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Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 12 (2004) 2239–2249

Synthesis and biological activities of some new dibenzopyranones and dibenzopyrans: search for potential oestrogen receptor agonists and antagonists

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Received 10 November 2003; accepted 10 February 2004

Abstract—Continuing our search for newer oestrogen agonists or antagonists and extending our work on the exploration of benzopyran related compounds, some new tricyclic molecules bridged between the active molecules of 3,4-diaryl chroman and 2,3-diaryl benzopyrans have been synthesised. Structural modifications at different positions with elements known to impart agonist or antagonist activities have been carried out to prepare the desired molecules. The target compounds were screened for their anti-osteoporotic (agonist) and anti-uterotrophic (antagonist) activities and were found to be moderately active.

1. Introduction

The steroid hormone oestrogens influence the growth, differentiation and functioning of many target tissues. They play an important role in the female reproductive system and recent clinical findings have firmly established that the extra reproductive range of oestrogens is quite broad. These steroid hormones and their receptors are intimately involved in bone density maintenance, in the central nervous system and the cardiovascular system in regulation of blood lipid profile.[1–3] However, oestrogen stimulation is also implicated in the development of cancers of breast and uterus, consequently, there is an urgent need for oestrogen pharmaceuticals that exhibit tissue selective effects. The recent exciting discoveries related to the structure and function of oestrogen receptors has greatly enhanced the prospects for the development of new ligands that may function as tissue selective oestrogen agonists or antagonists popularly known as (SERMs).[4,5]

Many oestrogen agonists and antagonists have been developed in the recent past as agents for regulating fertility, preventing and controlling hormone responsive breast cancers and in post-menopausal hormone replacement therapy for the treatment of osteoporosis. The first step in the appearance of these activities is mediated by the binding of hormonal ligands to the oestrogen receptors ERα or ERβ.[6] Tamoxifen, the anti-breast cancer drug functions as an antagonist in breast tissue, is agonist on bone in maintaining bone density in post menopausal women but possess some oestrogen like effects in uterus,[7] the anti-osteoporosis drug raloxifene, acts as an antagonist in both breast and uterine tissue but is agonist in bone.[8] Similarly, EM-800 is a pure antagonist in breast and uterus, but is agonist in skeletal system,[9] centchroman, the first non steroidal oral contraceptive shows antagonist profile in breast and uterus, but is agonist in bone.[10] Some newer molecules like bazedoxifene acetate, lasofoxifene, pipendoxifene etc. are in various stages of development as oestrogen antagonists or modulators.[11] 2,3-diarylbenzopyrans developed at our institute have also emerged as a novel class of SERMs and showed potent antioestrogenic, anti-implantation and anti-breast cancer activities[12,13] (Chart 1).

Dibenzo[*b*,*d*]pyranone and pyran pharmacophore constitutes an important core structure of many natural products showing antibacterial, antitumor, antiallergic, antibiotic and immunomodulating activities.[14,15] Δ^9 Tetrahydrocannabinol with 6,6-dimethyl pyran structure has been reported to exhibit abortifacient activity.[16a,b] Recently, several molecules, which incorporate these pharmacophores have been reported as a novel class of nonsteroidal progesterone agonists[17] and

Keywords: Dibenzopyranones; Dibenzopyrans; Oestrogen receptor; Agonists; Antagonists.
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doi:10.1016/j.bmc.2004.02.018

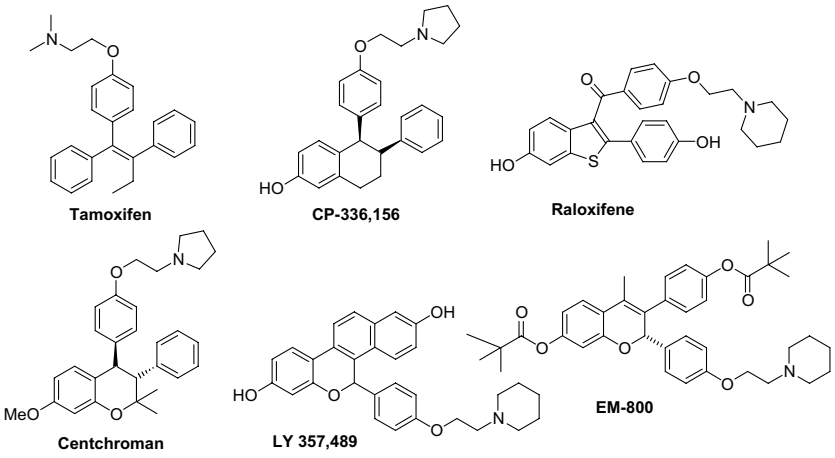
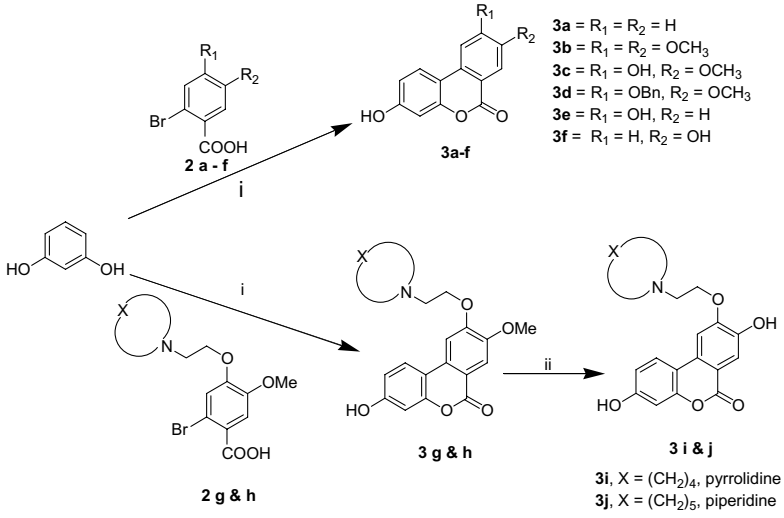


Chart 1. Structures of some potential oestrogen antagonists/SERMs.

endothelial cell proliferation inhibitors.[18] Encouraged with these findings and extending our work on benzopyran related molecules,[19–21] we envisaged the synthesis of some new tricyclic molecules bridged between the active molecules of 3,4-diaryl chroman and 2,3-diarylbenzopyrans and incorporating the dibenzopyranone and 6,6-dimethylbenzopyran core structures. Structural modifications at different positions with elements known to impart agonist or antagonist activities were carried out to prepare the target/desired molecules and also to study the structure–activity relationship.

1.1. Chemistry

Substituted dibenzopyranones (**3a**–**f**) with hydroxy/methoxy/benzyloxy substituent groups at 8- or 9-positions were prepared in good yields by the condensation of resorcinol with 4 and/or 5 hydroxy/methoxy/benzyloxy substituted 2-bromobenzoic acids (**2a**–**f**) in alkaline medium and copper sulphate catalyst (Hurtley reaction).[22] Similar condensation of resorcinol with 4-piperidino and pyrrolidinoethoxy-5-methoxy-2-bromo benzoic acids (**2g** and **2h**) formed the target molecules **3g** and **3h**. Demethylation with boron tribromide afforded the dihydroxy compounds **3i** and **3j**, respectively. The benzoic acids **2g** and **2h** with ethoxyamines at 4-position were prepared from 3-methoxy-4-hydroxy benzaldehyde (vanillin), which on acetylation formed acetylvanillin. Restricted bromination gave acetyl-6-bromovanillin as a single product. Reaction with hydroxylamine hydrochloride formed the oxime of acetyl-6-bromovanillin as crystalline colourless needles and its acetylation formed the acetate of the oxime in good yields. This was converted into its nitrile, 3-methoxy-4-acetoxy-6-bromobenzonitrile on boiling in acetic anhydride. Subsequent aqueous alkaline hydrolysis under reflux conditions yielded 5-methoxy-4-hydroxy-2-bromobenzoic acid, which on alkylation with 2-chloroethylpyrrolidine and 2-chloroethylpiperidine hydrochlorides formed the desired 4-aminoalkoxybenzoic acids **2g** and **2h** in good yields (Scheme 1).

