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Rapid synthesis of 4-benzylidene and 4-[bis-(4-methoxyphenyl)-methylene-2-substituted phenyl-benzopyrans as potential selective estrogen receptor modulators (SERMs) using McMurry coupling reaction

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Abstract—7-Methoxy-4-(4-methoxybenzylidene)-2-substituted phenyl-benzopyrans **I** and 4-[bis-(4-methoxyphenyl)-methylene-2-substituted phenyl-benzopyrans **II** carrying different alkylamino residues, designed as estrogen receptor (ER) binding ligands, were successfully synthesized through the McMurry coupling reaction of substituted benzaldehyde/substituted benzophenones and 2-hydroxyphenyl-7-methoxy-chroman-4-one in presence of lithium aluminum hydride and titanium (IV) chloride ($LAH–TiCl_4$). Self-coupling of carbonyl reactants led to the formation of several side products. The prototypes were evaluated for their relative binding affinity (RBA), as well as their estrogenic and antiestrogenic activities. High order of estrogenic activity (>50% gain) observed with compounds **3**, **7a**, **7b**, **7c**, **8**, and **10a** and also their partial estrogen antagonistic activity ($\geqslant$15%) at the uterine level points toward successful designing of the compounds. Compounds **4**, **7a**, **7b**, **7c**, and **10a** also possessed significant anticancer activity against human adenocarcinoma cell line (MCF-7 cell line) that may be related to their estrogen-dependent action.

The importance of selective estrogen receptor modulators (SERMs)[1–4] as contraceptive and in the treatment of various estrogen dependent diseases such as breast cancer, osteoporosis, Alzheimer's disease (AD), and coronary heart disease has led to the development of structurally diverse estrogen receptor binding molecules.

Four main types of SERMs that have been investigated are triphenylethylenes (including tamoxifen,[5] toremifene, droloxifene, and idoxifene), benzothiaphenes (e.g., raloxifene,[6] and arzoxifene), naphthalenes (nafoxidine), and benzopyrans (ormeloxifene[7] and related derivatives) (Fig. 1). Tamoxifen[5] is being used frequently as a drug for the prevention and treatment of breast cancer. Raloxifene[6], another SERM, is commonly used in the prevention and treatment of osteoporosis. Ormeloxifene,[8] which is a benzopyran-based SERM developed by our group, is the first non-steroidal, post coital, oral contraceptive.

Ormiloxifene is a racemate mixture and is marketed under the trade name Saheli. Use of ormeloxifene and its different analogues as antiosteoporotic and antibreast cancer agents is under investigation.[8]

However, the SERMs that have been introduced in the market are not totally free from several undesirable side effects such as increased incidence of endometrial cancer and hot flashes.[9,10] Therefore, further work in this area is needed to develop SERM for a specific disorder.

It was found that introduction of an aryl moiety at C-2 of a benzopyran nucleus leads to potent estrogen antagonists. Two such important compounds, CDRI-85/287[11] and EM-652,[12] are shown below in Figure 2.

Keywords: Antiestrogens; Estrogen antagonists; Antifertility; RBA.
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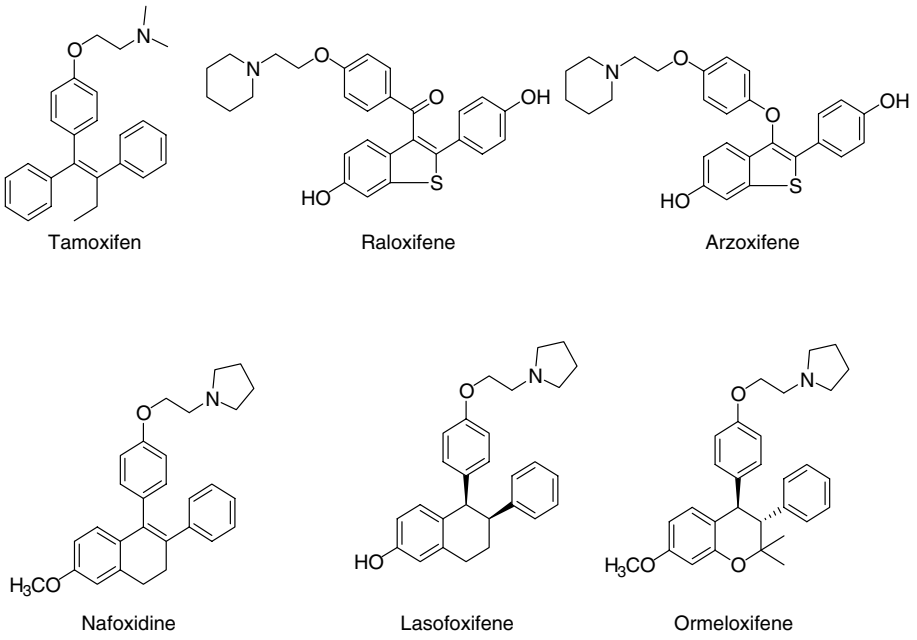


