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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In vivo evaluation of substituted 3-phenyl,7-methoxy-benzopyrans as modified estrogens

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Abstract Substituted 3-phenyl,7-methoxy-benzopyran derivatives and vitamin D_3 (cholecalciferol, **1**) were evaluated for their estrogen agonistic and antagonistic activities in immature female Sprague–Dawley rat model. The benzopyran derivatives **17** and **18**, which were made as hybrids of estrogen and vitamin D_3 (pseudo vitamin D_3 analogs), showed significant estrogen agonistic activity (up to 48%) and weak estrogen antagonistic activity (up to 6%) at 10 mg/kg, whereas vitamin D_3 showed significant estrogen agonistic (82%) and antagonistic activities (39%) at 10 mg/kg.

Keywords Vitamin D_3 · Estrogen agonists · Estrogen antagonist · Osteoporosis · Antiosteoporotic agents · Benzopyran · Drug research

Introduction

Osteoporosis is a disease characterized by depletion of bone mass and enhanced bone fragility, leading to increased risk of fractures (Doggrell, 2003). In women, osteoporosis sets in after menopause when the estrogen level drops. This indicates that estrogen is related to maintenance of bone mass. It has now been established that estrogen has a role in osteoporosis directly and also through an indirect mechanism involving vitamin D_3 (**1**) (Fig. 1) (Mizwicki *et al*., 2005). Regulation of calcium is governed mainly by the parathyroid hormone (PTH) and 1,25-$(OH)_2$-vitamin D_3 (**2**) (Fig. 2) (Lips, 2006). 1,25-$(OH)_2$-vitamin D_3 (**2**) helps absorption of calcium in the gut. This active metabolite is formed from vitamin D_3 (**2**) under the

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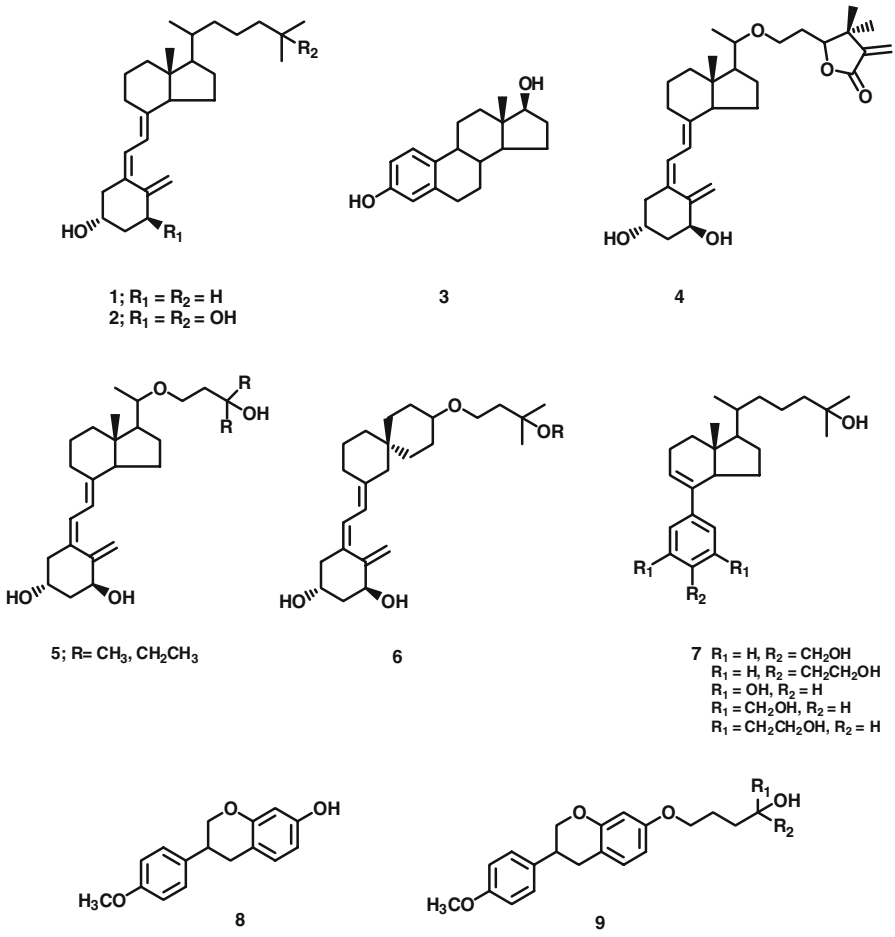