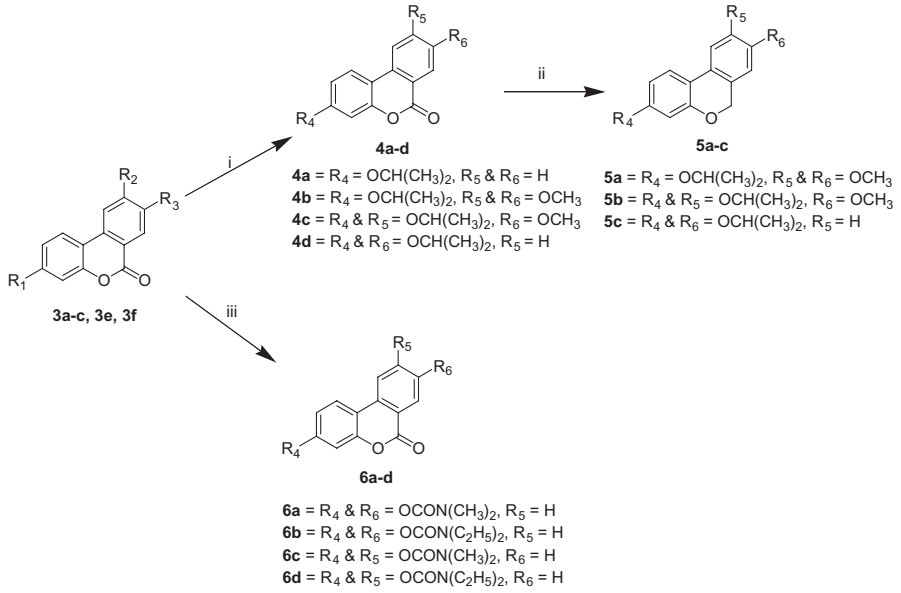


Scheme 1. Reagents and conditions: (i) $CuSO_4$/NaOH, heat; (ii) BBr_3, DCM, −78 °C.

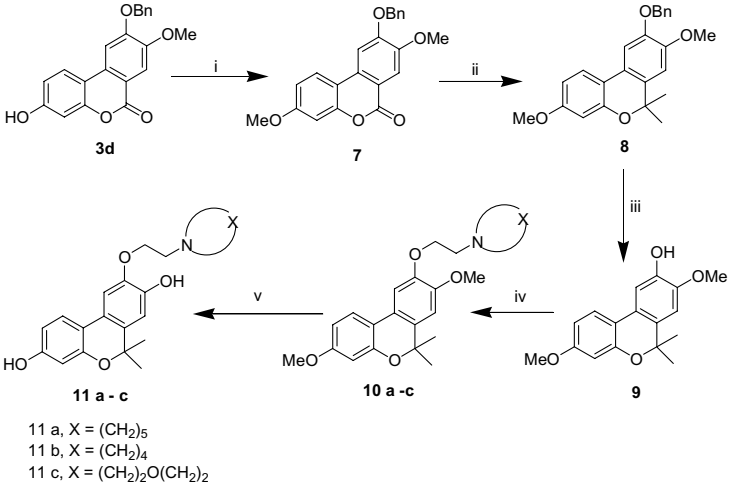
To prepare derivatives structurally related to ipriflavone a well known bone resorption inhibitor, alkylation of mono and dihydroxy benzopyranones (**3a**–**c**, **3e** and **3f**) with isopropylbromide resulted in the formation of crystalline mono and diisopropyl derivatives **4a**–**d** in excellent yields. Dibenzopyrans **5a**–**c** were prepared by the reduction of corresponding pyranones **4b** and **4d** with borane dimethyl sulfide complex at room temperature. Similarly, esterification of 3,8/3,9-dihydroxy benzopyranone (**3e** and **3f**) with *N*,*N*-dimethyl and *N*,*N*-diethyl carbamoyl chlorides formed the desired carbamoyl esters **6a**–**d** as crystalline products (Scheme 2).

Scheme 3 outlines the approach used to prepare the target molecules bearing structural similarity to centchroman. Methylation of hydroxy group in **3d** with iodomethane formed the 3,8-dimethoxy-9-benzyloxydibenzopyranone **7**, which on Grignard reaction with excess of methyl magnesium iodide in dry THF afforded the 6,6-dimethyl dibenzopyran **8** in good yield. Removal of the benzyl group at 9-position with Raney nickel in methanol produced the 3,8-dimethoxy-9-hydroxyl-6,6-dimethyl-dibenzopyran **9**, which was alkylated with different *t*-amino alkyl hydrochlorides to give the desired basic ethers **10a**–**c** in good yields. Finally, demethylation with boron tribromide formed the dihydroxy derivatives **11a**–**c**.

Following the methodology as reported for the coumestrol SERM[23], 3,8-dihydroxy dibenzopyranone **3e** was protected as its bis silyl ether with *t*-butyl dimethylsilyl chloride and subsequent DIBAL-H reduction of



Scheme 2. Reagents and conditions : (i) Isopropyl bromide, anhyd K_2CO_3, dry acetone; (ii) $(CH_3)_2S{\cdot}BH_3$/dry THF; (iii) dimethyl/diethyl carbamoyl chloride, pyridine, rt.



Scheme 3. Reagents and conditions: (i) Methyl iodide, anhyd K_2CO_3, dry acetone, (ii) methyl magnesium iodide, dry ether/dry THF, reflux, (iii) Raney nickel, methanol, H_2 (60 mm), (iv) 1-(2-chloroethyl) amines, anhyd K_2CO_3, dry acetone, (v) BBr_3, DCM, −78 °C.

the carbonyl function in dry THF at −95 °C in inert atmosphere formed the lactol **12** as white crystalline solid. On reaction with phenol at ambient temperature, lactol was converted into hemiacetal phenoxy derivative **13** obtained as oily residue, which showed more than 90% purity on TLC. Since it was highly unstable and decomposed on standing, was used immediately in the next reaction with 4-piperidinoethoxy and 4-pyrrolidinoethoxy phenyl magnesium bromides forming complex mixtures from which the target molecules **14a** and **14b** could be isolated in very low yields of 15–20% through column chromatography. Finally, removal of the silyl groups with iodine in methanol afforded the desired dihydroxy compounds **15a** and **15b** well characterised by spectral data (Scheme 4).

