Synthesis of 3-phenyl-4-phenylvinyl Benzopyranones and the Corresponding 2,2-dimethyl-benzopyrans with Structural Similarity to Estradiol, as Estrogen Receptor Ligands

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Abstract: 7-Methoxy-3-phenyl-4-phenylvinyl benzopyran-2-ones and the corresponding 2,2-dimethyl-benzopyrans, substituted with different alkylamino residues were synthesized. Except compound **13e**, all compounds showed high level of estrogen agonistic activity (>81 %) whereas, compounds **13 b-e** and **15a** showed significant estrogen antagonistic activity (>20 %). X-Ray analysis of a 7-methoxy-3-phenyl-4-phenylvinyl benzopyran-2-one derivative **13d** showed its structural resemblance to endogenous estrogen, 17β-estradiol. Estrogenic and antiestrogenic activities of these derivatives demonstrate their estrogen receptor (ER) binding ability. The lack of hydroxyl groups at appropriate positions resulted in poor Relative Binding Affinity (RBA).

Key Words: Antiestrogens, estrogen agonists, estrogen antagonists, estrogen receptor (ER), RBA, LBD(ligand binding domain).

1. INTRODUCTION

Synthesis of selective estrogen receptor modulators (SERMs) towards development of drugs for estrogen dependent disorders such as osteoporosis, breast cancer and as contraceptives, has gained importance in the recent past in light of their selectivity of action [1-3]. 3, 4-Diaryl benzopyran derivative Centchroman (**1**), developed by our group, has been introduced in the market as the first non-steroidal oral contraceptive [4]. It also possesses significant activity in the treatment of osteoporosis [5] and breast cancer [6]. The naturally occurring benzopyran derivative coumestrol (**2**) is known for its estrogenic activity [7]. Derivatives of benzopyaranone and benzopayran with methylene bridge between benzopyranone, benzopyaran nucleus and phenyl ring carrying basic alkyl amine residue **3**, showed potent estrogen receptor (ER) dependent activities [8, 9]. Substituted diphenylnaphthyl methanes **4**, reported by our group, showed potent estrogen receptor dependent antiimplantation activity [10]. Further, compounds with a vinyl spacer between phenyl rings, having alkyl amine chain, and basic skeleton responsible for their binding to estrogenic site of ER, as present in substituted diphenylnaphthyl alkenes **5**, also showed affinity for ER and SERM-like activities [11]. It is an interesting fact that beside structural modifications nature as well as orientation of alkyl amine chain residue affects markedly the antiestrogenic potency of the estrogen receptor ligands [12]. Considering all above observations, we have designed and synthesized substituted 7-methoxy-3-phenyl-4-phenylvinyl benzopyran-2-one derivatives **6** and 2,2-dimethyl-7-methoxy-3-phenyl-4-phenylvinyl benzopyran derivatives **7** substituted with different alkylamino groups at para position of phenyl ring D (Fig. (**1**)), to see the effect of vinyl spacer, which alters the orientation of phenyl ring carrying alkyl amine residue, on antiestrogenic activity of the molecules.

The synthesized compounds were evaluated for their relative binding affinities, estrogenic and antiestrogenic activities. A representative member of this series, compound **13d**, was analyzed by X-ray crystallography to study its molecular geometry. The structural correlation of newly synthesized benzopyranones with endogenous estrogen, 17β-estradiol (**8**) (Fig. (**2**)) has been made using molecular superimposition studies to simulate their structural features, since the physical and chemical parameters such as molecular thickness, molecular volume apolar surface area and nature of the hydroxy groupings, associated with endogenous estrogen 17β-estradiol, are generally considered essential for estrogen receptor modulation by ligands [13, 14]. In our previous studies, we have well correlated our drug molecule, Centchroman (**1**), with estradiol and other antiestrogenic molecules and found the same results [15].

2. CHEMISTRY

Synthesis of the desired compounds was carried out starting from 1-(2-hydroxy-4-methoxy-phenyl)-3-(4-hydroxy-phenyl)-propenone **10** [16], prepared in 40 % yield from 2-hydroxy-4-methoxy acetophenone **9** (Scheme **1**). Its condensation with phenyl acetic acid in acetic anhydride and triethyl amine gave 7-methoxy-3-phenyl-4{2-[4-acetoxy-phenyl]-vinyl}-benzopyran-2-one **11** in 86 % yield. Deacetylation of compound **11** was done in methanolic NaOH to give 7-methoxy-3-phenyl-4{2-[4-hydroxy-phenyl]-vinyl}-benzopyran-2-one **12** in 80 % yield. Condensation of compound **12** with 2-chloroethyl alkylamine hydrochloride in acetone in