Figure 1. Non-steroidal SERM molecules.

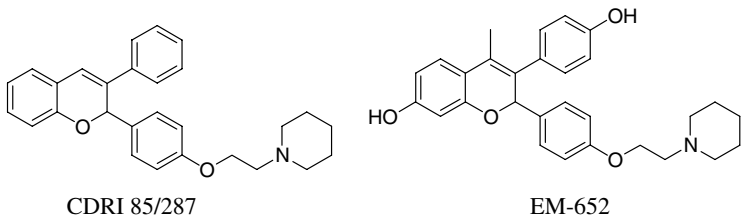
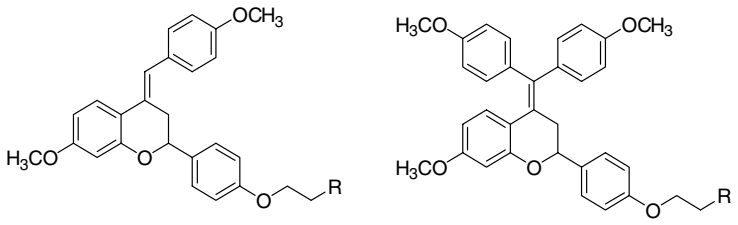


Figure 2.

Keeping in view all the above-mentioned compounds and their biological activities, it was thought worthwhile to design and synthesize compounds belonging to prototypes **I** and **II** (Fig. 3) which are reported herein.