2. Results and discussion

2.1. Bone resorption inhibitory activity

The in vitro bone resorption inhibitory activity of compounds **4a–d** and **5a–c** was determined in 18 days old foetal rat femora by measuring the inhibition of PTH (Parathyroid hormone) induced osteoclastic activity. Bone resorbing activity is expressed as percentage of released ^{45}Ca and the effect of test compounds as percent of corresponding control or *T*/*C* ratio as shown:

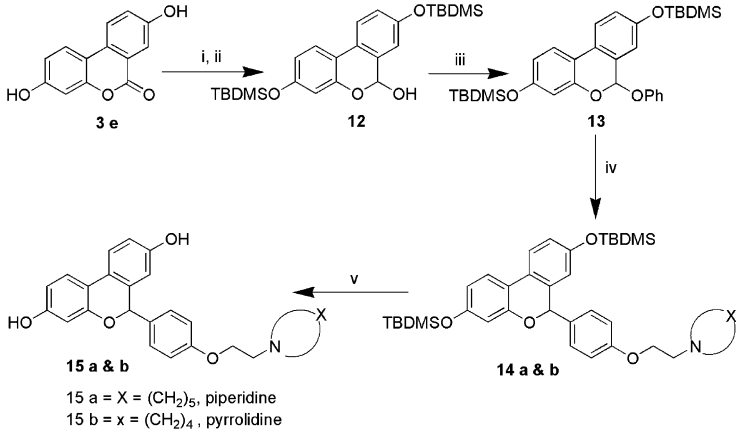
$$T/C \text{ ratio} = \frac{^{45}\text{Ca resorption in presence of PTH + test compound}}{^{45}\text{Ca resorption in presence of PTH + vehicle}}$$

The compounds showing *T*/*C* ratio of $\leqslant 0.6$ at $\leqslant 100$ micromolar (μM) concentration are considered to be active. DL-Centchroman and raloxifene were used as reference compounds. The biological results of all the compounds tested are shown in Table 1. As can be seen from the data, incorporation of isopropyl groups in dibenzopyranones and dibenzopyrans as in ipriflavone, the compounds showed inhibitory effect on bone resorption. The unsubstituted benzopyranone molecule **4a** exhibited resorption inhibition of 2.6%. Introduction of a second isopropyl group at position 8 or 9 marginally increased the anti-resorptive activity and its effect appeared to be position specific, compound **4c** with

Table 1. In vitro bone resorption inhibitory activity in PTH induced resorption of ^{45}Ca from rat fetal bones in culture

Compound no[a]	Final concentration (μM)[b]	% Resorption		*T*/*C* ratio	% Inhibition[d]
		PTH + vehicle[c] (*C*)	PTH + compound[c] (*T*)		
4a	100	34.51	33.60	0.973	2.64
4b	100	36.73	37.92	1.032	−3.2
4c	100	30.00	27.66	0.922	7.8
4d	100	35.41	34.43	0.972	2.8
5a	100	33.22	31.50	0.948	5.2
5b	100	35.57	33.86	0.952	4.9
5c	100	37.22	35.04	0.935	5.8
Raloxifene	100	35.46	23.41	0.661	33.9
DL-Centchroman	100	34.01	27.60	0.811	18.8

[a] Compounds were devoid of any oestrogenic/antioestrogenic activities upto 10 mg/kg po in OVX immature rat assay.
[b] In 0.1% DMSO or 1% methanol.
[c] The values are expressed as the means SEM.
[d] Denotes the ability of the test compounds to inhibit PTH induced ^{45}Ca resorption, computed as $(C–T) \times 100/C$, where *C* and *T* refer to control and treated animals.



Scheme 4. Reagents and conditions: (i) TBDMSCl, imidazole, DMF, (ii) DIBAL-H, −95 to −75 °C, (iii) 4-(2-piperidinoethoxy)/4-(2-pyrrolidinoethoxy) phenyl magnesium bromide, dry THF (v) I_2, methanol, rt.

isopropyl group at 9-position had increased inhibitory profile of 7.8% than compd **4d** with the same group at position 8. Substituting both or either the 8 and 9 positions with methoxy groups however, had negative effect and the compound **4b** was found to be bone resorptive. Conversion of lactone to pyran nucleus increased the inhibitory profile though slightly and compounds **5a–c** showed resorption inhibition in the range of 5–6%. Compounds **6a–d** bearing bis carbamoyl groups did not show any bone resorption inhibition and were also devoid of CNS activity.

3. Oestrogenic and antioestrogenic activities

The results of oestrogenic and antioestrogenic activity of basic amino ethers (Table 2) showed that dibenzopyranones **3i** and **3j** are weakly antioestrogenic and more oestrogenic with uterine weight gain of 21% and 25% over that of control. Conversion into dibenzopyrans **11a–c** by introduction of 6,6-dimethyl groups as in centchroman enhanced the antagonist character of only one compound **11c** to 24.72% and uterine weight gain was slightly lowered to 20%. The other two compounds were more oestrogenic than antioestrogenic. However, compounds **15a** and **b** bearing close homology to 2,3-diaryl benzopyrans were devoid of any antagonistic activity and exhibited only agonist profile causing an increase of uterine weight gain of more than 60% as compared to controls. The same compounds when tested for their post-coital contraceptive (anti-implantation) activity showed 100% inhibition of implantation at 10 mg/kg dose and the activity dropped to 50% and 60% when tested at a lower dose of 5 mg/kg. It is interesting to note that very recently some novel non steroidal phenanthrene ligands[24] have been reported to display only antagonistic activity, but our tricyclic molecules **15a** and **15b** though bearing close structural similarity to phenanthrene scaffold failed to show any antagonistic activity and were totally agonists.

From the biological activity results it may be concluded that modification of the parent benzopyranone to benzopyran nucleus by introducing dimethyl groups did confer some degree of antagonism in the molecules as seen in 3,4-diaryl chromans. The display of weak antagonistic character may be explained due to the planarity of the tricyclic ring system and smaller core structure than oestradiol. The absence of any D-ring substructure in the synthesised molecules substantiates its importance, which may be necessary for any molecule to adopt the requisite conformation essential for a proper receptor fit and antagonism. Agonist profile of compounds **15a** and **15b** is rather surprising. More work is in progress to modify the structures of active molecules so as to obtain compounds with potent tissue selective oestrogen agonistic or antagonistic activities.

4. Experimental

4.1. Chemistry

All the melting points were determined in open capillaries on a Toshniwal melting point apparatus and are uncorrected. The ^{1}H NMR was recorded on Brucker Avans DRX 300 (300 MHz, FT NMR) spectrometer using TMS as internal standard. The chemical shifts are expressed in δ (ppm) as values and coupling constants in Hz. Electron impact (EIMS) mass spectra were run on a JEOL-JMSD 300 instruments fitted with a direct inlet system. Elemental analyses were performed on elemental analyser EA-1108 and results were within $\pm$4% of theoretical values. The purity of the products was checked on precoated silica gel 60 F_{254} or aluminium oxide 60 F_{254} TLC plates and the spots were visualised by inspecting under short (254 nm) wavelength UV light or by the colours developed with iodine vapours. Coloumn chromatography was performed on kieselgel 60 (Merck) or basic aluminium oxide 90 (Merck).

Table 2. Receptor binding affinity and biological activity data of dibenzopyranones and dibenzopyrans

Compound no	Dose[a] (mg/kg body weight)	RBA % $E_2 = 100$	Oestrogenic activity[b] (% uterine weight gain)		Antioestrogenic activity (% inhibition)[c]		Post coital contraceptive activity[d]
Control			18.40				
E_2					94.5		
3i	10	0.21	22.39	21.66	91.96	2.63	Inactive
3j	10	0.26	23.04	25.20	85.45	9.57	Inactive
11a	10	0.011	22.10	20.10	89.36	5.45	Inactive
11b	10	0.035	20.41	10.9	86.61	8.34	Inactive
11c	10	0.09	22.10	20.10	71.13	24.72	Inactive
15a	10	0.72	30.01	63.04	99.56	Nil	100%
	5						50%
15b	10	0.59	31.63	71.90	98.38	Nil	100%
	5						60%

[a] Five animals were used for each dose test.

[b] Control group of animals received the vehicle alone. The values represent the mean uterine weight and activity expressed as percent increase over that of control used as basal value.

[c] Computed as $(E - T) \times 100/E$ where E and T refer to the mean uterine weights from animals treated with E_2 (17β-oestradiol)+vehicle and with given test compound along with E_2, respectively.

[d] Inactive = all rats pregnant.