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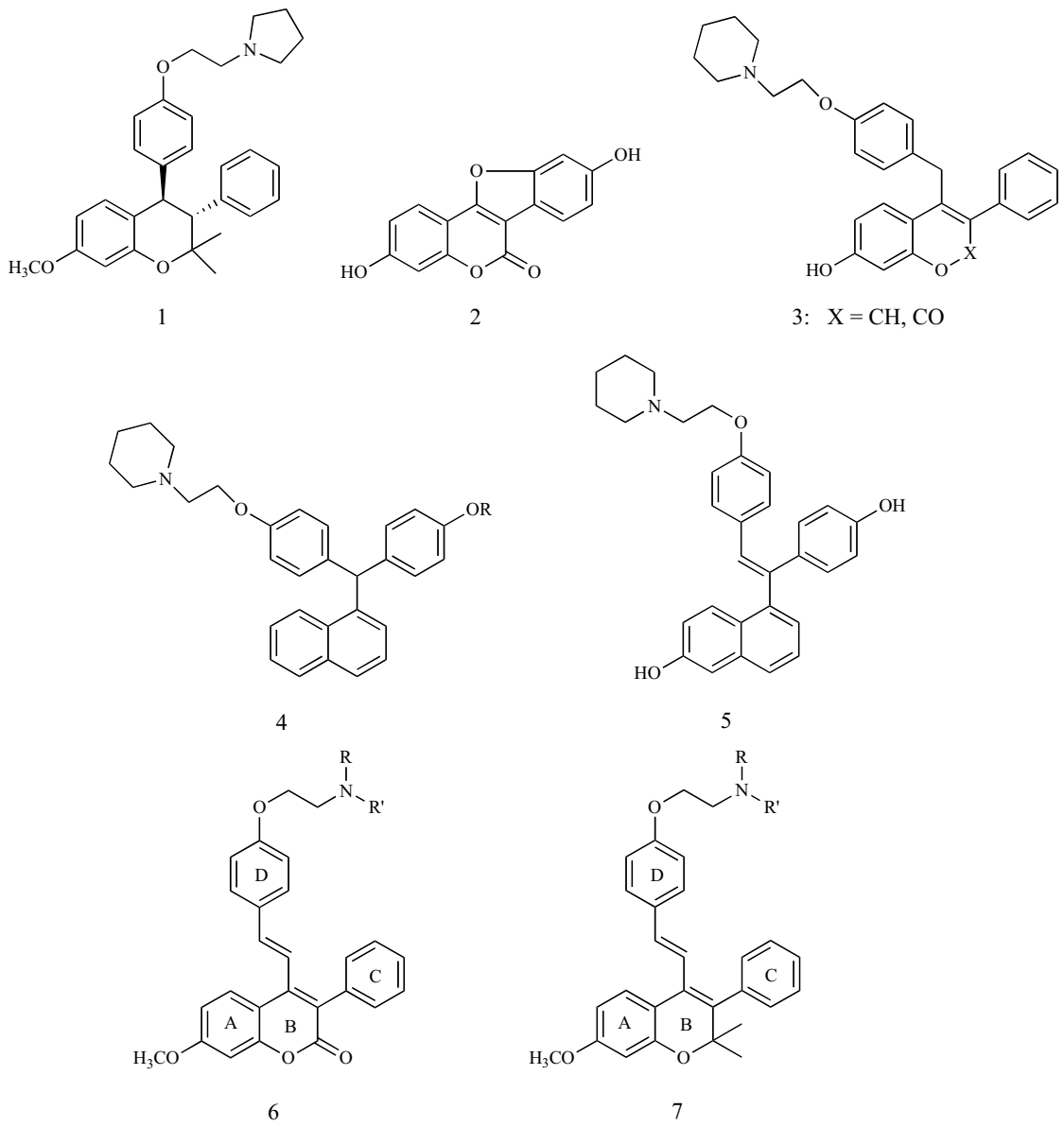


Fig. (1). Some selected non-steroidal Estrogen receptor ligands.

presence of K_2CO_3 under reflux gave the desired products **13** in 75-86 % yield.

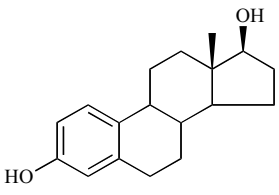


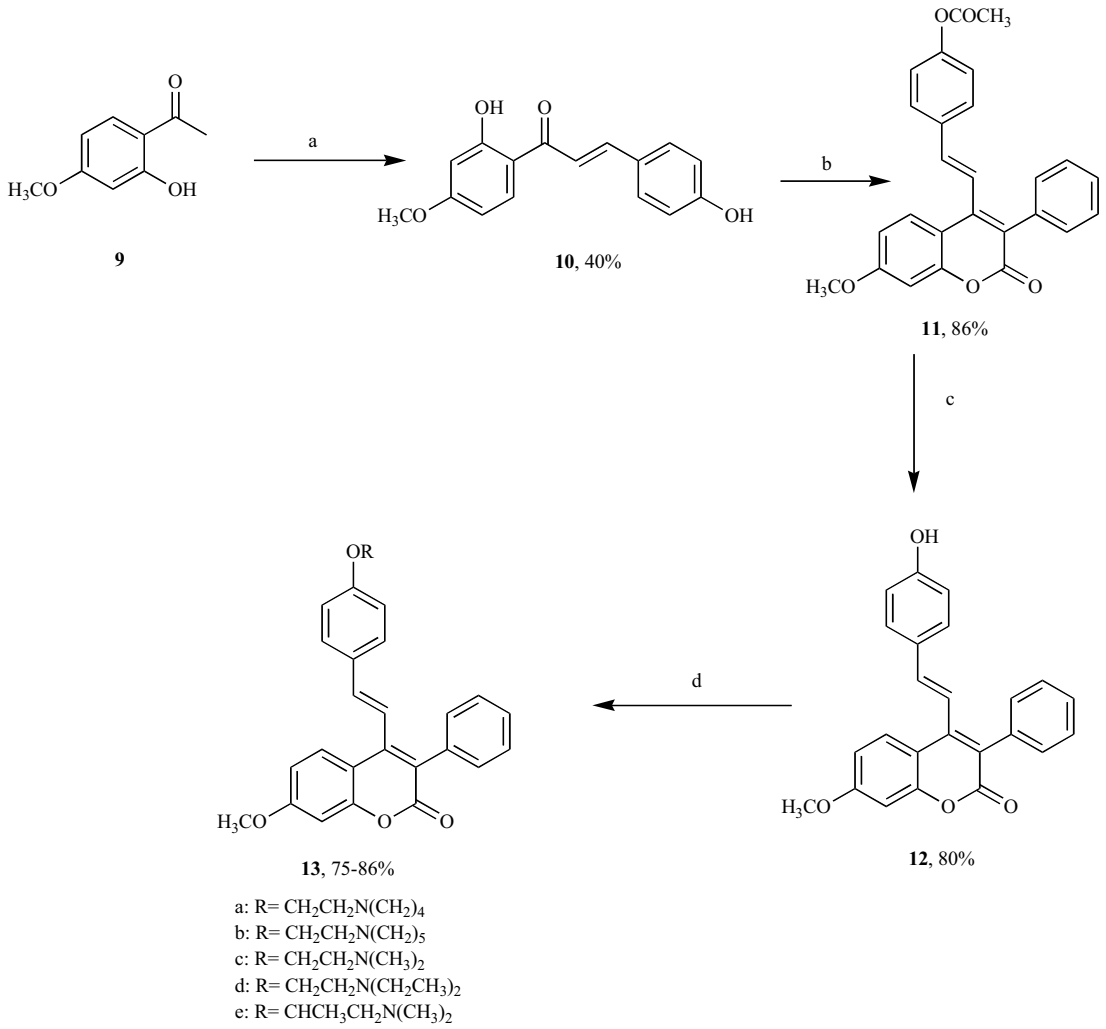
Fig. (2). 17β-estradiol.

