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Research paper

# Differentiation of skeletal osteogenic progenitor cells to osteoblasts with 3,4-diarylbenzopyran based amide derivatives: Novel osteogenic agents

Atul Gupta [a, *], Imran Ahmad [a], Jyoti Kureel [b], Aijaz A. John [b], Eram Sultan [c], Debabrata Chanda [c], Naresh Kumar Agarwal [d, f], Alauddin [d, f], Wahajuddin [d], S. Prabhaker [e], Amita Verma [a], Divya Singh [b]

[a] Medicinal Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, PO. CIMAP, Kukrail Road, Lucknow, 226015, India
[b] Division of Endocrinology, CSIR-Central Drug Research Institute, Sector-10, Jankipuram Extension, Lucknow, 226031, India
[c] Molecular Bioprospection Department, CSIR-Central Institute of Medicinal and Aromatic Plants, PO. CIMAP, Kukrail Road, Lucknow, 226015, India
[d] Division of Pharmacokinetics and Metabolism, CSIR-Central Drug Research Institute, Sector-10, Jankipuram Extension, Lucknow, 226031, India
[e] National Centre for Mass Spectrometry, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad, 500 007, India
[f] Department of Zoology, HNB Garhwal Central University, Badshahi Thaul Campus, Tehri Garhwal 249 199, Uttarakhand, India

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ABSTRACT

A series of 3,4-diarylbenzopyran based amide derivatives was synthesized and evaluated for osteogenic activity in *in vitro* and *in vivo* models of osteoporosis. Compounds **17a**, **21b**–**c** and **22a**–**b** showed significant osteogenic activity in osteoblast differentiation assay. Among the synthesized compounds, **22b** was identified as lead molecule which showed significant osteogenic activity at 1 pM concentration in osteoblast differentiation assay and at 1 mg $kg^{-1}$ body weight dose in estrogen deficient balb/c mice model. *In vitro* bone mineralization and expression of osteogenic marker genes *viz* BMP-2, RUNX-2, OCN, and collagen type 1 further confirmed the osteogenic potential of **22b**. Gene expression study for estrogen receptor α and β (ER-α and ER-β) in mouse calvarial osteoblasts (MCOs) unveiled that possibly **22b** exerted osteogenic efficacy via activation of Estrogen receptor-β preferentially. *In vivo* pharmacokinetic, estrogenicity and acute toxicity studies of **22b** showed that it had good bioavailability and was devoid of uterine estrogenicity at 1 mg $kg^{-1}$ and inherent toxicity up to 1000 mg $kg^{-1}$ body weight dose respectively.



## 1. Introduction

Osteoporosis is the most prevalent metabolic bone disease among men and women with an average age above 50 years [1,2]. It is defined as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to non-traumatic fractures [3–7]. The incidence of osteoporotic fractures increases with age. Osteoporosis is now considered as one of the major and growing health care problem around the world [8]. This disease occurs due to an imbalance in the process of bone remodeling which leads to exaggerated bone resorption [9–11]. Any defect in osteoprotegerin (OPG)/receptor activator NFκB ligand (RANKL)/receptor activator NFκB (RANK) system, a dominant mediator of osteoclastogenesis, leads to imbalance in bone remodeling [12,13]. Osteoprotegerin acts as a decoy receptor for the receptor activator NFκB ligand (RANKL) in osteoblast cells and prevents the RANK-RANKL interaction between osteoblast and osteoclast precursor cells which restrain maturation of premature osteoclast cells [14]. The production of osteoprotegerin is stimulated by estrogen in a dose and time dependent manner [15]. Saika et al. reported that estrogen induced increase in OPG is likely to involve its genomic action through estrogen receptor-α (ER-α) [16]. Many cytokines such as interleukins (IL-1, IL-6), TNF-α and monocyte/macrophage colony stimulating factor (M-CSF) involved in bone resorption are also known to be down regulated by estrogen [17]. Furthermore, estrogen is also implicated in stimulation, differentiation and activity of osteoblast cells in cultures [18,19].

* Corresponding author.
*E-mail address:* atisky2001@yahoo.co.in (A. Gupta).

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In postmenopausal women, the level of reproductive hormones fluctuates causing a series of immunological and metabolic alterations which leads to lack of adequate bone formation and increases bone resorption [20,21]. The use of estrogen replacement or hormone replacement therapy (ERT or HRT) improves the situation. However, these options have their limitations due to associated side effects especially in reproductive tissues [22,23]. The approved therapies for osteoporosis treatment are mainly focused on bone resorption inhibitors [24]. The recent observations reveal that bone resorption is accompanied by inhibited bone formation due to coupling of these two processes which represents the main disadvantage associated with bone resorption inhibitors [25–27]. The attention has now been focused on osteogenic agents for osteoporosis treatment [28–30].

In order to develop osteogenic drugs, 3-arylbenzopyran core which structurally simulates with tetracyclic nucleus of estran (e.g. 17β-estradiol) and stilbene core of diethylstilbestrol (DES) as well as resveratrol, has been realized as an important nucleus. This core is present in many naturally occurring osteogenic compounds such as geinstein (**1**), daidgein (**2**), formonentin (**3**), isoformonenetin (**4**), equol and medicarpin etc [31–37]. 3-arylbenzopyran core is also present in various synthetic compounds such as **5**–**10** (Fig. 1) [33–35,38–43]. These compounds possess potential osteogenic activity with minimal or no toxicity.