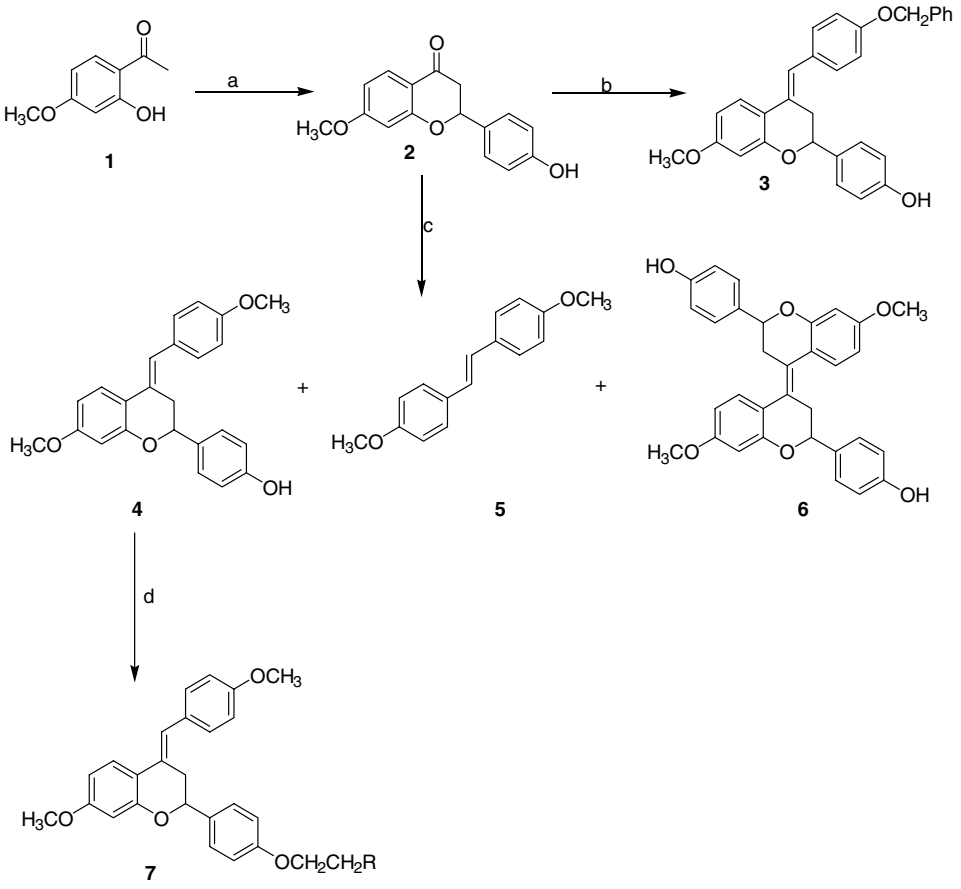
The synthesis of prototype **I** was initially attempted through Grignard reaction on 2-(4-hydroxyphenyl)-7-methoxy chroman-4-one **2**, prepared in 35% yield from 2-hydroxy-4-methoxy-acetophenone **1** and substituted benzyl bromide. This reaction led to a mixture of products containing 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)-propenone as a result of chromanone ring opening and possibly due to the formation of products with endo- and exocyclic double bonds, which could not be separated. Chromans and chromanones are known to undergo ring opening under Mg/THF.[13] The desired compounds were finally synthesized through the McMurry coupling reaction. When condensation of 4-benzyloxy benzaldehyde with 2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **2** was carried out in presence of Zn–$TiCl_4$ in dry THF at reflux temperature, the desired compound 4-[7-methoxy-4-(4-benzyloxybenzylidene)-chroman-2-yl]-phenol **3** was obtained in very poor yield along with dimeric product of reactants. It is reported that in McMurry reaction the efficacy of reaction depends on the reactivity of low valent titanium reagent which ultimately depends on its method of preparation.[14] Lithium aluminum hydride (LAH), a far stronger reducing agent as compared to zinc has been successfully used for such reactions. We therefore used LAH–$TiCl_4$ in place of Zn–$TiCl_4$ in anhydrous THF. Thus, reductive coupling of compound **2** with 4-methoxybenzaldehyde was carried out with LAH–$TiCl_4$ in dry THF under inert reaction condition giving compound **4** in satisfactory yield. In this reaction, dimeric products of 4-methoxybenzaldehyde (**5**) and 2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **2** **(6)** were formed as byproducts. Dimer of 4-methoxybenzaldehyde was isolated as white solid which was a mixture of cis- and trans isomers, whereas dimer of 2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **2** (**6**) appeared to be unstable and was detected only through mass spectroscopy of partly purified sample. Condensation of compound **4** with 1-(2-chloroalkyl) amine hydrochloride in acetone in the presence of anhydrous K_2CO_3 under reflux gave the desired product **7** (prototype **I**) (Scheme 1).[20]

The synthesis of prototype **II** was carried out through the McMurry coupling reaction using 4,4′-dimethoxy benzophenone and 2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **2** (Scheme 2).[20] In this case, besides the desired compound **8**, dimers of chromanone **2** (**6**) and 4,4′-dimethoxybenzophenone (**9**) were formed as



Prototype **I** Prototype **II**

Figure 3.