Grignard reaction on **12**, with methyl magnesium iodide followed by acid treatment, gave the hydroxyl benzopyran **14** in 70 % yield, which is unstable. Condensation of compound **14** with 2-chloroethyl alkylamine hydrochloride in acetone in presence of K_2CO_3 under reflux gave the desired products **15** in 78-85 % yield **(**Scheme **2**).

3. X-RAY AND COMPUTATIONAL STUDY

3.1. Ligand's Structural Study [17]

After several attempts, only diffraction quality crystals of the compound **13d** were obtained by its slow recrystallization from ethylacetate-hexane. X-ray crystallographic analysis revealed that the flexible diethylamino ethyl part of the molecule (Fig. (**3**)) was severely disordered. The molecule contains a planar chromen-2-one ring that consists of a methoxy group substituted at C8; a phenyl group at C3 and diethyl vinyl phenoxy ethylamine substituted at C4. Rings B

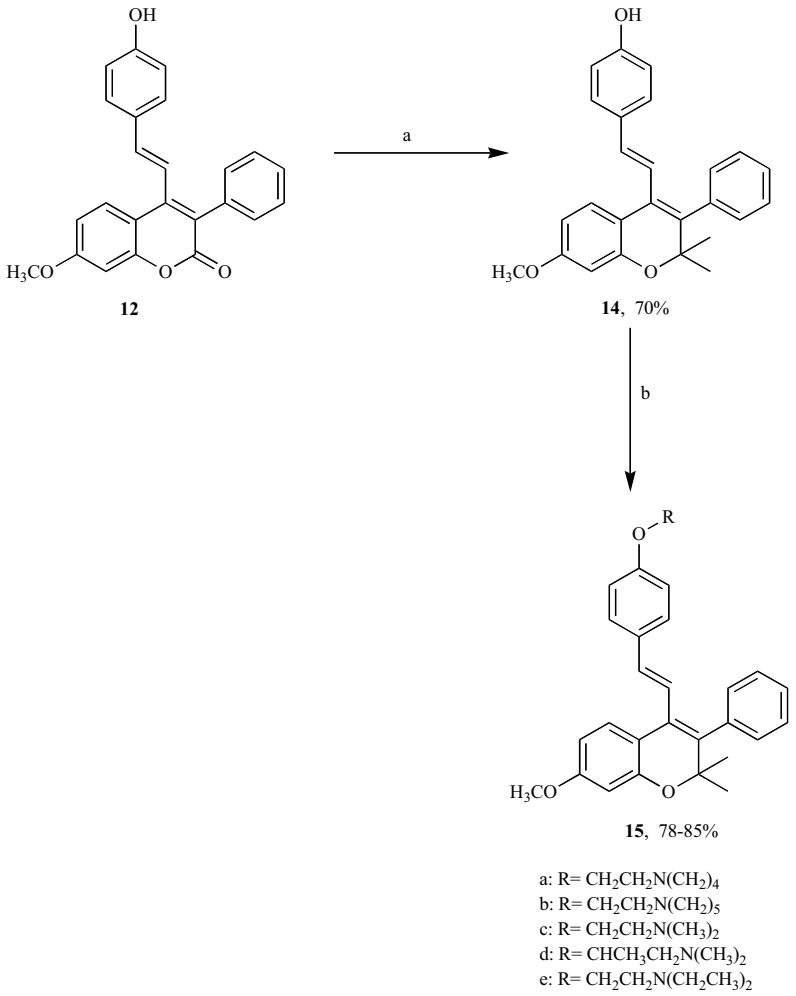


Scheme 1. (a) 4-Hydroxybenzaldehyde, dry piperidine, dry benzene (b) Phenylacetic acid, Et_3N, Ac_2O (c) 2% NaOH-MeOH (d) 2-Chloroethyl alkylamine hydrochloride, K_2CO_3, dry acetone.

and D are anti to each other across vinyl double bond (Fig. (**3**)). The x-ray study of compound showed the orientation of the phenyl rings in space which was useful in the structural correlation of the compounds with endogenous estrogen, 17β-estradiol (**8**).