Fig. 1 Ligands for estrogen and vitamin D receptors

influence of estradiol (**3**) (Fig. 1) (Castillo *et al*., 1977). Also, estrogen is responsible for adjustment of a setpoint at which lowered level of calcium in serum triggers release of parathyroid hormone (PTH) to utilize calcium from bone to maintain calcium level (Christakos and Prince, 2003). Thus, vitamin D_3 (**2**) and estrogen are both required for maintenance of bone (Holick, 2006).

Furthermore, the study made by Demirpence *et al*. (1994) showed antiestrogenic effect of vitamin D_3 (**2**) as measured by its inhibitory action on estrogen-induced growth of MCF-7 cells (antiproliferative activity). The results of that study suggested that the ligand binding, dimerization, and ligand-dependent transactivating domains (DEF domains) of estrogen receptor (ER) were required for antiestrogenic effect of vitamin D_3 (**1**). It also suggested that vitamin D receptor (VDR) may play a part in imparting the binding of the ER to the estrogen response element (ERE). Therefore, there could be either direct or an indirect interaction between VDR and ER.

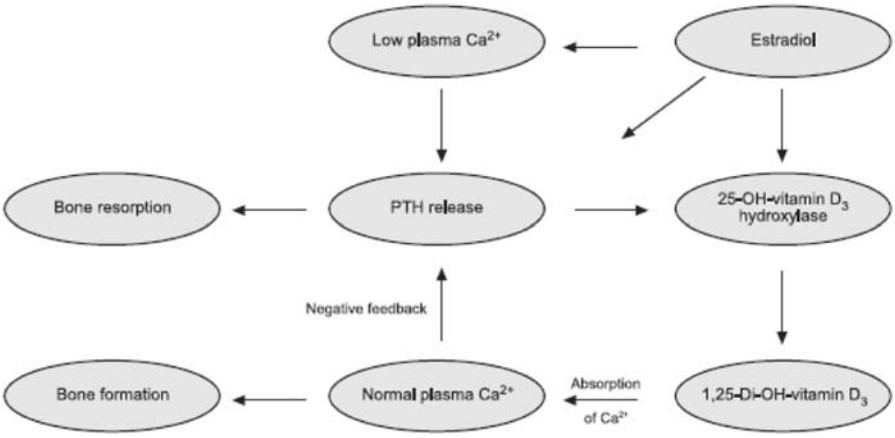


Fig. 2 Interplay of estrogens and vitamin D_3 in bone maintenance (Ray and Gupta, 2006)

To study the similarities in their biological functions, we reported a review of literature on structure–function relationship between vitamin D_3 (**2**) and potent endogenous estrogen, estradiol (**3**) (Ray and Gupta, 2006).

In our approach to see whether estrogens also cross-react with vitamin D receptor (VDR) in terms of biological activities, an attempt was made towards preparation of compounds as modified estrogen for bone-selective antiproliferative activities. The designed compounds will have a properly designed estrogen binding subunit along with a residue similar to that present in vitamin D_3 (**2**) to encourage cross-reactivity with both estrogen and vitamin D receptors (VDR).

Structurally, vitamin D_3 (**2**) has highly flexible side-chain as well as triene sterol structure (**3**) (Fig. 1) (Yamada *et al*., 2003). Stereochemistry of vitamin D_3 side-chain also plays an important role in its biological responses (Yamada *et al*., 2003). Several structural modifications have been made in the side-chain of vitamin D_3, which include flexible to rigid nature of side-chain (**4**–**6**) (Fig. 1) (Saito *et al*., 2004; Schepens *et al*., 2004). Other structural modification involves replacement of highly flexible *seco*-triene system of vitamin D_3 by a benzene ring, which led to compound **7** (Fig. 1), showing affinity for vitamin D receptor (Posner *et al*., 1995; Kenzler *et al*., 1996).