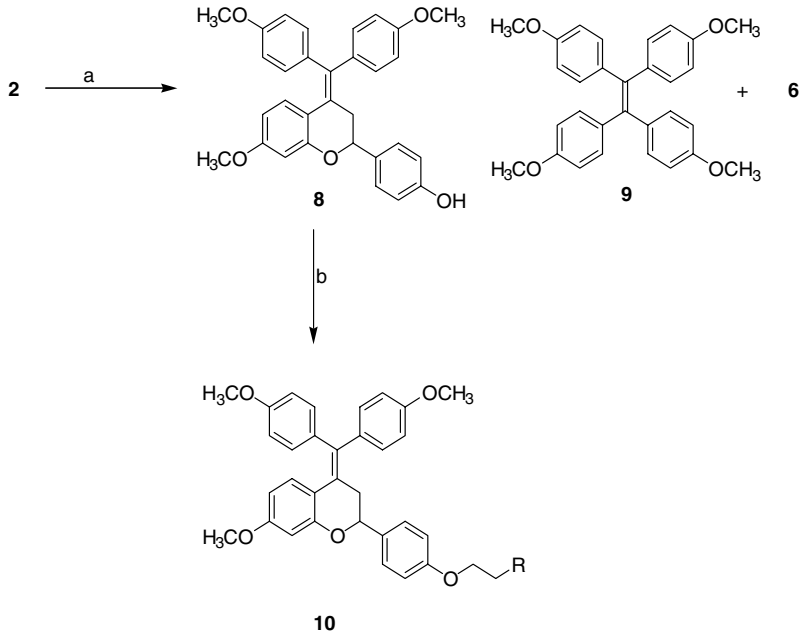


Scheme 1. Reagents: (a) 4-hydroxybenzaldehyde, dry piperidine, dry benzene, reflux; (b) 4-benzyloxybenzaldehyde, Zn–$TiCl_4$, dry THF, reflux; (c) 4-methoxybenzaldehyde, $TiCl_4$–LAH, dry THF, reflux; (d) 2-chloroethyl alkylamine hydrochloride, K_2CO_3, dry acetone, reflux. For structure **7**, R = (a) NC_4H_8, (b) NC_5H_{10}, (c) $N(CH_3)_2$.

byproducts. Dimer **9** was isolated as crystalline solid, whereas dimer **6** seemed to be unstable and was detected through mass spectroscopy. Condensation of compound **8** with 1-(2-chloroalkyl) amine hydrochloride in acetone in the presence of anhydrous K_2CO_3 under reflux gave the desired product **10** (prototype **II**).

Estrogen receptor binding affinity, estrogenic, antiestrogenic, and antiproliferative activity data of compounds are shown in Table 1. Compounds **3**, **7a**, **7b**, **7c**, **8**, and **10a** administered orally showed high estrogenic activity (>50% gain) and also possessed estrogen antagonistic activity ($\geqslant$15%). Compounds **4**, **7a**, **7b**, **7c**, and **10a** possessed significant anticancer activity similar to that of Tamoxifen against MCF-7 cancer cell line.

In conclusion, this study presents an efficient method for the preparation of 4-[7-methoxy-4-(4-methoxy-benzylidene)-chroman-2-yl]-phenol and 4-[bis-(4-methoxyphenyl)-methylene]-7-methoxy-chroman-2-yl]-phenol substituted with different alkyl amine chains, using the McMurry coupling reaction. Significant estrogenic and anti-estrogenic activities exhibited by these compounds point to their ability to bind to estrogen receptor as such or as possible hydroxy metabolite. Their low RBA values are likely due to the absence of free phenolic groups



Scheme 2. Reagents: (a) 4,4′-dimethoxybenzophenone, $TiCl_4$–LAH, dry THF, reflux; (b) 2-chloroethyl alkylamine hydrochloride, K_2CO_3, dry acetone, reflux. For structure **10**, R = (a) NC_5H_{10}, (b) $N(CH_3)_2$.

Table 1. Biological activity data

Compound	Dose (oral) (mg/kg/day)	Estrogen antagonistic activity[16]		Estrogen agonistic activity[16]		RBA[15] % of E_2	Antiproliferative activity[17] IC_{50} (μM)
		Uterine weight[a] (mg)	Inhibition[b] (%)	Uterine weight[a] (mg)	Gain[c] (%)		
Vehicle	10	18.20 ± 0.50		18.20 ± 0.50			
EE	0.02	102.60 ± 2.20		102.60 ± 2.20	464		
TAM	3	52.30 ± 5.30	49	46.32 ± 2.92	155	2[g]	5.09
Vehicle	10	15.00 ± 0.57		15.00 ± 0.57			
EE	0.02	66.35 ± 0.44		66.35 ± 0.44	342		
3	10	46.33 ± 2.16[e]	29	38.30 ± 3.66[g]	153	0.02	Inactive
Vehicle	10	14.00 ± 1.52		14.00 ± 1.52			
EE	0.02	85.00 ± 0.57		85.00 ± 0.57	507		
4	10	88.33 ± 10.47	—	17.33 ± 2.40[f]	24	0.013	8.77
8	10	70.33 ± 6.88[d]	17	26.00 ± 2.30[g]	86	0.097	Inactive
Vehicle	10	16.03 ± 0.99		16.03 ± 0.99			
EE	0.02	108.43 ± 4.14		108.43 ± 4.14	576		
7a	10	92.30 ± 13.17[d]	15	39.57 ± 4.75[g]	147	0.095	7.39
7b	10	104.4 ± 1.00[d]	4	24.63 ± 0.90[g]	54	0.095	4.39
7c	10	85.40 ± 2.88	21	46.30 ± 1.61	189	0.006	2.00
Vehicle	10	19.60 ± 0.44		19.60 ± 0.44			
EE	0.02	106.96 ± 7.05		106.96 ± 7.05	445		
10a	10	81.70 ± 4.00[e]	26	52.50 ± 4.60[f]	168	<0.001	6.68
10b	10	—	ND	—	ND	<0.001	Inactive

ND, not determined; EE = 17α-ethynylestradiol; E_2 = 17β-estradiol.