3.2. Docking Studies Protocol

Docking, molecular dynamics, energy minimization and molecular graphics works were performed on a silicon graphics octane workstation. All the ligand structures were constructed using the Molecular Builder (Insight II) and the energy minimizations by Discover software. Reference protein coordinates used for docking were taken from X-ray structure deposited in protein data bank. The genetic algorithm of AutoDock 3.0.5 has been employed for docking the benzopyranone derivative into the active sites of the receptor. Autodock helps to narrow the conformational possibilities and to identify the structure. The original procedure developed for AutoDock used Monte Carlo simulated annealing (SA) technique for configurational exploration with the rapid energy evaluation using grid based molecular affinity potentials. Docking procedure involves the following steps-: The protein target and the ligand were prepared for docking using the AutoDock 3.0.5 and autodocktools. All the "heteroatoms", including water molecules and ions were removed from the original files. The macromolecule first needs polar hydrogens to be added and then partial atomic charges to be assigned (Kollman charges). The atomic solvation parameters were assigned using ADDSOL utility in AutoDock 3.0.5 program. The ligand molecules were checked for polar hydrogens and assigned for Gasteiger-Huckel partial atomic charges. Flexible torsion was defined with the help of Autotors. This allowed the conformational search of ligand during the process of docking. The PDBQ file was created for the ligand. Using Autogrid Algorithm the 3D maps of 0.375 Å spacing was centered on the active site for whole protein using Autogrid algorithm to evaluate the interaction energies between the ligand and the LBD(ligand binding domain) of



Scheme 2. (a) CH_3I, Mg, Diethyl ether (b) 2-Chloroethyl alkylamine hydrochloride, K_2CO_3, dry acetone.

the estrogen receptor. In this Autogrid program the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The affinity and electrostatic potential grid were calculated for each type of atom in the ligands. A series of docking parameters were set on. Atom types, generations, energy evaluations, and GA runs were set of 27000, 250000 and 100 respectively. The Lamarckian genetic algorithm (LGA) method was used with the default parameters as suggested by Autodock. Finally the docked of ligand-receptor complexes were selected according to the criteria of interacting energies combined with the geometrical matching quality. These complexes were used to have a comparative account of activity and their structural conformations. The total binding energy and corresponding inhibition constant between the compound and receptor was calculated according to the algorithm in the AutoDock 3.0.5 program.

3.3. *In-Silico* Docking Studies

Presuming that molecular geometry of compound **13d** and the corresponding piperidino compound **13b** would be similar, superimposition of **13b** with endogenous estrogen, 17β-estradiol (**8**) was carried out which is shown in (Fig. (**4**)). A close structural resemblance between the two molecules could be seen, particularly of the regions containing 3-

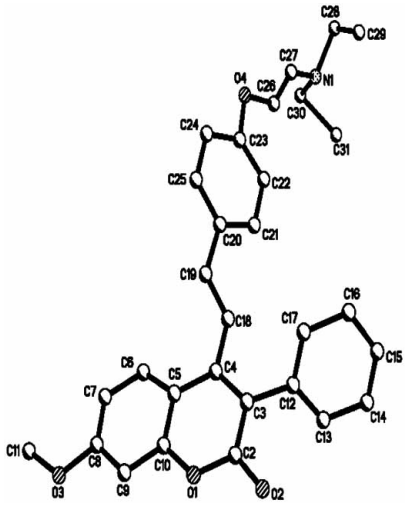


Fig. (3). X-ray Structure of Compound **13d**.

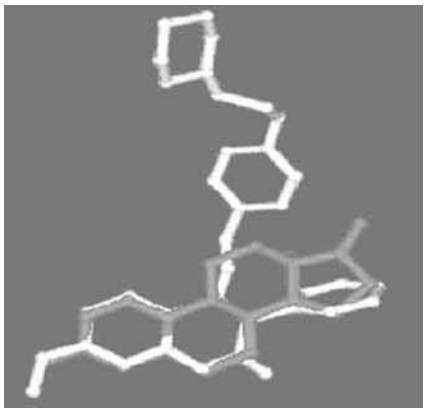


Fig. (4). Superimposition of **13b** with 17β-estradiol.

phenylbenzopyranone and A, D rings of estradiol having hydroxy groups, responsible for binding to estrogenic binding subsite of ER. The benzopyranone nucleus and phenyl ring C of the synthesized componds correspond to the A, B and D rings of the steroidal framework of the 17β-estradiol while the position of phenyl ring D of the synthesized compounds, having basic alkylamino chain, corresponds to the 11β position of 17β-estradiol.

Regarding the scope of molecular modeling studies, from both the docking experiment and biological activity, it is evident that the docking scores indeed correlated with biological activity of these compounds. For docking purpose, we have used autodock while for superimposition and rms deviation calculation, we have used insight II (Accelyrs). The r.m.s fit between superimposed **13b** and 17β-estradiol (**8**) is 0.02.

4. BIOLOGY

4.1. Estrogen Receptor Binding Affinity [18]

The relative binding affinity (RBA) of the compounds for estrogen receptor was determined by competition assay, employing radio labelled 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 20–21 days old rat uteri. Aliquots of the uterine cytosol (200 µl concentration 1 uterus per ml) prepared in TEA buffer (10 mM TRIS, 1.5 mM EDTA, 0.02% sodium azide, pH 7.4) were incubated in triplicate with a fixed concentration of radio labelled 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 were briefly vortexed and allowed to stand for 15 min. DCC was precipitated by centrifugation (800 g×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 verses 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.

4.2. Estrogen Agonistic Activity [19]

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 3 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, 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.

4.3. Estrogen Antagonistic Activity [19]

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 17 α-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.

5. RESULT AND DISCUSSION

RBA and estrogenic, antiestrogenic activities of aminoalkylated derivatives of 7-methoxy-3-phenyl-4-phenylvinyl benzopyran-2-ones and corresponding the 2,2-dimethylbenzopyrans are given in Table **1**.