4.1.1. 3-Hydroxy-dibenzo[*b*,*d*]pyran-6-one (3a).[22] Brown amorphous solid, yield, 56%, mp 235 °C; MS: *m*/*z* 212 (M^+); ^{1}H NMR ($CDCl_3$) δ: 6.4–6.7 (m, 2H, H-2 and H-4), 7.5–7.6 (t, 1H, H-8), 7.78–7.81 (t, 1H, H-9), 7.90–7.98 (m, 2H, H-7 and H-10), 8.33–8.35 (d, 1H, H-1).

4.1.2. 8,9-Dimethoxy 3-hydroxy-dibenzo[*b*,*d*]pyran-6-one (3b). Yield 58%, mp > 280 °C; MS: *m*/*z* 272 (M^+); ^{1}H NMR ($CDCl_3$) δ: 3.88 (s, 3H, OCH_3), 6.39 (s, 1H, H-4), 6.73–6.79 (dd, 1H, H-2), 7.42–7.46 (dd, 1H, H-9), 7.57 (s, 1H, H-7), 8.00–8.04 (d, 1H, H-1), 8.11–8.16 (d, 1H, H-10).

4.1.3. 3,9-Dihydroxy-8-methoxy-dibenzo[*b*,*d*]pyran-6-one (3c). Yield 290 mg (56%), mp > 280 °C; MS: *m*/*z* 258 (M^+); ^{1}H NMR ($CDCl_3$) δ: 3.9 (s, 3H, OCH_3), 6.72–6.73 (d, 1H, H-4), 6.79–6.85 (dd, 1H, H-2), 7.40 (s, 1H, H-10), 7.50 (s, 1H, H-7), 7.88–7.92 (d, 1H, H-1).

4.1.4. 9-Benzyloxy-3-hydroxy-8-methoxy-dibenzo[*b*,*d*]-pyran-6-one (3d). Yield 80%, mp > 300 °C; MS: *m*/*z* 348 (M^+); ^{1}H NMR ($CDCl_3$) δ: 3.91 (s, 3H, OCH_3), 5.49 (s, 2H, OCH_2), 6.86–6.87 (d, 1H, H-4), 6.94–6.98 (dd, 1H, H-2), 7.49–7.69 (m, 7H, H-7, H-10 and Ar-H), 7.93 (s, 1H, H-7), 8.26–8.30 (d, 1H, H-1).

4.1.5. 3,9-Dihydroxy-dibenzo[*b*,*d*]pyran-6-one (3e). Yield 66%, mp > 280 °C; MS: *m*/*z* 228 (M^+); ^{1}H NMR (DMSO-d_6) δ: 6.72–6.73 (d, 1H, H-4), 6.80–6.86 (dd, 2H, H-2 and H-8), 7.45 (s, 1H, H-10), 7.79–8.08 (m, 2H, H-1 and H-7).

4.1.6. 3,8-Dihydroxy-dibenzo[*b*,*d*]pyran-6-one (3f). Yield 58%, mp > 300 °C; MS: *m*/*z* 228 (M^+); ^{1}H NMR ($CDCl_3$) δ: 6.95–6.96 (d, 1H, H-4), 7.01–7.06 (dd, 1H, H-2), 7.52–7.57 (dd, 1H, H-9), 7.73–7.75 (d, 1H, H-7), 8.22–8.27 (d, 1H, H-1), 8.31–8.35 (d, 1H, H-10), 10.38 (s, 1H, 8-OH), 10.45 (s, 1H, 3-OH).

4.1.7. 3-Hydroxy-8-methoxy-9-(2-pyrrolidin-1-yl-ethoxy)-dibenzo[*b*,*d*]pyran-6-one (3g). Yield 48%, mp 196 °C; MS: *m*/*z* 355 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.70–1.80 (m, 4H, $(CH_2)_2$), 2.50–2.57 (m, 4H, $N(CH_2)_2$), 2.88–3.08 (t, 2H, NCH_2), 3.98 (s, 1H, OCH_3), 4.30–4.36 (t, 2H, OCH_2), 6.73–6.79 (d, 1H, H-4), 6.84–6.86 (dd, 1H, H-2), 7.53 (s, 1H, H-7), 7.64 (s, 1H, H-10), 8.18–8.22 (s, 1H, H-1), 9.13 (s, 1H, OH).

4.1.8. 3-Hydroxy-8-methoxy-9-(2-piperidin-1-yl-ethoxy)-dibenzo[*b*,*d*]pyran-6-one (3h). Yield 50%, mp 205 °C; MS: *m*/*z* 369 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.4–1.5 (m, 6, $(CH_2)_3$), 2.47–2.51 (m, 4H, $N(CH_2)_2$), 2.71–2.76 (t, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 4.30–4.36 (t, 2H, OCH_2), 6.72–6.74 (d, 1H, H-4), 6.79–6.85 (dd, 1H, H-2), 7.5 (s, 1H, H-7), 7.71 (s, 1H, H-10), 8.18–8.23 (dd, 1H, H-2).

4.1.9. 3,8-Dihydroxy-9-(2-pyrrolidin-1-yl-ethoxy)-dibenzo[*b*,*d*]pyran-6-one (3i). A solution of compound **3f** (355 mg, 1 mM) in dry dichloromethane was cooled to −78 °C. Boron tribromide (1.0 M soln in CH_2Cl_2; 5 equiv 0.6 mL) was added dropwise via syringe and the solution stirred at −78 °C for 15 min, thereafter, it was allowed to come to rt and left overnight. The reaction mixture was poured in ice water, and extracted with dichloromethane. Removal of excess solvent gave a sticky solid, which was crystallised from chloroform/methanol as white crystalline solid, yield 210 mg, mp 214 °C; MS: *m*/*z* 341 (M^+); ^{1}H NMR (DMSO-d_6) δ: 1.70–1.80 (m, 4H, $(CH_2)_2$), 2.50–2.57 (m, 4H, $N(CH_2)_2$), 2.88–3.08 (t, 2H, NCH_2), 4.30–4.36 (t, 2H, OCH_2), 6.73–6.79 (d, 1H, H-4), 6.84–6.86 (dd, 1H, H-2), 7.53 (s, 1H, H-7), 7.64 (s, 1H, H-10), 8.18–8.22 (s, 1H, H-1), 9.13 (s, 1H, OH). Anal. Calcd for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10. Found C, 66.52; H, 5.45; N, 4.14.

4.1.10. 3,8-Dihydroxy-9-(2-piperidin-1-yl-ethoxy)-dibenzo[*b*,*d*]pyran-6-one (3j). Yield 60%, mp 184 °C; MS: *m*/*z* 355 (M^+); ^{1}H NMR (DMSO-d_6) δ: 1.4–1.5 (m, 6, $(CH_2)_3$), 2.47–2.51 (m, 4H, $N(CH_2)_2$), 2.71–2.76 (t, 2H, NCH_2), 4.30–4.36 (t, 2H, OCH_2), 6.72–6.74 (d, 1H, H-4), 6.79–6.85 (dd, 1H, H-2), 7.5 (s, 1H, H-7), 7.71 (s, 1H, H-10), 8.18–8.23 (dd, 1H, H-2). Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found C, 67.61; H, 6.01; N, 4.13.