[a] Values represent means ± SEM of a minimum of six observations in each group.

[b] Percent of 17α-ethynylestradiol per se treated group.

[c] Percent of vehicle control group.

[d] $P < 0.05$.

[e] $P < 0.01$ versus corresponding EE per se treated group; compound **4** exhibited an additive effect on ethnylestradiol induced uterine weight gain, but lacked any estrogen antagonistic activity.

[f] $P < 0.05$.

[g] $P < 0.01$, versus corresponding vehicle control group, all other relevant comparisons were statistically not significant, g = Ref. 18, TAM = tamoxifene.

at the estrogen receptor binding subsite. Free phenolic groups present in compounds **4** and **8** are possibly at the anti-estrogen binding subsite. A preliminary study of their anti-proliferative activity, showing promising activity, suggests a more detailed investigation of these prototype molecules as anticancer agents.

Acknowledgments

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References and notes

1. (a) A. Gruber, C. J.; Tschugguel, W.; Schneeberger, C.; Huber, J. C. *N. Eng. J. Med.* **2002**, *346*, 304; (b) Grese, T. A.; Dodge, J. A. *Annu. Rep. Med. Chem.* **1996**, *19*, 181.
2. Riggs, B. L.; Hartmann, L. C. *N. Eng. J. Med.* **2003**, *348*, 618.
3. Pearce, S. T.; Jordan, V. C. *Crit. Rev. Oncol./Heamatol.* **2004**, *50*, 3.
4. (a) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 883; (b) Ray, S.; Dwivedy, I. *Adv. Drug Res.* **1997**, *29*, 171; (c) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 1081.
5. (a) Andersson, M.; Storm, H. H.; Mouridsen, H. J. *Acta Oncol.* **1992**, *31*, 259; (b) Anzai, Y.; Holinka, C. F.; Kuramoto, H.; Gurpide, E. *Cancer Res.* **1989**, *49*, 2362.5; (c) Sacco, A.J.; Gendre, I. Tamoxifene, Beyond the antiestrogens. In Jhon A Kellen, Ed.; 1996, pp. 59–92.
6. (a) Black, L. J.; Goode, R. L. *Life Sci.* **1980**, *26*, 1453; (b) Black, L. J.; Sato, M.; Rowley, E. R., et al. *J. Clin. Invest.* **1994**, *93*, 63; (c) Ettinger, B.; Black, D. M.; Mitlak, B. H., et al. *J. Am. Med. Assoc.* **1999**, *282*, 637.
7. (a) Ray, S.; Tandon, A.; Dwivedy, I.; Wilson, S. R.; O'Neil, J. P.; Katzenellenbogen, J. A. *J. Med. Chem.* **1994**, *37*, 696; (b) Kamboj, V. P.; Ray, S.; Dhawan, B. N. *Drugs Today* **1992**, *28*, 227.
8. (a) Singh, M. M. *Med. Res. Rev.* **2001**, *21*, 302; (b) Misra, N. C.; Nigam, P. K.; Gupta, R.; Agarwal, A. K.; Komboj, V. P. *Int. J. Caner* **1989**, *43*, 781.
9. Fisher, B.; Costantino, J. P.; Wickerham, D. L.; Redmond, C. K.; Kavanah, M.; Cronin, W. M.; Vogel, V.; Robidoux, A.; Dimitrov, N.; Atkins, J.; Daly, M.; Wieand, S.; Tan-Chiu, E.; Ford, L.; Wolmark, N. *J. Natl. Cancer Inst.* **1998**, *90*, 1371.
10. Walsh, B. W.; Kuller, L. H.; Wild, R. A.; Paul, S.; Farmer, M.; Lawrence, J. B.; Shah, A. S.; Anderson, P. W. *J. Am. Med. Assoc.* **1998**, *279*, 1445.
11. Saeed, A.; Sharma, A. P.; Durani, N.; Jain, R.; Durani, S.; Kapil, R. S. *J. Med .Chem.* **1990**, *33*, 3210, 3216, 3211.
12. Gauthier, S.; Caron, B.; Cloutier, J.; Dory, Y. K.; Favre, A.; Larouche, D.; Mailhot, J.; Ouellet, C.; Schwerdtfeger, A.; Leblanc, G.; Martel, C.; Simard, J.; Mérand, Y.; Bélanger, A.; Labrie, C.; Labrie, F. *J. Med. Chem.* **1997**, *40*, 2117.
13. Katritzy and Rees, Comprehensive Heterocyclic Chemistry, Vol. 3, Chapter 2.22, British Cataloguing in Publication Data, pp. 573–883.
14. McMurry, J. E. *Chem. Rev.* **1989**, *89*, 1513.