Most of the amino alkylated compounds synthesized, except **13e**, showed significant estrogen agonistic activity (>81 %). In general the 2,2-dimethyl benzopyran derivatives were more potent estrogen agonists as compared to the corresponding benzopyranone derivatives whereas benzopyranone compounds were more potent as estrogen antagonists. As, we anticipated that addition of a vinyl spacer between phenyl ring D and basic benzopyranone and benzopyran skeleton, responsible for their binding to estrogenic site of ER, will increase interactions of the amino group in the antibinding site of the receptor leading to improved antiestrogenic activity of the compounds, synthesized compounds did not show improved antiestrogenic activity over the parent drug, ormeloxifene (centchroman). The estrogenic and antiestrogenic data of the compounds show that dimethyl aminoethyl chain residue is well accomodated in the LBD of the estrogen receptor. In general, there is no difinite structure

Table 1.

Compd. No.	Dose (oral) mg/kg/day	Estrogen Antagonistic Activity		Estrogen Agonistic Activity		RBA % of E_2
		Uterine Weight[a] (mg)	Inhibition[b] %	Uterine Weight[a] (mg)	Gain[c] %	
Vehicle	10	16.70±2.40		16.70±2.40		
EE	0.02	104.60±6.70		104.60±6.70	526	
13a	10	97.00±6.92[d]	7	39.50±0.15	137	0.018
Vehicle	10	19.60±0.44		19.60±0.44		
EE	0.02	106.96±7.05		106.96±7.05	446	
13b	10	59.90±3.10	44	46.40±3.80	137	0.06
13c	10	51.70±1.90	52	44.65±0.51	128	ND
13d	10	81.70±4.00[e]	24	52.50±4.60	168	ND
Vehicle	10	17.90±0.00		17.90±0.00		
EE	0.02	80.60±5.00		80.60±5.00	350	
13e	10	58.30±2.90[e]	28	22.90±3.20[f]	28	0.06
Vehicle	10	14.00±1.52		14.00±1.52		
EE	0.02	85.00±0.57		85.00±0.57	507	
15a	10	65.00±4.70[e]	24	25.30±1.20[g]	81	0.0152
Vehicle	10	20.00±1.52		20.00±1.52		
EE	0.02	121.70±2.90		121.70±2.90	509	
15b	10	117.50±5.82	3	40.60±1.50[g]	103	<0.001
15c	10	107.36±11.69	12	111.16±4.92[g]	456	<0.001
15d	10	110.66±1.97[d]	9	57.66±1.66[g]	188	ND
15e	10	107.60±5.50	12	61.26±2.60[g]	206	<0.001
Vehicle	10	18.10±1.14		18.10±1.14		
EE	0.02	109.50±1.22		109.50±1.22	505	
Centchroman	10	35.39±3.13[e]	67	46.33±3.82[f]	156	15.2

ND = Not determined; EE= 17α-Ethynylestradiol; E_2= 17β-Estradiol, [a]Values represents mean ±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, [f]P<0.05, [g]P<0.01, *versus* corresponding vehicle control group, all other relevant comparisons were statistically not significant. The values of Centchroman **1**, a marketed drug, are given for comparison with compounds tested.

activity relationship (SAR) in this series however, biological data suggests the high potential of both benzopyranone as well as benzopyran derivatives to interact with estrogen receptors. The observed low RBA values are likely due to lack of free hydroxyl groups at appropriate positions for receptor binding. Such hydroxyl groups are possibly getting generated on metabolism, resulting in the observed estrogen agonist and antagonist activities.

The molecular frame work of the benzopyranone derivatives determined by X-Ray studies and the endogenous estrogen, 17β-estradiol, shows a close structural resemblance between the two molecules, particularly of the regions containing 3-phenylbenzopyranone and A, D rings of estradiol having hydroxy groups, responsible for binding to estrogenic binding subsite of ER. The benzopyranone nucleus and phenyl ring C of the componds correspond to the A, B and D rings of the steroidal framework of the 17β-estradiol while the position of phenyl ring D of the synthesized compounds, having basic alkylamino chain, corresponds to the 11β position of 17β-estradiol.

6. CONCLUSION

In conclusion, significant estrogen agonist and antagonist activities exhibited by these amino alkyl derivatives of

7-methoxy-3-phenyl-4-phenylvinyl benzopyran-2-ones and corresponding 2,2-dimethyl-benzopyrans show the ability of these molecules to interact with the estrogen receptors as such or as a possible hydroxy metabolite. The biological data of the compounds suggests the high potential of both benzopyranone as well as benzopyran derivatives as estrogen receptor ligands. Their further evaluation as SERMs could be of interest in the designing of drugs for estrogen dependent disorders.

7. EXPERIMENTAL

Anhydrous reactions were performed under an inert atmosphere, the set-up assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reactant and solvents were obtained commercially and were used as such or purified and dried by standard means. The reported melting points were determined in open capillaries and are uncorrected. The ^{1}H NMR was recorded on Brucker Avans DRX 200 (200 MHz, FT NMR) spectrometer using TMS as internal standard. The chemical shifts are expressed in δ (ppm) as values and coupling constants in Hz. Multiplicities are described by the following abbreviations: s for singlet, d for doublet, t for triplet, bs for broad singlet. Mass spectra (FAB) were recorded on Jeol JMS-D-300 spectrometer. The infrared spectra were recorded in KBr and neat on a Perkin Elmer model 881. Elemental analyses were carried out on a Carlo-Erba EA 1108 instrument and results were within ±0.4% of theoretical values. The purity of the products was checked on precoated silica gel 60 F254 or aluminium oxide 60 F254 TLC plates and the spots were visualised by inspecting under short (254 nm) wavelength UV light or by the colours developed with iodine vapours. Column chromatography was performed on silicagel 60 (Merck) or basic aluminium oxide 90 (Merck).

