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3-Piperidylethoxypterocarpan: A potential bone anabolic agent that improves bone quality and restores trabecular micro-architecture in ovariectomized osteopenic rats

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ABSTRACT

A series of new 6*H*-benzofuro[*3*, *2-c*]chromenes (BFC, pterocarpans) with structure-activity relationships were investigated for their potential use in osteoporosis treatment. One of the BFCs 3-piperidylethoxypterocarpan **20** promotes osteoblast differentiation and mineralization at a dose as low as 1 pM via activation of ER/P38MAPK/BMP-2 pathway. When evaluated for *in-vivo* osteogenic activity in female Sprague-Dawley rats, BFC **20** increased bone mineral density and new bone formation, compared with control at 1.0 and 10.0 mg/kg/body weight by oral gavage for 30 days. The compound was devoid of any uterotrophic effect and led to the new bone formation in adult ovariectomized osteopenic rats. BFC **20** compound also inhibited bone resorption by reducing Ovx induced increase in urinary CTx, thus exhibiting both bone anabolic and anti-catabolic action. Finally, BFC **20** treatment to Ovx rats led to improved trabecular microarchitectural restoration and exhibited therapeutic potential as a dual acting anti-osteoporotic agent for the management of osteoporosis.

1. Introduction

Osteoporosis is characterized by low bone mass and microarchitectural bone tissue deterioration with increased risk of fractures (Canalis, 2013; Chen and Sambrook, 2012; Consensus development conference, 1991.). Prevalence of osteoporosis is on the rise in both the developed and the developing countries. There are an estimated 200 million osteoporotic women worldwide (Facts and statistics, osteoporosis general, 2017). Osteoporosis is more prevalent in postmenopausal women due to estrogen deficiency. Hyperparathyroidism and vitamin D deficiency remain the other causes of osteoporosis in men and premenopausal women. Bone is composed of osteoblasts and osteocytes which are the bone forming cells and osteoclasts which are the bone resorbing cells. When there is an imbalance between the bone resorption and bone formation, the risk of osteoporosis sets in (Tabatabaei-Malazy et al., 2017).

Pharmacological agents for the treatment of osteoporosis are classified in two categories anti-resorptive and anabolic agents. These include bisphosphonates (BPs) which are recommended as first-line treatment of osteoporosis (Tabatabaei-Malazy et al., 2017). BPs inhibit bone resorption by accelerating osteoclast apoptosis but

Abbreviations: BFC, 6*H*-benzofuro[*3,2-c*] chromenes; BMD, bone mineral density; MAR, mineral apposition rate; BFR, bone formation rate; SERM, selective estrogen receptor modulators; PTH, parathyroid hormone; MAPK, mitogen-activated protein kinases; ALP, alkaline phosphatase; OVx, ovariectomized; TFSP, tibio-fibula separating point; μ-CT, micro computed tomography; B.Ar, cortical mean cross section; BV/TV, percentage bone volume; Cs.Th., cross-sectional cortical thickness; T.Ar, periosteal area; T.Pm, periosteal perimeter; BMCs, Bone marrow cells; ER, Estrogen Receptor; BMP-2, Bone Morphogenetic Protein; Tb. Pf, Trabecular pattern factor; Tb. Sp, Trabecular separation; SMI, Structure model index; DA, Degree of anisotropy.

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are associated with side effects like jaw osteonecrosis, arterial fibrillation and acute renal failure (Salari and Abdollahi, 2012; Aynardi and Ilyas, 2013; Reid, 2013). Denosumab, a human monoclonal antibody, is another approved anti-resorptive agent which targets RANKL and thereby leads to osteoclast inactivation and apoptosis. Side effects include serious infections, skin reaction and hypercholesterolemia (Facts and statistics, osteoporosis general, 2017, Rosen et al., 2017a,b,c). Hormone replacement therapy is also effective for prevention of osteoporosis in postmenopausal women but side effects include the risk of breast cancer, cardiac event, and stroke (Lindsay et al., 1980; Watts et al., 2010). Selective estrogen receptor modulators (SERMs) like raloxifene and bazedoxifene though effective anti-resorptives are again associated with several side effects (Salari et al., 2011; Rosen et al., 2017a,b,c). Parathyroid hormone (PTH) is the only FDA-approved osteoanabolic drug that promotes bone formation and reduces fracture risks but is not considered as the first line of therapy owing to the cost of PTH analogs and subcutaneous (SC) route of administration (Iniguez-Ariza and Clarke, 2015; Rosen et al., 2017a,b,c). Other upcoming anabolic treatments include anti-sclerostin antibodies like romosozumab and blosozumab which are directed against sclerostin, a wnt signaling antagonist (Cosman et al., 2016). The beneficial effects have been shown in osteoporosis and also in other conditions like rheumatoid arthritis, osteoarthritis and type 2 diabetes mellitus (MacNabb et al., 2016). Side effects include elevated liver enzymes and injection site reactions (Iniguez-Ariza and Clarke, 2015). Thus adequate therapy for osteoporosis is a challenge worldwide.