4.1.11. 3-Isopropyloxy-dibenzo[*b*,*d*]pyran-6-one (4a). To a solution of **3a** (228 mg; 1 mM) in dry acetone (60 mL) was added anhydrous potassium carbonate (276 mg; 2 mM) and isopropyl bromide (0.19 mL; 2 mmol). The reaction mixture was stirred and refluxed (70 °C) for 24 h. After completion of the reaction, the flask was cooled, and the contents were filtered and the residue washed with acetone (5 mL × 3). The filtrate was concentrated and the crude product was chromatographed over silica gel column eluting the pure compound with ethyl acetate–hexane (2%), yield 295 mg (94.5%), mp 78 °C; MS: *m*/*z* 254 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.38–1.40 (d, 6H, $(CH_3)_2$), 4.52–4.58 (m, 1H, CH), 6.4–6.7 (d, 1H, H-4), 6.90–6.95 (dd, 1H, H-2), 7.46–7.54 (t, 1H, H-8), 7.74–7.78 (t, 1H, H-9), 7.92–7.96 (d, 1H, H-7), 7.98–8.02 (d, 1H, H-10), 8.34–8.38 (d, 1H, H-1). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55. Found C, 75.78; H, 5.70.

4.1.12. 3-Isopropyloxy 8,9-dimethoxy-dibenzo[*b*,*d*]pyran-6-one (4b). Yield 88.28%, mp 218 °C; MS: *m*/*z* 314 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.38–1.40 (d, 6H, $(CH_3)_2$), 3.99 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 4.35–4.67 (m, 1H, CH), 6.8 (s, 1H, H-4), 6.90–6.95 (d,1H, H-2),7.32 (s, 1H, H-7), 7.77 (s, 1H, H-10), 7.79 (d, 1H, H-1). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found C, 67.97; H, 5.85.

4.1.13. 3,9-Diisopropyloxy-8-methoxy-6*H*-dibenzo[*b*,*d*]-pyran-6-one (4c). Yield 80%, mp 196 °C, MS: *m*/*z* 342

(M^+); ^1H NMR ($CDCl_3$) δ: 1.37–1.40 (m, 12H, $(CH_3)_2\times2$), 4.0 (s, 3H, OCH_3), 4.76–4.66 (m, 2H, CH×2), 6.84 (s, 1H, H-4), 6.88 (m, 2H, H-2 and H-7), 7.15 (s, 1H, H-10), 7.35–7.50 (d, 1H, H-1). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.16; H, 6.48. Found C, 70.49; H, 6.61.

4.1.14. 3,8-Diisopropyloxy-6*H*-dibenzo[*b*,*d*]pyran-6-one (4d). Yield 94.5%, mp 98 °C; MS: *m*/*z* 312 (M^+); ^1H NMR ($CDCl_3$) δ: 1.36–1.39 (m, 12H, $(CH_3)_2\times2$), 3.92–4.07 (m, 2H, CH×2), 6.84 (s, 1H, H-4), 6.88 (d,1H, H-2), 7.30–7.34 (dd, 1H, H-10), 7.34 (d, 1H, H-7), 7.75–7.88 (m, 2H, H-9 and H-1). Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found C, 70.28; H, 6.62.

4.1.15. 3-Isopropyloxy-8,9-dimethoxy-6*H*-dibenzo[*b*,*d*]-pyran (5a). To a mixture 3-isopropyloxy 8,9-dimethoxy dibenzopyranone (314 mg; 1 mM) in borane methyl sulfide complex (0.5 mL; 2 M solution in THF) was left overnight in dry RB flask (50 mL) with guard tube. After the completion of reaction, the reaction flask was cooled in ice-bath and quenched with cold saturated solution of ammonium chloride (5 mL) with stirring. The reaction mixture was refluxed on water bath with ethanol (5 mL) for 10 min. The ethanol was distilled off and the product extracted with dichloromethane (10 mL). The organic layer was washed with water (3 mL×3), dried Na_2SO_4 and concentrated. The product was recrystallised in ethyl acetate–hexane to yield the product as pure white crystals, yield 81%, mp 220 °C; MS: *m*/*z* 300 (M^+); ^1H NMR ($CDCl_3$) δ: 1.33–1.40 (m, 6H, $(CH_3)_2$), 3.84 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.70–6.71 (d, 1H, H-4), 6.78–6.82 (dd, 1H, H-2), 7.5 (s, 1H, H-10), 7.7 (s, 1H, H-7), 8.14–8.18 (d, 1H, H-1). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found C, 72.01; H, 6.75.

4.1.16. 3,9-Diisopropyloxy-8-methoxy-6H-dibenzo-[*b*,*d*]pyran (5b). Yield 79.2%, mp 96 °C; MS: *m*/*z* 328 (M^+); ^1H NMR ($CDCl_3$) δ: δ 1.36–1.39 (m, 12H, $(CH_3)_2\times2$), 4.54–4.64 (m, 1H,CH×2), 4.66–4.76 (s, 2H, OCH_2), 6.84–6.85 (s, 1H, H-4) 6.88–6.89 (d, 1H, H-2), 7.30–7.34 (dd, 1H, H-10), 7.75–7.76 (d, 1H, H-7), 7.82–8.19 (m, 2H, H-9 and H-1). Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found C, 73.41; H, 7.15.

4.1.17. 3,8-Diisopropyloxy-6*H*-dibenzo[*b*,*d*]pyran (5c). Yield, 75%, mp 64 °C; MS: *m*/*z* 298 (M^+); ^1H NMR ($CDCl_3$) δ: 1.38–1.39 (m, 12H, $(CH_3)_2\times2$), 4.55–4.67 (m, 2H, CH×2), 4.52–4.66 (s, 2H, OCH_2), 6.84–6.85 (s, 1H, H-4) 6.88–6.89 (d, 1H, H-2), 7.30–7.34 (dd, 1H, H-10), 7.75–7.76 (d, 1H, H-7), 7.82–8.19 (m, 2H, H-9 and H-1). Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found C, 76.54; H, 7.63.

4.1.18. 3,8-Bis(dimethylcarbamoyl)dibenzo[*b*,*d*]pyran-6-one (6a). To a solution of **3e** (228 mg; 1 mM) in dry pyridine (3 mL) was added *N*,*N*-dimethyl carbamoyl chloride (0.5 mL). The reaction mixture was stirred for 40 h at room temperature and quenched with ice cold water (5 mL). The precipitated solid was filtered and crystallised, yield 60%, mp 214 °C; MS: *m*/*z* 370 (M^+), 105, 72; ^1H NMR ($CDCl_3$) δ: 3.04 (s, 6H, $(CH_3)_2$), 3.13 (s, 6H, $(CH_3)_2$), 7.07 (d, 1H, H-7), 7.36 (d, 1H, H-4), 7.63 (dd, 1H, H-2), 7.84–8.09 (m, 3H, H-9, H-10 and H-1). Anal. Calcd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56. Found C, 61.39; H, 4.94; N, 7.31.

4.1.19. 3,8-Bis(diethylcarbamoyl)dibenzo[*b*,*d*]pyran-6-one (6b). Yield 77%, mp 140 °C; MS: *m*/*z* 426 (M^+) ($CDCl_3$) δ: 1.20 (t, 6H, $(CH_3)_2$), 1.25 (t, 6H, $(CH_3)_2$), 3.04 (q, 4H, $(CH_2)_2$), 3.13 (q, 4H, $(CH)_2$), 7.07 (d, 1H, H-7), 7.36 (d, 1H, H-4), 7.63 (dd, 1H, H-2), 7.84–8.09 (m, 3H, H-9, H-10 and H-1). Anal. Calcd for $C_{23}H_{26}N_2O_6$; C, 64.78; H, 6.15; N, 6.57. Found C, 64.97; H, 6.46; N, 6.71.

4.1.20. 3,9-Bis(dimethylcarbamoyl)dibenzo[*b*,*d*]pyran-6-one (6c). Yield 65%, mp 214 °C; MS: *m*/*z* 370 (M^+); ^1H NMR ($CDCl_3$) δ: 3.04 (s, 6H, $(CH_3)_2$), 3.18 (s, 6H, $(CH_3)_2$), 7.07 (d, 1H, H-8), 7.36 (d, 1H, H-4), 7.63 (dd, 1H, H-2), 7.84–8.09 (m, 3H, H-7, H-10 and H-1). Anal. Calcd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56. Found C, 61.66; H, 4.83; N, 7.55.