7.1. 1-(2-Hydroxy-4-methoxy-phenyl)-3-(4-hydroxy-phenyl)-propenone (10)

To a solution of 2-hydroxy-4-methoxyacetophenone **9** (1.66 g, 0.01 mol) and 4-hydroxybenzaldehyde (1.22 g, 0.01 mol) in dry benzene (100 ml) was added piperidine (0.1 ml). The reaction mixture was refluxed for 32 hr, removing water azeotropically. The reaction mixture was then cooled and washed with water. The organic layer was separated, dried over anhydrous sodium sulphate and concentrated. The residue was chromatographed over a column of silica gel eluting with the ethyl acetate-hexane to afford compound **10**.

Yield: 40%, m.p.152-55^{0}C [lit. m.p. [16] 150-53^{o}C].

Fab Mass: 270, 271 (270+1); IR (cm^{-1}): 3259, 1636, 1599, 1374, 1225, 833; ^{1}H NMR (δ): 3.85(s, 3H, OCH_3), 6.79(d, J = 8.50 Hz, 2H, ArH), 7.37(s, 1H, OH), 7.43(d, J = 15.35 Hz, 1H, CH), 7.54(d, J = 8.49 Hz, 2H, ArH), 7.84(m, 3H, ArH & CH), 7.90 (d, J = 8.61 Hz, 1H, ArH).

7.2. 7-Methoxy-3-phenyl-4{2-[4-acetoxyphenyl]vinyl}-benzopyran-2-one (11)

A mixture of 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)-propenone **10** (5.40 g, 0.02 mol) and phenylacetic acid (3.72 g, 0.02 mol) in acetic anhydride (8ml) and triethylamine (3ml) was refluxed under anhydrous condition for 12 hr. The reaction mixture was poured on to ice; the separated product was collected and crystallized from benzene-methanol to yield pure compound **11**.

Yield: 86 %, m.p. 190-92^{o}C.

Fab Mass: 413; IR (cm^{-1}): 1762, 1708, 1610, 1206, 756; ^{1}H NMR (δ): 2.22(s, 3H, CH_3), 3.82(s, 3H, OCH_3), 6.82(d, J = 10.50Hz, 1H, CH), 6.85(d, J = 7.00Hz, 2H, ArH), 6.98(d, J = 8.40Hz, 2H, ArH), 7.25(m, 8H, ArH & CH), 7.70(d, J = 8.34Hz, 1H, ArH); Anal. Calcd. for $C_{26}H_{20}O_5$; C, 75.73; H, 4.85. Found: C, 75.62; H, 5.23.

7.3. 7-Methoxy-3-phenyl-4{2-[4-hydroxyphenyl]vinyl}-benzopyran-2-one (12)

Compound **11** (4.12g, 0.01 mol) was stirred with 2% methanolic sodium hydroxide (15ml) at room temperature for 30 min. After completion of reaction, yellow solid precipitated out on acidification with 5% HCl. The solid was crystallized from methanol-benzene to give pure compound **12**.

Yield: 80 %, m.p. 235-37^{o}C.

Fab Mass: 370; IR (cm^{-1}): 3415, 1673, 1611, 1278, 1150, 875; ^{1}H NMR (δ): 3.91(s, 3H, OCH_3), 6.61(d, J = 16.80Hz, 1H, CH), 6.83(m, 5H, ArH & CH), 7.17(d, J = 8.10Hz, 2H, ArH), 7.38(m, 5H, ArH), 7.86(d, J = 9.60Hz, 1H, ArH); Anal. Calcd. for $C_{24}H_{18}O_4$; C, 77.84; H, 4.86. Found: C, 78.03; H, 4.62.

7.4. Synthesis of 7-Methoxy-3-phenyl-4{2-[4-(2-substituted aminoethoxy)-phenyl]-vinyl}-benzopyran-2-one(13a-13e)

7-Methoxy-3-phenyl-4{2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-vinyl}-benzopyran-2-one (13a)

To a solution of **12** (0.37 g, 0.001 mol) in dry acetone, anhydrous potassium carbonate and 2-chloroethyl pyrrolidine hydrochloride (0.25 g, 0.002 mol) were added. The solution was heated under reflux for 6hr. The reaction mixture was filtered and was evaporated to dryness. The residue was taken into ethylacetate, washed with water, dried over anhydrous sodium sulphate and concentrated. The crude material was then chromatographed over a column of silicagel eluting with the methanol-chloroform (3:97) to afford compound **13a**.

Yield: 75%, m.p. 128-30^{o}C.

Fab Mass: 468 (467+1); IR (cm^{-1}): 3435, 1714, 1603, 1259; ^{1}H NMR (δ): 1.81(bs, 4H, CH_2), 2.62(bs, 4H, CH_2), 2.76(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.11(t, 2H, OCH_2), 6.73(d, J = 17.0Hz, 1H, CH), 6.87(m, 5H, ArH & CH), 7.25(d, J = 7.34Hz, 2H, ArH), 7.37(m, 5H, ArH), 7.83(d, J = 8.66Hz, 1H, ArH); Anal. Calcd. for $C_{30}H_{29}NO_4$; C, 77.09: H, 6.21; N, 2.99. Found: C, 77.24; H, 6.33; N, 3.21.

Compounds **13b -13e** were prepared following the above procedure as described for compound **13a**.

7-Methoxy-3-phenyl-4{2-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-vinyl}-benzopyran-2-one (13b)

Yield: 78 %, m.p. 126-28^{o}C.