Furthermore, 3-phenyl-benzopyran nucleus, as present in Equol (**8**), forms the basis of several selective estrogen receptor modulators (SERMs) such as ormeloxifene, CDRI-85/287, and EM-800 (Ray and Diwedy, 1997). Equol (**8**) has affinity towards estrogen receptors (ER-α and ER-β) (Muthyala *et al*., 2004). In the present work, owing to their mild estrogenic action and effective affinity with estrogen receptors, we have chosen this isoflavonoid as basic nucleus, which was incorporated with modified side-chains similar to that present in vitamin D_3 and its synthetic analogs. The following compounds of type **9** were designed to achieve this objective. This study presents the in vivo estrogen agonistic and estrogen antagonistic activities of these 3-phenyl,7-methoxy-benzopyran-based hybrids of estrogen and vitamin D_3 analogs, and vitamin D_3 in immature female Sprague–Dawley rat model.

Materials and methods

Chemistry

The target benzopyran derivatives of type **9** were prepared through known procedure starting with condensation of resorcinol **10** and 4-methoxy phenylacetic acid (**11**) in presence of BF_3-OEt_2 through Friedel–Craft acylation reaction, yielding 1-(2,4-dihydroxyphenyl)-2-(4-methoxy-phenyl) ethanone (**12**) in 62% yield, as shown in Scheme 1 (Gupta and Ray, 2007). Compound **12** was alkylated with benzyl bromide in presence of anhydrous potassium carbonate in dry acetone, giving 1-(4-benzyloxy-2-hydroxy-phenyl)-2-(4-methoxy-phenyl) ethanone (**13**) in 72% yield. Compound **13** was then condensed with paraformaldehyde in aqueous solution of sodium hydroxide, which gave 7-benzyloxy-3-(4-methoxy-phenyl)-chroman-4-one (**14**) in 40% yield. Catalytic reduction of chromanone **14** using 10% palladium charcoal in methanol at 50 psi pressure afforded corresponding hydroxy chroman **15**. Alkylation of **15** with ethyl-4-bromobutyrate in presence of anhydrous potassium carbonate in dry acetone at reflux temperature furnished 4-[3-(4-methoxy-phenyl)-chroman-7-yloxy] butyric acid ethyl ester (**16**). Grignard reaction on compound **16** with alkylmagnesium halide reagents in dry THF–OEt_2 system yielded compounds **17** and **18** in 94% yield. All the synthesized compounds were identified by ^{1}H nuclear magnetic resonance (NMR), fast-atom bombardment (FAB) mass spectroscopy (MS), and infrared (IR) spectroscopy.