Nature plays an inevitable role in the voyage of drug discovery. Natural products such as sciadopitysin (Fig. 1) (Lee et al., 2006) and manglieside B (Kiem et al., 2008) have been isolated from traditional medicinal plants and studied for their bone-conserving effects by stimulation of alkaline phosphatase (ALP) activity. Psoralen, a furocoumarin natural product shows the stimulatory effect on the local new bone formation and promotes osteoblast differentiation through up-regulating the BMP-2 and BMP-4 genes (Tang et al., 2011).

Apart from these, isoflavones like genistein and daidzein (Fig. 1) attenuate the bone loss by increasing the bone mineral density (BMD) and bone mineral content (BMC) in ovariectomized (OVx) rats without any estrogenic action in the uterus. Based on these isoflavones, ipriflavone was synthesized and developed for the treatment of osteoporosis (Fig. 1) (Tsuda et al., 1986). There are reports that ipriflavone reduces bone loss in rats (Cecchini et al., 1997) and humans (Gennari et al., 1997). A 4-y multicenter trial, however, showed that ipriflavone had no effect on BMD in postmenopausal women (Alexandersen et al., 2001). Despite these conflicting observations, Ipriflavone is approved for the treatment of involutional osteoporosis in some European countries and in Japan with the trade names Iprosten, Osteofix, and Osten (Kelly et al., 2006).

During our drug development program on the management of osteoporosis and bone-related disorders, the extracts made from the stem bark of *Butea monosperma* showed stimulation of bone formation in ovariectomized rats (Goel et al., 2014; Maurya et al., 2009). Activity-guided analysis of the extracts revealed the presence of four methoxy isoflavonoids and a pterocarpan medicarpin, which were found to stimulate osteoblast functions via various mitogen-activated protein kinases (MAPK) but did not activate estrogen receptors (ERs) (Bhargavan et al., 2009). We have demonstrated that medicarpin exhibited *in vitro* (osteoblast mineralization and differentiation) and *in vivo* anti-osteoporotic activity (Bhargavan et al., 2012; Tyagi et al., 2012a,b; Goel et al., 2012, 2013, 2015). Based on the scaffolds known in the literature for anti-osteoporotic activity (Fig. 1), we rationally designed and synthesized a series of 6*H*-benzofuro[*3*, *2-c*]chromenes by incorporating different hydrophobic moieties. In this manuscript, we report a new dual bone anabolic and anti-catabolic agent 3-piperidylethoxy-pterocarpan, which increased bone mineral density, mineral apposition rate and bone formation rate in female Sprague-Dawley rats and restores trabecular bone microarchitecture in adult Ovx osteopenic rats. The detailed mechanism of action, structure-activity relationships and *in-vitro* and *in-vivo* studies are described.

2. Materials and methods

2.1. Chemicals and reagents

All fine chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Cell culture media and supplements were purchased from Invitrogen (Carlsbad, CA). All antibodies for western blot analysis were obtained from Cell Signaling Technologies (USA). ECL kit was purchased from Amersham Pharmacia. pGL3 2x ERE pS2-luc, pSG5 mERα and pSG5-hERβ were generous gifts from Prof. M. G. Parker, Imperial Cancer Research Fund, London, UK and pRL-luc was obtained from Promega, USA. Dual Luciferase Assay System was procured from Promega, USA.

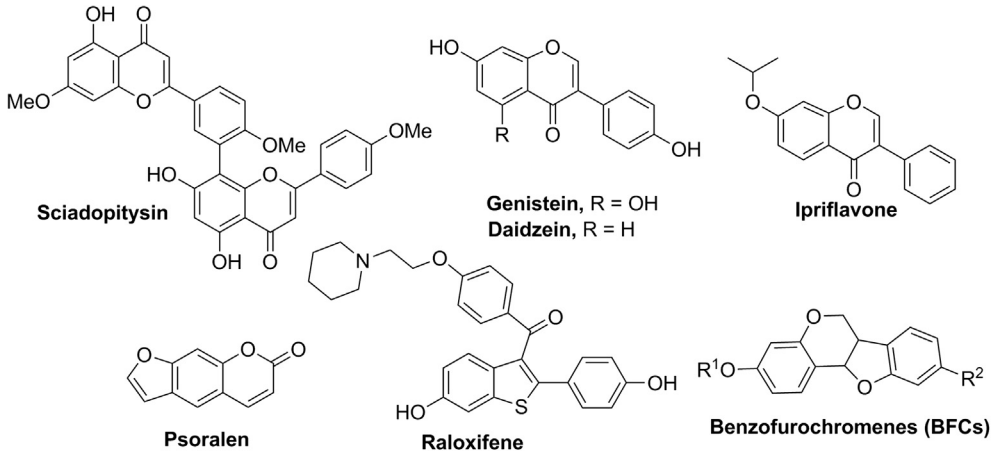

Fig. 1. Structures of anti-osteoporotic molecules.