Fab Mass: 482, 504 (481+23); IR (cm^{-1}): 1710, 1625, 1599, 1352, 1259, 1025; ^{1}H NMR(δ): 1.61(bs, 6H, CH_2),

2.51(m, 4H, NCH_2), 2.78(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.10(t, 2H, OCH_2), 6.65(d, J = 16.70 Hz, 1H, CH), 6.87(m, 5H, ArH & CH), 7.24(d, J = 7.00 Hz, 2H, ArH), 7.37(m, 5H, ArH), 7.82(d, J = 8.62 Hz, 1H, ArH); Anal. Calcd. for $C_{31}H_{31}NO_4$; C, 77.34; H, 6.44; N, 2.91. Found: C, 77.24; H, 6.72; N, 3.02.

7-Methoxy-3-phenyl-4{2-[4-(2-dimethylaminoethoxy)-phenyl]-vinyl}-chromen-2-one (13c)

Yield: 80 %, m.p. 156-58°C.

Fab Mass: 442, 464 (441+23); IR (cm^{-1}): 1704, 1620, 1597, 1256; ^1H NMR(δ): 2.62(bs, 6H, CH_3), 2.76(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.11(t, 2H, OCH_2), 6.73(d, J = 17.0 Hz, 1H, CH), 6.87(m, 5H, ArH & CH), 7.25(d, J = 7.34 Hz, 2H, ArH), 7.37(m, 5H, ArH), 7.83(d, J = 8.66 Hz, 1H, ArH); Anal. Calcd. for $C_{28}H_{27}NO_4$; C, 76.19: H, 6.12; N, 3.17. Found: C, 76.42; H, 6.28; N, 3.41.

7-Methoxy-3-phenyl-4{2-[4-(2-diethylaminoethoxy)-phenyl]-vinyl}-benzopyran-2-one (13d)

Yield: 80 %, m.p. 106-08°C.

Fab Mass: 470, 492(469+23); IR (cm^{-1}): 1710, 1625, 1598, 1359, 1261, 1060; ^1H NMR(δ): 1.07(t, 6H, CH_3), 2.63(q, 4H, NCH_2), 2.87(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.05(t, 2H, OCH_2), 6.65(d, J = 16.72 Hz, 1H, CH), 6.88(m, 5H, ArH & CH), 7.25(d, J = 8.83 Hz, 2H, ArH), 7.38(m, 5H, ArH), 7.83(d, J = 8.70 Hz, 1H, ArH); Anal. Calcd. for $C_{30}H_{31}NO_4$; C, 76.76; H, 6.60; N, 2.99. Found: C, 77.83; H, 6.72; N, 3.11.

4{2-[4-(2-Dimethylamino-1-methyl-ethoxy)-phenyl]-vinyl}-7-Methoxy-3-phenyl-chromen-2-one (13e)

Yield: 80 %, m.p. 98-100°C.

Fab Mass: 456; IR (cm^{-1}): 1705, 1628, 1597, 1256, 1012; ^1H NMR(δ): 1.24(s, 3H, CH_3), 2.52(bs, 6H, CH_3), 2.76(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.21(m, 1H, OCH), 6.68(d, J = 17.2 Hz, 1H, CH), 6.72(m, 5H, ArH & CH), 7.25(d, J = 7.23 Hz, 2H, ArH), 7.37(m, 5H, ArH), 7.83(d, J = 8.40 Hz, 1H, ArH); Anal. Calcd. for $C_{29}H_{29}NO_4$; C, 76.48; H, 6.37; N, 3.07. Found: C, 76. 81; H, 6.52; N, 3.35.

7.5. 2,2-Dimethyl-7-methoxy-3-phenyl-4[4-hydroxy-phenyl]-vinyl}-benzopyran (14)

To a stirred solution of Mg (0.32 g, 0.014 mol) and CH_3I (0.87 ml, 0.014 mol) in dry diethyl ether, was added **12** (1.00 g, 0.003 mol) dissolved in dry THF. Reaction mixture was allowed to stir at room temperature for 3 hr. After completion of reaction, solvent was evaporated. Residue was taken into ethyl acetate and washed with saturated solution of ammonium chloride followed with water. Organic layer was dried over sodium sulphate and concentrated to dryness. Oily residue was then purified over silicagel using ethyl acetate-hexane (5:95)as eluent to yield pure compound **14**.

Yield: 70 %, m.p. Oil.

Fab Mass: 384; IR (cm^{-1}): 3308, 1602, 1596, 1250, 1155, 782; ^1H NMR (δ): 1.20(s, 3H, CH_3), 1.50(s, 3H, CH_3), 3.78(s, 3H, OCH_3), 6.68(d, J = 14.70Hz, 1H, CH), 7.01(m, 5H, ArH & CH), 7.10(d, J = 7.89Hz, 2H, ArH), 7.42(m, 5H, ArH), 7.65(d, J = 8.20Hz, 1H, ArH).

7.6. Synthesis of 2,2-dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-substituted aminoethoxy)-phenyl]-vinyl}-benzopyran (15a-15e)

Compounds **15a-15e** were synthesized from compound **14**, following the same procedure as described for **13a.**

2,2-Dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-pyrrolidin-1-yl-ethoxyphenyl]-vinyl}-benzopyran (15a)

Yield: 82%, m.p. (of oxalate salt) 210-12°C.

Fab Mass: 481; IR (cm^{-1}): 1606, 1252, 1145, 802; ^1H NMR (δ): 1.20(s, 3H, CH_3), 1.50(s, 3H, CH_3), 1.78(bs, 4H, CH_2), 2.72(bs, 4H, NCH_2), 2.77(t, 2H, NCH_2), 3.80(s, 3H, OCH_3), 4.20(t, 2H, OCH_2), 6.68(d, J = 15.42Hz, 1H, CH), 7.01(m, 5H, ArH & CH), 7.10(d, J = 7.90Hz, 2H, ArH), 7.45(m, 5H, ArH), 7.66(d, J = 8.00Hz, 1H, ArH); Anal. Calcd. for $C_{32}H_{35}NO_3$; C, 79.80; H, 7.32; N, 2.91. Found: C, 79.64; H, 7.71; N, 3.00.