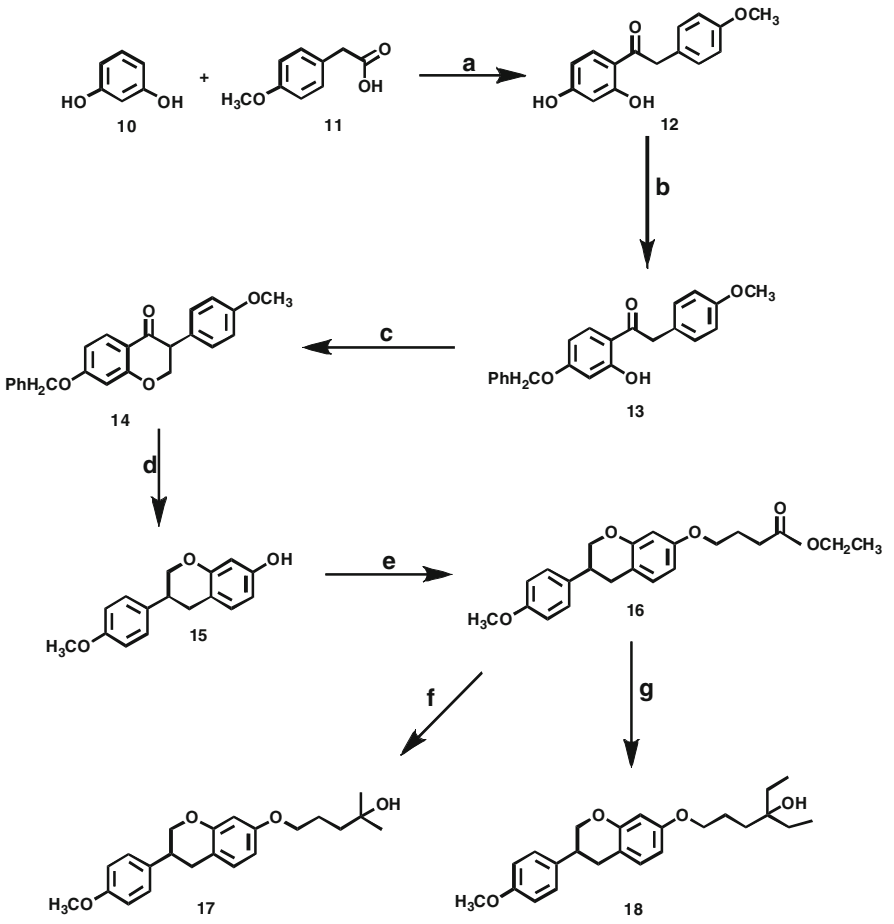
Biology

Estrogen agonistic activity (Ghosh *et al*., 2001)

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and, after postoperative 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, serving 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. Increase in uterine fresh weight was taken as parameter for evaluation of estrogen agonistic activity in comparison with rats of vehicle control group. Theobjective was to evaluate estrogen agonistic effect of the compounds on the uterus.

Estrogen antagonistic activity (Ghosh *et al*., 2001)

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and, after postoperative rest for 7 days, were randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg/kg dose of 17α-ethynylestradiol (EE) in 10% ethanol–



Scheme 1 (a) BF_3–OEt_2,Heat, (b) $C_6H_5CH_2Br$, K_2CO_3, dry acetone, (c) HCHO, aq NaOH, (d) Pd/C, H_2, CH_3OH, (e) ethyl-4-bromobutyrate, K_2CO_3, dry acetone, (f) CH_3MgI, diethyl ether, (g) CH_3CH_2MgI, Et_2O–THF

distilled water once daily for three consecutive days on days 28–30 of age by oral route. A separate group of animals receiving only EE (0.02 mg/kg) in 10% ethanol–distilled water for similar duration, as 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 increase in uterine fresh weight was taken as parameter for evaluation of estrogen antagonistic effect of the compounds.

Inhibition in uterine weight in case of estrogen antagonistic action has been calculated in comparison with respective EE per se treated group. For estrogen agonistic activity, uterine weight gain was calculated with respect to the corresponding vehicle control group.