4.1.21. 3,9-Bis(diethylcarbamoyl)dibenzo[*b*,*d*]pyran-6-one (6d). Yield 79%, mp 140 °C; MS: *m*/*z* 426 (M^+); ^1H NMR ($CDCl_3$) δ: 1.20 (t, 6H, $(CH_3)_2$), 1.25 (t, 6H, $(CH_3)_2$), 3.04 (q, 4H, $(CH_2)_2$), 3.13 (q, 4H, $(CH)_2$), 7.07 (d, 1H, H-8), 7.36 (d, 1H, H-4), 7.63 (dd, 1H, H-2), 7.84–8.09 (m, 3H, H-7, H-10 and H-1). Anal. Calcd for $C_{23}H_{26}N_2O_6$; C, 64.78; H, 6.15; N, 6.57. Found C, 64.99; H, 6.14; N, 6.53.

4.1.22. 9-Benzyloxy-3,8-dimethoxy-dibenzo[*b*,*d*]pyran-6-one (7). To a solution of **3d** (3.48 g, 0.01 mol) in dry acetone (50 mL) was added anhydrous potassium carbonate (2.76 g, 0.02 mol) and iodomethane (2.5 mL, 0.02 mol) and the mixture was refluxed in inert atmosphere for 24 h. After the completion of the reaction as shown by TLC, the solution was cooled, filtered and excess of solvent was removed. The oily residue obtained was dissolved in ethyl acetate and with slow addition of hexane, the desired product was separated as crystalline yellow solid, yield 2.84 g, mp 190 °C; MS: *m*/*z* 362 (M^+); ^1H NMR ($CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 3.99 (s, 6H, $2\times OCH_3$), 5.34 (s, 2H, OCH_2); 6.83–6.89 (d, 1H, H-4), 6.94–6.98 (dd, 1H, H-2); 7.26–7.56 (m, 7H, ArH); 7.68–7.74 (d, 1H, H-1).

4.1.23. 9-Benzyloxy-3,8-dimethoxy-6,6-dimethyl-dibenzo-[*b*,*d*]pyran (8). A solution of **7** (3.62 g; 0.1 mol) in dry THF (100 mL) was added dropwise over 15 min to a freshly prepared solution of methyl magnesium iodide at room temperature. The reaction mixture was allowed to stir as such for 1 h and then refluxed at 70 °C for 6 h. After completion of the reaction as checked by TLC, the

solution was cooled and poured into a mixture of concd HCl (2 mL) and ice with vigorous stirring. The product separated as sticky solid was extracted with benzene (200 mL), washed with water, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over silica gel column eluting with ethyl acetate/ hexane to obtain a white crystalline solid, yield 2.8 g, mp 152 °C; MS: m/z 376 (M^+). ^1H NMR ($CDCl_3$) δ: 1.56 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2), 6.48–6.52 (d, 1H, H-4), 6.52–6.58 (dd, 1H, H-2), 7.30–7.82 (m, 8H, Ar-H).

4.1.24. 3,8-Dimethoxy-9-hydroxy-6,6-dimethyl dibenzo-[*b*,*d*]pyran (9). To a solution of **8** (3.76 mg; 0.1 mol) in methanol (50 mL) was added Raney nickel (1.0 g). The reaction mixture was hydrogenated at 60 psi with shaking for 4 h. After the completion of reaction, the mixture was filtered through Celite and washed with methanol (5 mL × 3).The filtrate was concentrated and the residue crystallised in methanol to obtain compound **9** as white crystalline solid, yield 3.0 g, mp 126 °C; MS: m/z 286 (M+), 271, 256, 135; ^1H NMR ($CDCl_3$) δ: 1.56 and 1.60 (s, s, 6H, $CH_3 \times 2$), 3.80 (s, 3H, 8-OCH_3), 3.92 (s, 3H, 3-OCH_3), 5.58 (s, 1H, OH), 6.39 (s, 1H, H-4), 6.49–6.54 (dd, 1H, H-2), 6.68 (s, 1H, H-7), 7.36 (s, 1H, H-10), 7.45–7.50 (dd, 1H, H-1).

4.1.25. 3,8-Dimethoxy-9-piperidinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (10a). To a solution of compound **9** (286 mg; 1 mM) in dry acetone (50 mL) was added anhydrous potassium carbonate (276 mg; 2 mM) and 1-(2-chloroethyl)piperidine hydrochloride (368 mg; 2 mmol). The reaction mixture was refluxed with stirring at 70 °C for 24 h. After the completion of reaction, the solution was cooled and the contents filtered. The filtrate was concentrated to give an oily residue, which was chromatographed over basic alumina coloum eluting the product with ethyl acetate/hexane, yield 320 mg, mp 118 °C; MS: m/z 397 (M^+), ^1H NMR ($CDCl_3$) δ: 1.60–1.70 (m, 12H, $(CH_2)_3$ and $CH_3 \times 2$), 2.51–2.56 (m, 4H, $N(CH_2)_2$), 2.81–2.87 (t, 2H, N-CH_2), 3.80 (s, 3H, 8-OCH_3), 3.98 (s, 3H, 3-OCH_3), 4.18–4.25 (t, 2H, OCH_2), 6.49–6.50 (d, 1H, H-4), 6.55–6.60 (d, 1H, H-2), 6.70–7.06 (s, 2H, H-7 and H-10), 7.47–7.51 (d, 1H, H-1).

4.1.26. 3,8-Dimethoxy-9-pyrrolidinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (10b). Yield 62%, mp 124 °C; MS: m/z 383 (M^+), ^1H NMR ($CDCl_3$) δ: 1.49 and 1.50 (s, $3H \times 2$, $2 \times CH_3$), 1.79–1.86 (m, 4H, $(CH_2)_2$), 2.64–2.67 (m, 4H, $N(CH_2)_2$), 2.95–3.02 (t, 2H, NCH_2), 3.80 (s, 3H, 3-OCH_3), 3.99 (s, 3H, 8-OCH_3), 4.19–4.26 (t, 2H, OCH_2), 6.49–6.50 (d, 1H, H-4), 6.55–6.59 (dd, 1H, H-2), 6.60 (s, 1H, H-7), 7.16 (s, 1H, H-10), 7.46–7.51 (d, 1H, H-1).

4.1.27. 3,8-Dimethoxy-9-morpholinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (10c). Yield 62%, mp 126 °C; MS: m/z 399 (M^+), ^1H NMR ($CDCl_3$) δ: 1.60–1.70 (s, s, 6H, $CH_3 \times 2$), 2.50–2.54 (m, 4H, $N(CH_2)_2$), 3.68–3.80 (m, 4H, $(CH_2)_2$) 3.90 (s, 3H, OCH_3), 3.98–4.22 (t, 2H, OCH_2), 6.49–6.51 (d, 1H, H-4), 6.55–6.60 (dd, 1H, H 2), 6.71 (s, 1H, H- 7), 7.16 (s, 1H, H-10), 7.46–7.51 (d, 1H, H-1).