2,2-Dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-vinyl}-benzopyran (15b)

Yield: 80 %, m.p.(of oxalate salt) 168-70°C.

Fab Mass: 495, 518 (495+23); IR (cm^{-1}): 1615, 1585, 1479, 1360, 1255, 1031, 805; ^1H NMR(δ): 1.20(s, 3H, CH_3), 1.52(s, 3H, CH_3), 1.60(bs, 6H, CH_2), 2.55(m, 4H, NCH_2), 2.69(t, 2H, NCH_2), 3.80(s, 3H, OCH_3), 4.12(t, 2H, OCH_2), 6.68(d, J = 13.78Hz, 1H, CH), 6.91(m, 5H, ArH & CH), 7.15(d, J = 7.09Hz, 2H, ArH), 7.45(m, 5H, ArH), 7.65(d, J = 8.31Hz, 1H, ArH); Anal. Calcd. for $C_{33}H_{37}NO_3$; C, 79.97: H, 7.52; N, 2.83. Found: C, 79.84; H, 7.33; N, 3.01.

2,2-Dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-dimethylamino-ethoxy)-phenyl]-vinyl}-benzopyran (15c)

Yield: 78 %, m.p.(of oxalate salt) 184-86°C.

Fab Mass: 455; IR (cm^{-1}): 1602, 1595, 1480, 1250, 782; ^1H NMR(δ): 1.20(s, 3H, CH_3), 1.50(s, 3H, CH_3), 2.58 (bs, 6H, CH_3), 2.62 (t, 2H, NCH_2), 3.88 (s, 3H, OCH_3), 4.20 (t, 2H, OCH_2), 6.68(d, J = 14.24Hz, 1H, CH), 7.01(m, 5H, ArH & CH), 7.14(d, J = 7.80Hz, 2H, ArH), 7.42(m, 5H, ArH), 7.58(d, J = 8.20Hz, 1H, ArH); Anal. Calcd. for $C_{30}H_{33}NO_3$; C, 79.09: H, 7.30; N, 3.07. Found: C, 79.23; H, 7.39; N, 3.22.

2,2-Dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-dimethylamino-1-methyl-ethoxy)-phenyl]-vinyl}-benzopyran (15d)

Yield: 80 %, m.p.(of oxalate salt) 175-76°C.

Fab Mass: 469; IR (cm^{-1}): 1610, 1580, 1450, 1246, 1018, 792; ^1H NMR(δ): 1.20(s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.50(s, 3H, CH_3), 2.62(bs, 6H, CH_3), 2.76(t, 2H, NCH_2), 3.78(s, 3H, OCH_3), 4.21(m, 1H, OCH), 6.58(d, J = 15.00Hz, 1H, CH), 7.01(m, 5H, ArH & CH), 7.10(d, J = 7.81Hz, 2H, ArH), 7.45(m, 5H, ArH), 7.52(d, J = 7.90Hz, 1H, ArH).; Anal. Calcd. for $C_{31}H_{35}NO_3$; C, 79.28: H, 7.51; N, 2.98. Found: C, 78.96; H, 7.43; N, 3.11.

2,2-Dimethyl-7-methoxy-3-phenyl-4{2-[4-(2-diethylamino-ethoxy)-phenyl]-vinyl}-benzopyran (15e)

Yield: 80 %, m.p.(of oxalate salt) 190-92°C.

Fab Mass: 483, 483(506+23); IR (cm^{-1}): 1600, 1575, 1498, 1350, 1261, 1050, 798; ^1H NMR(δ): 1.10(t, 6H, CH_3),

1.20(s, 3H, CH_3), 1.50(s, 3H, CH_3), 2.68(q, 4H, NCH_2), 2.78(t, 2H, NCH_2), 3.90(s, 3H, OCH_3), 4.05(t, 2H, CH_2), 6.68(d, J = 14.76Hz, 1H, CH), 7.00(m, 5H, ArH & CH), 7.12(d, J = 7.81Hz, 2H, ArH), 7.32(m, 5H, ArH), 7.49(d, J = 7.92Hz, 1H, ArH); Anal. Calcd. for $C_{32}H_{37}NO_3$; C, 79.47: H, 7.71; N, 2.90. Found: C, 79.23; H, 7.51; N, 3.18.

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[17] The crystal data of 13d: $C_{30}H_{31}NO_4$, M = 469.56, triclinic, P 2(1)/n, a = 17.587(3) Å, b = 8.3620(1) Å, c = 20.052(3) Å, β = 115.96(1)°, V = 2651.4(7) Å^3, Z = 4, D_c = 1.176 g cm^{-3}, μ (Mo-Kα) = 0.078 mm^{-1}, F(000) = 1000, rectangular block, Brownish yellow, size = 0.425 x 0.3 x 0.225 mm, 5870 reflections measured (R_{int} = 0.0414), 4632 unique, wR_2 = 0.3965 for all data, conventional R = 0.1103 [$(\Delta/\sigma)_{max}$ = 000)] on F-values of 1403 reflections with $I>2\sigma(I)$, S = 1.087 for all data and 319 parameters. Unit cell determination and intensity data collection (2θ= 50°) was performed on a Bruker P4 diffractometer at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2. Since the flexible diethylamino ethyl part of the molecule was severely disordered only isotropic refinement could be done. Rest of the molecule was refined anisotropically. Programs: XSCANS [Siemens Analytical X-ray Instrument Inc.: Madison, Wisconsin, USA 1996], SHELXTL-NT [Bruker AXS Inc.: Madison, Wisconsin, USA 1997].

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