In our approach to investigate novel osteogenic agents, 3,4-diarylbenzopyran based amide derivatives **15a**–**c**, **16a**–**c**, **21a**–**c** and **22a**–**c** were designed and synthesized. To achieve the targeted osteogenic activity, the proposed molecules were designed to have 3-arylbenzopyran core for their binding with estrogen receptors (ER) and a long chain alkylamide bearing phenyl group at position 4 of 3-arylbenzopyran core for induction of mixed estrogen agonistic and antagonistic activity. The synthesized compounds were evaluated for osteogenic activity in osteoblast differentiation assay using mouse calvarial osteoblast cells (MCOs). The osteogenic potential of most active compound **22b** was further confirmed by bone mineralization activity and osteogenic gene expression analysis by qPCR. Following *in vitro* osteogenic activity data, **22b** was further evaluated for osteogenic activity in *in vivo* estrogen deficient balb/c mice model for osteoporosis. The effect of **22b** on trabecular bone volume (BV/TV; %), trabecular number (Tb.N), and trabecular separation (Tb.Sp), trabecular thickness (Tb.Th) was evaluated. Furthermore, **22b** was evaluated for its pharmacokinetic profile, estrogenicity and acute toxicity studies in animal models.

### 1.1. Chemistry

The synthesis of designed compounds was started with the synthesis of 2,2-dimethyl-4-(4-hydroxyphenyl)-7-methxoy-3-phenyl-2*H*-benzopyran (**13**) and 2,2-dimethyl-4-(4-hydroxyphenyl)-7-methxoy-3-phenyl-3*H*-benzopyran (**18**) from 3-methoxyphenol (**11**) and 4-hydroxybenzoic acid (**12**) following reported methodology [44]. For synthesis of **16a**–**c** and **17a**–**c**, **13** was alkylated with different bromo alkyl esters in presence of anhydrous $K_2CO_3$ in dry acetone which gave corresponding ester derivatives (**14**) in 76–80% yields (Scheme 1). On basic hydrolysis with 10% NaOH in methanol at reflux, compound **14** yielded **15** in quantitative yield. Subsequently, **15** was treated with 2-methylaminopyridine or *N*-methyl-*N*-butylamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxybenzotrizole (HOBt) in dry dimethylformamide (DMF) at room temperature to yield target amides, **16** and **17**, in 69–75% yields.

Similarly, **21a**–**c** and **22a**–**c** were synthesized through alkylation of **19** with different alkyl esters followed by basic hydrolysis to synthesize corresponding acid derivatives (**20**) in 82–88% yield following above protocol used for synthesis of **16** and **17** (Scheme 2). The target amides, **21** and **22**, were achieved in 68–74% yields, through reaction of **20** with 2-methylaminopyridine or *N*-methyl-*N*-butylamine using EDC and HOBt in dry DMF at room temperature.

Synthesized compounds were characterized using NMR, IR and mass spectrometry. The trans orientation of two aryl groups present at C-3 and C-4 of 3,4-diaryl-3,4-dihydrobenzopyran core was ascertain with the help of coupling constants (J values). The

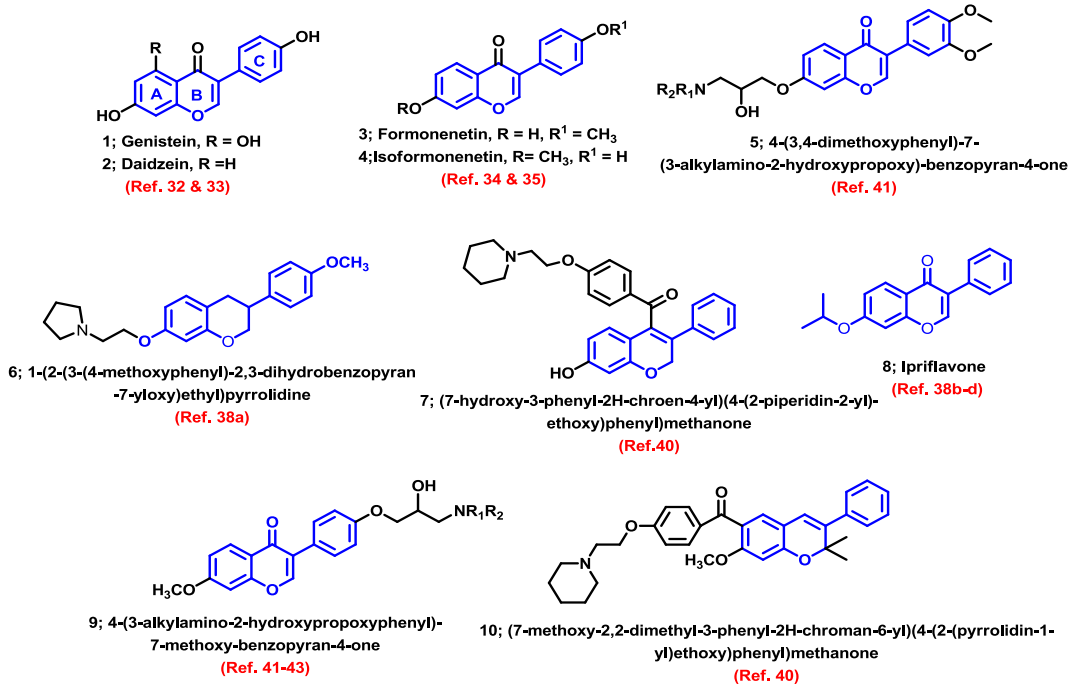


**Fig. 1.** Some 3-arylbenzopyran based osteoprotective agents.