Results and discussion

The above discussion has shown direct or indirect interplay of estrogens and vitamin D_3 in maintenance of bones, cellular proliferation, and other biological activities. Vitamin D_3 not only acts as anti-bone-resorptive agent, but also helps in bone formation, whereas estrogens are involved in vitamin D_3 metabolism and also act as anti-bone-resorptive agents. It is noteworthy that selective estrogen receptor modulators (SERMs) are anti-bone-resorptive agents, similar to many steroidal and nonsteroidal estrogens. To validate our hypothesis of cross-reactivity of vitamin D_3 with estrogen as well as vitamin D receptors for bone maintenance and cellular proliferation, we first time evaluated in vivo estrogen agonistic and estrogen antagonistic activities of vitamin D_3 (cholecalciferol, **1**) in immature female Sprague–Dawley rat model. Vitamin D_3 showed both significant estrogen agonistic (82%) as well as estrogen antagonistic activity (39%), which highlights its ability to interact with estrogen receptors. Since the aim of this study was to design and synthesize modified estrogens as bone-selective antiproliferative agents, we therefore evaluated the synthesized benzopyran derivatives **17** and **18**, which were made as hybrids of estrogen and vitamin D_3 (pseudo vitamin D_3 analogs) for estrogen agonistic, antagonistic activities. The designed compounds (**17** and **18**), which incorporate 3-phenyl-benzopyran nucleus as present in Equol **8**, and several SERMs such as Ormeloxifene, CDRI-85/287, and EM-800, were expected to have mixed estrogen agonistic and antagonistic activities through their interaction with estrogen receptor (ER) similar to Equol, ormeloxifene, and raloxifene. Estrogen agonistic and antagonistic activities were determined in vivo using EE as standard, whereas ormeloxifene and raloxifene, selective estrogen receptor modulators used for treatment of osteoporosis, were used as standard for comparisons of their estrogen agonistic/antagonistic activities with newly synthesized compounds **17** and **18**. Tested compounds showed significant estrogen agonistic activity (up to 48%) and weak estrogen antagonistic activity (up to 6%). Interestingly, during in vivo evaluation of these compounds in rats, we did not observe any apparent toxicity of these compounds. The biological activities of the tested compounds are presented in Table 1.

Conclusion

In summary, the newly designed substituted 3-phenyl,7-methoxy-benzopyran derivatives, which were made as hybrids of estrogen and vitamin D_3 (pseudo vitamin D_3 analogs), showed significant estrogen agonistic activity (up to 48%) and weak estrogen antagonistic activity (up to 6%) at 10 mg/kg, whereas vitamin D_3 showed significant estrogen agonistic (82%) and antagonistic activity (39%) at 10 mg/kg. As anticipated significant in vivo estrogen agonistic and estrogen antagonistic activities of vitamin D_3 (cholecalciferol, **1**) highlight its ability to interact with estrogen receptors. In comparison with vitamin D_3, raloxifene, and ormeloxifene, the synthesized benzopyran derivatives showed low order of activities, which prevented our further experimentation. Further investigation of such molecules could be of interest for the design of drugs for management of bone disease such as osteoporosis.

Table 1 Estrogen antagonistic and agonistic activities of the test compounds

Compound no.	Dose (mg/kg/day)	Route of administration	Schedule (day of treatment)	Estrogen antagonistic activity		Estrogen agonistic activity	
				Uterine weight[a] (mg)	Inhibition[b] (%)	Uterine weight[a] (mg)	Gain[c] (%)
Vehicle	10	Oral	1–3	18.10 ± 1.14		18.10 ± 1.14	
EE	0.02	Oral	1–3	108.30 ± 8.30		108.30 ± 8.30[g]	
Ormeloxifene	10	Oral	1–3	35.90 ± 3.13[e]	67	46.33 ± 3.82[g]	156
Raloxifene	10	Oral	1–3	46.60 ± 4.90[e]	57	34.40 ± 3.20[g]	90
Vehicle	10	Oral	1–3	22.23 ± 1.20		22.23 ± 1.20	
EE	0.02	Oral	1–3	128.96 ± 1.66		128.96 ± 1.66[g]	480
Vitamin D_3	10	Oral	1–3	78.13 ± 10.95[e]	39	43.90 ± 2.89[f]	82
Vehicle	10	Oral	1–3	14.00 ± 1.50		14.00 ± 1.50	
EE	0.02	Oral	1–3	85.00 ± 0.57		85.00 ± 0.57[g]	507
17	10	Oral	1–3	79.70 ± 3.90[d]	6	18.33 ± 1.30[f]	31
18	10	Oral	1–3	82.30 ± 5.20	3	20.70 ± 2.00[g]	48

EE 17α-Ethynylestradiol

[a] Values represents mean ± standard error on the mean (SEM) of minimum of six observations in each group

[b] Percentage of EE per se treated group

[c] Percentage 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

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