4c**)

A mixture of ethyl 2-(4-*l*(-)-3,4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.15 g, 0.33 mmol) and hydrazine hydrate (2 ml, 64.2 mmol) in alcohol (20 ml) was refluxed for 7 h. The solvent was distilled off to give an oil, which was crystallized from ethanol to give product.

Yield: 0.123 g (84.57%); m.p.: 125°C; $[\alpha]_D^{20}$: (C = 1, MeOH): –146.218; IR (KBr, cm^{-1}): 1507, 1618, (ArH), 1678 (C=O), 1216 (OMe), 2930 (CH), 1382 (*gem*-dimethyl), 3442 (NH), 3758 (NH_2); ^{1}H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.12 (d, 1H, monobenzylic H, J = 12 Hz), 4.29 (d, 1H, dibenzylic H, J = 12 Hz), 6.35–7.51 (m, 12H, ArH), 1.73 (d, 3H, $CHCH_3$) 4.61 (q, 1H, $CHCH_3$), 7.26 (s, 1H, NH); MS *m/z*: 446. Anal.: ($C_{27}H_{30}O_4N_2$), C, H, and N.

Ethyl 2-(4-*d*(+)-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (**2d**)

A mixture of *d*(+)3,4-*trans*-2,2-dimethyl-3-phenyl-4-[4-hydroxy phenyl]-7-methoxychroman (0.2 g, 0.56 mmol), anhydrous potassium carbonate (0.8 g, 5.7 mmol), and ethyl 2-bromopropionate (0.39 ml, 3.0 mmol) in dry acetone (10 ml) was refluxed for 4 h. Potassium carbonate was filtered off and the solvent was distilled off. The residue was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and concentrated to give the desired product.

Yield: 0.170 g (66.52%); $[\alpha]_D^{20}$: (C = 1, MeOH): +190.566; IR (neat, cm^{-1}): 1449, 1505, 1586, 1615 (ArH), 1744 (ester), 1235 (OMe), 2987 (CH), 1377 (*gem*-dimethyl); ^{1}H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.76 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.2 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 1.1 (t, 3H, CH_2CH_3), 4.1 (q, 2H, CH_2CH_3), 1.5 (d, 3H, $CHCH_3$), 4.6 (q, 1H, $CHCH_3$); MS *m/z*: 460. Anal.: ($C_{29}H_{32}O_5$) C and H.

2-[4-(*d*(+)-*trans*-3,4-dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid (**3d**)

A mixture of ethyl 2-(4-*d*(+)-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.4 g, 0.87 mmol), and sodium hydroxide (0.8 g, 20 mmol) in alcohol (20 ml) was refluxed for 1.5 h. The solvent was distilled off. The reaction mixture was acidified with 5% HCl solution and extracted with ethyl acetate, washed with water to pH 7, dried over anhydrous sodium sulfate, then ethyl acetate was distilled off to give an oil, which was crystallized from methanol to give the desired product.

Yield: 0.325 g (86.52%); m.p.:150°C; $[\alpha]_D^{20}$: (C = 1, MeOH): +200; IR (KBr, cm^{-1}): 1447, 1506, 1585, 1609 (ArH), 1725 (C=O), 1231 (OMe), 2983 (CH), 1350 (*gem*-dimethyl), 3402 (OH); ^{1}H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.1 (d, 1H, monobenzylic H, J = 12 Hz), 4.1 (d, 1H, dibenzylic H, J = 12 Hz), 6.3–7.2 (m, 12H, ArH), 4.6 (q, 1H, $CHCH_3$), 1.5 (d, 3H, $CHCH_3$); MS *m/z*: 432. Anal.: ($C_{27}H_{28}O_5$), C and H.

2-[4-(*d*(+)-*trans*-3,4-Dihydro-2,2-dimethyl-3-phenyl-7-methoxybezopyran-4-yl)-phenoxy] propionic acid hydrazide (**4d**)

A mixture of ethyl 2-(*d*(+)-4-*trans*-2,2-dimethyl-3-phenyl-7-methoxychroman-4-yl)-phenoxy-propanoate (0.15 g, 0.33 mmol) and hydrazine hydrate (2 ml, 64.2 mmol) in alcohol (20 ml) was refluxed for 7 h. The solvent was distilled off to give an oil.

Yield: 0.115 g (79.07%); $[\alpha]_D^{20}$: (C = 1, MeOH): +187.40; IR (neat, cm^{-1}): 1452, 1507, 1617 (ArH), 1671 (C=O), 1231 (OMe), 2996 (CH), 1374 (*gem*-dimethyl), 3327 (NH), 3658 (NH_2); ^{1}H NMR (δ, $CDCl_3$): 1.2 (s, 3H, *gem*-dimethyl), 1.3 (s, 3H, *gem*-dimethyl), 3.77 (s, 3H, MeO), 3.12 (d, 1H, monobenzylic H, J = 12 Hz), 4.29 (d, 1H, dibenzylic H, J = 12 Hz), 6.35–7.36 (m, 12H, ArH), 1.51 (d, 3H, $CHCH_3$), 4.6 (q, 1H, $CHCH_3$), 7.26 (s, 1H, NH); MS *m/z*: 446. Anal.: ($C_{27}H_{30}O_4N_2$), C and H.

Biochemical and biological methods

Receptor affinity (Katzenellenbogen *et al*., 1973; Salman *et al*., 1983)

Relative binding affinities (RBA) for uterine cytosol 17-β-estradiol receptors obtained from immature Chares Foster rats, 21–25 days old, were determined by competition assay, employing dextran-coated charcoal (DCC) for separation of unbound steroids according to the method of Korenman, as modified by Katzenellenbogen, and are listed in Table 1.

Estrogenic activity (Salman *et al*., 1983; Kar *et al*., 1967)

Estrogenic activity of the compounds, reported in Table 1, was evaluated in immature rats (25–30 g) as assessed by uterine weight gain. The compounds were administered subcutaneously once daily over a 3-day period in 0.2 ml saline/propylene glycol (1:1, v/v).

Estrogen antagonistic activity (Kar *et al*., 1967)

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and, after postoperative rest for 7 days, randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg/kg does of 17α-ethynylestradiol in 10% ethanol-distilled water once daily for 3 consecutive days on days 28–30 of age by oral route. A separate group

of animals receiving only 17α-ethynylestradiol (0.02 mg/kg) in 10% ethanol-distilled water for similar duration were used for comparison. At autopsy on day 31 of age vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed, and fixed for histology and histomorphometry using image analysis. Inhibition of ethynylestradiol-induced cornification of vaginal epithelium, increase in uterine fresh weight, total uterine and endometrial area, and uterine luminal epithelial cell height were taken as parameters for evaluation of estrogen antagonistic effect of the compounds. Compounds exhibiting potent estrogen antagonistic activity in this assay were identified for further development as anti-implantation agents.

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