15. Dhar, J. D.; Dwivedi, A.; Srivastava, A.; Setty, B. S. *Contraception* **1994**, *49*, 609.
16. Ghosh, R.; Kamboj, V. P.; Singh, M. M. *Contraception* **2001**, *64*, 261.
17. (a) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Acad. Sci.* **1990**, *82*, 1107; (b) Srivastava, S.; Sharma, R.; Balapure, A. K. *Ind. J. Pharm.* **2004**, *36*, 238.
18. Sudo, K.; Munsmaj, F. J.; Katzenellenbogen, B. S. *Endocrinology* **1983**, *112*, 425.
19. *Selected Physical data*:
Compound **3**: Yield: 20%, mp138–140 °C. FABMS: 450; IR (cm^{-1}): 3383, 2945, 1606, 1509, 1238, 836; ^1H NMR (δ): 2.84 (m, 1H, CH_2), 3.15 (dd, 1H, CH_2), 3.79 (s, 3H, OCH_3), 4.90 (dd, 1H, CH), 5.07(s, 1H, CH), 6.48 (d, $J = 2.40$ Hz, 1H, ArH), 6.57 (dd, 1H, ArH), 6.84 (d, $J = 8.40$ Hz, 2H, ArH), 6.94 (d, $J = 8.70$ Hz, 2H, ArH), 7.32 (m, 8H, ArH), 7.89 (d, $J = 8.70$ Hz, 1H, ArH). Anal. Calcd for $C_{30}H_{26}O_4$: C, 80.00; H, 5.78. Found: C, 80.25; H, 6.02.
Compound **4**: Yield: 30%, mp 162–164 °C. FABMS: 374; IR (cm^{-1}): 3398, 2943, 1601, 1352, 1242, 1165, 1024, 836; ^1H NMR (δ): 2.86 (t, 1H, CH), 3.15 (dd, 1H, CH), 3.83 (s, 6H, 2× OCH_3), 4.89 (dd, 1H, CH), 6.49 (s, 1H, CH), 6.57 (d, $J = 8.81$ Hz, 1H, ArH), 6.85 (m, 5H, ArH), 7.29 (m, 4H, ArH), 7.60 (d, $J = 8.60$ Hz, 1H, ArH); ^{13}C NMR (δ): 32, 54, 76, 100, 107, 112, 114, 118.4, 124, 125.8, 126.6, 127.2129.4, 130.1, 155, 156.4, 157, 159.2. Anal. Calcd for $C_{24}H_{22}O_4$: C, 77.00; H, 5.88. Found: C, 76.81; H, 5.93.
Compound **7a**: Yield: 85%, mp of oxalate salt 183–185 °C. FABMS: 472(M+1); IR (cm^{-1}): 2950, 2832, 1603, 1510, 1244, 1030; ^1H NMR (δ): 1.39 (br s, 4H, $2CH_2$), 2.49 (br s, 4H, $2CH_2$), 2.75 (t, 2H, CH_2), 2.83 (dd, 1H, CH), 3.09 (dd, 1H, CH), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.08 (t, 2H, CH_2), 4.84 (dd, 1H, CH), 6.42 (s, 1H, CH), 6.51 (d, $J = 8.21$ Hz, 1H, ArH), 6.84 (m, 5H, ArH), 7.15 (d, $J = 8.06$ Hz, 2H, ArH), 7.27 (d, $J = 8.04$ Hz, 2H, ArH), 7.53 (d, $J = 8.64$ Hz, 1H, ArH). Anal. Calcd for $C_{30}H_{33}NO_4$: C, 76.43; H, 7.01; N, 2.97. Found: C, 76.51; H, 7.33; N, 3.21.
Compound **7b**: Yield: 82%, mp 93–95 °C. FABMS: 486 (M+1).
Compound **7c**: Yield: 80%, mp of oxalate salt 138–140 °C. FABMS: 535.
Compound **8**: Yield: 35%, mp 182–184 °C. FABMS: 480; IR (cm^{-1}): 3430, 2969, 1643, 1601, 1317, 1255, 1162, 849, 767; ^1H NMR (δ): 2.80 (br s, 2H, CH_2), 3.78 (s, 9H, 3× OCH_3), 5.16 (dd, 1H, CH), 5.29 (s, 1H, OH), 6.14 (d, 1H, $J = 3.60$ Hz, ArH), 6.43 (s, 1H, ArH), 7.01 (m, 13H, ArH). Anal. Calcd for $C_{31}H_{28}O_5$: C, 77.50; H, 5.83. Found: C, 77.82; H, 5.72.
Compound **10a**: Yield: 84%, mp 167–169 °C. FABMS: 591; IR(cm^{-1}): 2933, 1603, 1506, 1383, 1248, 1166, 1028, 832: ^1H NMR (δ): 1.43 (br s, 6H, $3CH_2$), 2.51 (br s, 4H, $2CH_2$), 2.68 (t, 2H, CH_2), 2.81 (dd, 1H, CH), 3.12 (dd, 1H, CH), 2.80 (br s, 2H, CH_2), 3.78 (s, 9H, 3× OCH_3), 5.16 (dd, 1H, CH), 6.16 (d, $J = 3.60$ Hz, 1H, ArH), 6.44 (s, 1H, ArH), 7.14 (m, 13H, ArH). Anal. Calcd for $C_{38}H_{41}NO_5$: C, 77.16; H, 6.94; N, 2.37. Found: C, 77.23; H, 7.02; N, 2.53.
Compound **10b**: Yield: 75%, mp 170–172 °C. FABMS: 566 (M+1).
20. Experimental:
General procedures for the synthesis of 1-(2-{4-[7-methoxy-4-(4-methoxybenzylidene)-chroman-2-yl]-phenoxy}-ethyl)-alkylamines (**7**):
Synthesis of 4-[7-methoxy-4-(4-methoxybenzylidene)-chroman-2-yl]-phenol (**4**):
Under an inert atmosphere of N_2, a stirred mixture of THF (60 ml) and $TiCl_4$ (5 ml, 8.65 g, 0.05 mol) at 0 °C was