4.1.28. 3,8-Dihydroxy-9-piperidinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (11a). To a solution of compound **10a** (397 mg; 1 mM) in anhydrous dicholromethane (5 mL) cooled to −78 °C, was added dropwise boron tribromide (1.0 M soln in hexane, 5 mL, 5 mM) via syringe under N_2 atmosphere. The reaction mixture was allowed to come to rt and left overnight with stirring. The mixture was quenched with methanol and stirred for 30 min. Excess of solvent was removed under reduced pressure and the crude mixture was neutralised with a satd sodium bicarbonate solution. It was extracted with ethyl acetate (5 × 20 mL) and the organic layer washed with brine, dried over Na_2SO_4 and concentrated. The residue obtained was chromatographed over basic alumina column eluting with ethyl acetate/ hexane, yield 220 mg, mp 168 °C; MS: m/z 369 (M^+); ^1H NMR ($CDCl_3$) δ: 1.56–1.67 (m, 12H, $(CH_2)_3$ and $2 \times CH_3$), 2.44–2.46 (m, 4H, $N(CH_2)_2$), 2.6–2.7 (t, 2H, N-CH_2), 4.2 (t, 2H, OCH_2), 6. 5 (d, 1H, H-4), 6.55 (d, 1H, H–2), 6.70–7.06 (s, 2H, H-7 and H–10), 7.47–7.51 (d, 1H, H-1), 10.23 (s, 1H, 8-OH), 10.41 (s, 1H, 3-OH). Anal. Calcd for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found C, 71.55; H, 7.46; N, 3.83.

4.1.29. 3,8-Dihydroxy-9-pyrrolidinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (11b). Yield 54%, mp 171 °C; MS: m/z 355 (M^+); ^1H NMR ($CDCl_3$) δ: 1.49 and 1.50 (s, s, $3H \times 2$, $2 \times CH_3$), 1.73–1.85 (m, 4H, $(CH_2)_2$), 2.64–2.67 (m, 4H, $N(CH_2)_2$), 2.99–3.12 (t, 2H, NCH_2), 4.16–4.26 (t, 2H, OCH_2), 6.49–6.50 (d, 1H, H-4), 6.55–6.57 (dd, 1H, H-2), 6.63 (s, 1H, H-7), 7.19 (s, 1H, H-10), 7.49–7.51 (d, 1H, H-1). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found C, 71.04; H, 7.22; N, 3.99.

4.1.30. 3,8-Dihydroxy-9-morpholinoethoxy-6,6-dimethyl dibenzo[*b*,*d*]pyran (11c). Yield 55%, mp 163 °C. MS: m/z 371 (M^+), ^1H NMR ($CDCl_3$) δ: 1.64–1.67 (s, s, 6H, $CH_3 \times 2$), 2.44–2.49 (m, 4H, $N(CH_2)_2$), 3.46–3.48 (m, 4H, $(CH_2)_2O$), 6.41–6.47 (d, 1H, H-4), 6.51–6.56 (dd, 1H, H-2), 6.67 (s, 1H, H-7), 6.69 (s, 1H, H-10), 7.41–7.46 (d, 1H, H-1), 10.11 (s,1H, 8-OH), 10.23 (s, 1H, 3-OH). Anal. Calcd for $C_{21}H_{25}NO_5$: C, 67.91; H, 6.78; N, 3.77. Found C, 67.92; H, 6.82; N, 3.82.

4.1.31. 3,8-(Di-(*t*-butyldimethyl)silyloxy)dibenzo[*b*,*d*]-pyran-6-ol (12). A solution of bis silylated compound **3e** (886 mg; 2 mM) in dry toluene (20 mL) was cooled to −75 °C and treated dropwise with DIBAL-H (1.0 M cyclohexane soln, 1.2 mL; 1.2 mM) at a rate that maintained the internal temperature below −70 °C. The mixture was stirred for 4 h as the temperature gradually rose to rt. It was then was quenched with MeOH (3 mL) and 10% aqueous citric acid solution (1.2 mL). After dilution with CH_2Cl_2 (20 mL), the mixture was washed

with satd solution of potassium sodium tartarate (2×10 mL) and the aqueous layer was extracted with CH_2Cl_2 (2×20 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. Chromatography over silica gel column and eluting with ethyl acetate/hexane yielded white crystalline solid, yield 620 mg, mp 82 °C; MS: m/z 445 (M^+); ^{1}H NMR ($CDCl_3$) δ: 0.25 (s, 12H, $Si(CH_3)_2\times2$), 1.01 (s, 18H, $C(CH_3)_3\times2$), 3.0–3.11 (d, $J = 8$ Hz, 1H, OH), 6.28–6.32 (d, $J = 8$ Hz, 1H, H of lactol ring), 6.58–6.61 (m, 2H, H-4 and H-2), 6.73–6.74 (d, 1H, H-7), 6.89–7.03 (dd, 1H, H-9), 7.51–7.85 (m, 2H, H-10 and H-1).

4.1.32. 3,8-[Di-(*t*-butyldimethyl)silyloxy]-6-phenoxydibenzo[*b*,d]pyran (13). To a solution of compound **12** (445 mg, 1 mM) in dry CH_2Cl_2 (5 mL) was added phenol (490 mg; 5 mM) and anhydrous $MgSO_4$ (600 mg, 5 mM) and the reaction mixture was stirred at room temperature for 4 h. On completion it was diluted with CH_2Cl_2 (10 mL) and washed with 5% aqueous solution of sodium hydroxide (5×10 mL), then with water (3×5 mL). The organic layer was dried over Na_2SO_4, concentrated and used as such for the next reaction. Yield 210 mg; FAB MS: m/z, 534 (M^+); ^{1}H NMR ($CDCl_3$) δ: 0.10 (s, 12H, $Si(CH_3)_2\times2$), 1.06 (s, 18H, $C(CH_3)_3\times2$), 6.30–6.33 (d, 1H, H of lactol), 6.40–6.45 (m, 3H, H-2, H-4 and H-7), 6.70–6.71 (dd, 1H, H-9), 6.97–7.06 (m, 5H, ArH), 7.43–7.58 (m, 2H, H-1 and H-10).

4.1.33. 3,8-[Di-(*t*-butyldimethyl)silyloxy]-6-[4-[2-(1-piperidino)ethoxy]-phenyl]dibenzo[*b*,*d*]pyran (14a). To a stirred solution of compound **13** (200 mg; 0.38 mM) in dry toluene (5 mL) at 0 °C was added dropwise a solution of 4-[2-(1-piperidino) ethoxy]-phenyl magnesium bromide [prepared from [4-(2-(1-piperidino)ethoxy]-phenyl bromide (250 mg; 0.8 mM), Mg (22 mg; 0.88 mM), 1,2-dibromoethane (0.03 mL; 0.4 mM in dry THF, 10 mL)]. The reaction mixture was stirred at 0 °C for 4 h and then at room temperature for 24 h. Excess of THF was removed under vacuo and the product was extracted in ether (3×10 mL), washed with water (3 mL×3), dried over Na_2SO_4 and concentrated. Chromatography over a silica gel column and eluting with ethyl acetate/hexane afforded pale coloured oil, yield 200 mg, MS: m/z 645 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.42–1.64 (m, 6H, $(CH_2)_3$), 2.47–2.56 (m, 4H, $N(CH_2)_2$), 2.68–2.74 (t, 2H, N-CH_2), 3.88–3.94 (t, 2H, OCH_2), 6.31–6.34 (d, 1H, H of lactol), 6.50–6.56 (m, 3H, H-2, H-4 and H-7), 6.72–6.74 (dd, 1H, H-9), 7.01–7.68 (m, 5H, ArH and H-10), 7.88 (d, 1H, H-1).

4.1.34. 3,8-[Di-(*t*-butyldimethyl)silyloxy]-6-[4-[2-(1-pyrrolidino)ethoxy]-phenyl]dibenzo[*b*,*d*]pyran (14b). Oil, yield 155 mg, MS: m/z 631 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.42–1.64 (m, 4H, $(CH_2)_2$), 2.47–2.56 (m, 4H, $N(CH_2)_2$), 2.68–2.74 (t, 2H, N-CH_2), 3.88–3.94 (t, 2H, OCH_2), 6.31–6.34 (d, 1H, H of lactol), 6.50–6.56 (m, 3H, H-2, H-4 and H-7), 6.72–6.74 (dd, 1H, H-9), 7.01–7.68 (m, 5H, ArH and H-10), 7.88 (d, 1H, H-1).