treated with LAH (1 g, 0.03 mol). The resulting black slurry was allowed to reflux for an hour, then the reaction mixture was gradually cooled to 0 °C. To this mixture was added a mixture of 2-hydroxy-7-methoxy-chroman-4-one **2** (0.50 g, 0.002 mol) and 4-methoxybenzaldehyde (0.95 g, 0.007 mol) in dry THF. The reaction mixture was refluxed at 70 °C for 3 h. On completion of reaction, the mixture was poured onto 10% aqueous solution of potassium carbonate. The whole content was then filtered through silica gel under suction. Filtrate was extracted with ethyl acetate, the organic layer was dried over sodium sulfate and concentrated to oily residue. The crude material was chromatographed over silica gel using ethyl acetate–hexane (7:93) as eluent to yield (30%) pure compound **4**.[19]

Synthesis of 1-(2-{4-[7-Methoxy-4-(4-methoxy-benzylidene)-chroman-2-yl]-phenoxy}-ethyl)-pyrrolidine (**7a**, R = NC_4H_8):

To a solution of 4-[7-methoxy-4-(4-methoxybenzylidene)-chroman-2-yl]-phenol (**4**) (0.374 g, 0.001 mol) in dry acetone, anhydrous potassium carbonate (1.0 g) and 2-chloroethylpyrrolidine hydrochloride (0.26 g, 0.002 mol) were added. The solution was heated under reflux for 6 h. The reaction mixture was filtered and evaporated to dryness. The residue was taken into ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and concentrated. The crude material was then chromatographed over a column of silica gel eluting with methanol–chloroform (3:97) to afford compound **7a** in 85 % yield. Pure compound **7a** was then treated with oxalic acid to obtained the oxalate salt of compound **7a**.[19]

General procedure for the synthesis of 1-[2-(4-{4-[bis-(4-methoxyphenyl)-methylene]-7-methoxy-chroman-2-yl}-phenoxy)-ethyl]-alkylamine (**10**):

Synthesis of 4-[bis-(4-methoxyphenyl)-methylene]-7-methoxy-chroman-2-yl]-phenol (**8**):

The title product, compound **8**,[19] was prepared using the same procedure as for the preparation of derivative **4** using 4,4′-dimethoxybenzophenone (0.726 g, 0.003 mol) instead of 4-methoxybenzaldehyde. Compound **8** was obtained in 35% yield.

Synthesis of 1-[2-(4-{4-[bis-(4-methoxyphenyl)-methylene]-7-methoxy-chroman-2-yl}-phenoxy)-ethyl]-piperidine (**10a**, R = NC_5H_{10}):

The title product**10a**[19] was prepared using the same procedure as for the preparation of derivative **7a** using derivative **8** (0.48 g, 0.001 mol) and 2-chloroethylpiperidine hydrochloride (0.04 g, 0.002 mol) instead of 2-chloroethylpyrrolidine hydrochloride. Compound **10a** was obtained in 84%.

Estrogen receptor binding affinity[15]

The relative binding affinity (RBA) of the compounds for the estrogen receptor was determined[15] by competition assay, employing radiolabeled estradiol (^{3}H-E_2) as the reference compound. The test ligands and (^{3}H-E_2) were incubated (4 °C) with cytosol estrogen receptors obtained from immature 20 to 21-day-old rat uteri. Aliquots of the uterine cytosol (200 μl concentrated 1 uterus per ml) prepared in TEA buffer (10 mM Tris, 1.5 mM EDTA, and 0.02% sodium azide, pH 7.4) were incubated in triplicate with a fixed concentration of radiolabeled estradiol with or without various concentrations of the competitor substance dissolved in 60 μl of the TEA buffer containing DMF as co-solvent (final concentration of DMF in the incubation medium never exceeded 5%) for 18 h at 4 °C. At the end of this period, dextran-coated charcoal (DCC) (5% Norit 0.5% dextran) suspension in 100 μl of TEA buffer was added into each tube, which was briefly vortexed and allowed to stand for 15 min. DCC was precipitated by centrifugation ($800g \times 10$ min) and the supernatants counted for radioactivity in 10 ml of a dioxane-based scintillation fluid. RBA of the text compound was computed from a graph plotted between percent bound radioactivity versus log concentration of the test substance. At 50% inhibition, log of the competitor concentration relative to that of estradiol gave the affinity of the test compound to estrogen receptor relative to estradiol. This when multiplied with 100 gave the percentage value designated as RBA.

Estrogen agonistic activity[16]

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention once daily for three consecutive days on days 28–30 of age by oral route. A separate group of animals received only the vehicle for similar duration served as control. At autopsy 24 h after the last treatment on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, and weighed. Premature opening of vagina, cornification of vaginal epithelium, and increase in uterine fresh weight were taken as parameters for evaluation of estrogen agonistic activity in comparison to rats of vehicle control group. The objective was to evaluate estrogen agonistic effect of the compounds on the uterus and vagina.

Estrogen antagonistic activity[16]

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg kg^{-1} dose 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^{-1}) 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. Inhibition in ethynylestradiol induced cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen antagonistic effect of the compounds.

Antiproliferative activity assay of compounds[17]

The test produce for the evaluation of antiproliferative cytotoxic activity in vitro was accomplished following method proposed by Skehan et al.[17a] Briefly, the confluent flask of MCF-7 cells was trypsinized using 0.05% Trypsin–EDTA solution in PBS, 10^4 cells/well plated in a 96-welled flat-bottomed plate in 200 μl Minimum Essential Medium, pH 7.4, and allowed to attach for 24 h at 37 °C in a humidified CO_2 incubator essentially according to Srivastava et al.[17b] The test compounds were dissolved in appropriate solution (ethanol/DMSO), added at specified concentration, and further incubated for 48 h as before. The cells were fixed with chilled 10% TCA and incubated for 1 h at 4 °C. The supernatant was then discarded and the cells washed five times with deionized water and air-dried. One Hundred microliters of 0.4% (w/v) sulforhodamine B (SRB) in 1% acetic acid was added to each well and incubated at room temperature for 30 min. Unbound SRB was removed by five washes with 1% acetic

acid and the plate was air-dried. Two hundred microliters of unbuffered Tris base, pH 10.5, was added to extract the bound stain for 5 min and the OD read at 560 nM in plate reader. IC_{50} values were determined with respect to Tamoxifen as a positive control.