4.1.35. 3,8-Dihydroxy-6-[4-(2-(1-piperidino)ethoxyphenyl]dibenzo [b,d]pyran (15a). To a solution of compound **15** (100 mg; 0.07 mM) in dry methanol (1 mL) was added 5% methanolic solution of I_2 (2 mL). The reaction mixture was refluxed on water bath for 0.5 h. Methanol was evaporated off in vacuo and the product extracted in ether. The organic layer was washed with water (2 mL×3), dried (Na_2SO_4), concentrated and chromatographed over basic alumina (MeOH–$CHCl_3$) to give white solid, yield 43.4%; MS: m/z 417 (M^+), 333 (M^+–84), 303, 227, 165, 90; ^{1}H NMR ($CDCl_3$) δ: 1.42–1.56 (m, 6H, $(CH_2)_3$), 2.42–2.54 (m, 4H, $N(CH_3)_2$), 2.82–2.88 (t, 2H, $N(CH_2)_3$), 3.98–4.04 (t, 2H, OCH_2), 5.65 (s, 1H, OH), 5.80 (s, 1H, OH), 6.31 (s, 1H, H of pyran ring), 6.44–6.51 (m, 3H, H-2, H-4 and H-7), 6.78–7.01 (m, 6H, ArH, H-10 and H-1). Anal. Calcd for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found C, 74.82; H, 6.55; N, 3.41.

4.1.36. 3,8-Dihydroxy-6-[4-(2-(1-pyrrolidino)ethoxyphenyl]dibenzo[*b*,*d*]pyran (15b). Yield 40%; MS: m/z, 403 (M^+); ^{1}H NMR ($CDCl_3$) δ: 1.42–1.56 (m, 4H, $(CH_2)_2$), 2.42–2.54 (m, 4H, $N(CH_3)_2$), 2.82–2.88 (t, 2H, N $(CH_2)_3$), 3.98–4.04 (t, 2H, OCH_2), 5.65 (s, 1H, OH), 5.80 (s, 1H, OH), 6.31 (s, 1H, H of pyran ring), 6.44–6.51 (m, 3H, H-2, H-4 and H-7), 6.78–7.01 (m, 6H, ArH, H-10 and H-1). Anal. Calcd for $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found C, 74.43; H, 6.31; N, 3.52.

5. Biology

5.1. Anti-implantation activity

Anti-implantation activity was evaluated in adult Sprague–Dawley rats by the method given in literature[13]. The compounds were administered at 10 mg/kg per oral dose as aqueous gum acacia suspension in day 1–5 schedule. On the 11th day of the test, rats of both the control and treated groups were laparotomised and their uteri examined for implantation sites. The results were considered positive when implantation sites were totally absent in both the uterine horns.

5.2. Oestrogenic activity

Immature rats of Sprague–Dawley strain (21 day old, weighing 22–30 g) were divided into different groups (each group comprising five rats) and were orally administered the test compound in graded doses once daily for three consecutive days. The control group of animals were treated with the vehicle alone. Uterine weight and status of vaginal opening were noted at the time of autopsy, that is, 24 h after the last treatment. The oestrogenic activity was assessed by uterine weight gain expressed as percentage increase as compared to control, which was used as basal value.[12]

5.3. Antioestrogenic activity

In antioestrogen assay[12] different groups of rats were given daily, 17β-oestradiol (0.1 μg in olive oil) by subcutaneous route along with graded doses (5–15 μg) of compounds for three consecutive days. The injections were given at two different sites in order to avoid any mutual interference in absorption of the oestrogen and of the test compound. One group of rats served as control and received E_2 and vehicle alone. On day 4 that is, 24 h after last treatment, the animals were autopsied. The uterine tissue was removed, made free of fat and fluid and weighed on a torsion balance. Antioestrogenic activity was computed on the basis of decrease in uterine weight in the experimental animals (receiving test compd+vehicle) as compared to that in the control group receiving E_2 + vehicle alone. Inhibition was expressed as percent inhibition of oestradiol induced increase in uterine wet weight.

5.4. Anti-osteoporotic activity (in vitro antiresorptive assay)

The in vitro assay of anti-resorptive activity using ^{45}Ca pre-labelled rat fetal bone was done according the literature method.[25] Three month old Sprague–Dawley female rats (180–220 g) maintained at standard conditions (22 ± 1 °C) with alternate 12 h light/dark periods and free access to pellet diet and tap water were used throughout the study. Females were mated to males of proven fertility. 250 μCi/300 μL of $^{45}CaCl_2$ was administered subcutaneously to each rat on day 18 of pregnancy and labelled femur and radio-ulna bones were isolated 48 h thereafter under sterile conditions. Bones were cultured in 300 μL of the BGJb medium supplemented with antibiotic, antifungal and buffer (pH 7.3) for 24 h. The bones were washed twice with PBS and transferred to BJGb medium containing PTH (0.4 μM) and these cultured for 96 h in the presence or absence of test compound (100 μM) or the vehicle (0.1% ethanol/DMSO) in 300 μL of BGJb, medium. Contralateral femur of each fetus served as corresponding control. On termination of the culture, bones were transferred to 0.1 N HCl for 24 h. Radioactivity due to ^{45}Ca in the spent medium collected at 48 and 96 h of culture and the HCl extracts at 96 h of culture were quantified by liquid scintillation spectrophotometer. Bone resorbing activity was expressed as percentage of released ^{45}Ca and the effect of test compounds as percent of corresponding control or T/C ratio.

5.5. Receptor binding affinity

Immature (21 day old) female rats were sacrificed 24 h after a single injection (S.C.) of E_2 (1 μg/rat). Their uteri were homogenised in 10 mM Tris–HCl buffer (containing 1.5 M EDTA and 0.2% sodium azide) and cytosol was prepared by centrifuging the homogenate at 105,000 × g at 4 °C.

The relative binding affinity (RBA) of the compounds for oestrogen receptor was determined by competition assay,[13] employing ^3H-oestradiol (^3H-E_2) as the radioligand. The test ligands and ^3H-E_2 are incubated at 4 °C with cytosol oestrogen receptors obtained from uteri of immature oestradiol-primed (1 μg/rat 24 h before autopsy) 20–21 days old rats. Aliquot of uterine cytosol (200 μL; 2 uterine per mL) prepared in TEA buffer (10 mM Tris, 1.5 mM EDTA, 0.02% sodium azide, pH 7.4) are incubated in duplicate with a fixed concentration of ^3H-E_2 in the absence or presence of various concentrations of the competitor substance dissolved in 30 μL of the TEA buffer containing DMF as co-solvent (final concentration of DMF in the incubation mixture never exceeded 5%) for 18 h at 4 °C. At the end of this period, dextran coated charcoal (5% Norit 0.5% dextran) suspension in 100 μL of TEA buffer is added to each tube, briefly vortexed and allowed to stand for 15 min at 4 °C. The mixture is centrifuged at 800 g for 10 min and the supernatants counted for radioactivity in 10 mL of a dioxane-based scintillation fluid. RBA of the test compound is 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 17β-oestradiol, gives the affinity of the test compound to oestrogen receptor relative to oestradiol. This when multiplied with 100 gives the percentage value designated as RBA.

Acknowledgements

Jaya Pandey and Ashok K. Jha are thankful to CSIR New Delhi for the award of Senior Research fellowships.

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