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Review

Promoting osteoblast-mediated bone formation: a more promising approach for natural products to treat osteoporosis

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ABSTRACT

Osteoporosis is a systemic skeletal disorder characterized by reduced bone mass, compromised bone microstructure, and an increased risk of fractures, primarily due to excessive osteoclast-mediated bone resorption relative to osteoblast-mediated bone formation. While current anti-osteoporosis drugs, such as bisphosphonates and denosumab, predominantly focus on reducing bone resorption, osteoanabolic approaches are essential for restoring bone microarchitecture and ultimately reducing fracture risk. Traditional Chinese medicines (TCMs) and their active ingredients have long been used in China for osteoporosis prevention and treatment. This review provides a comprehensive evaluation of the effects and molecular mechanisms of 65 natural products across 24 categories on osteoblast-mediated bone formation. These compounds promote bone formation by regulating key transcription factors (RUNX2 and Osterix) and signaling pathways, including WNT/β-catenin, bone morphogenic protein (BMP), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), oxidative stress, autophagy, and epigenetic regulation. Notably, certain natural products [e.g., icariin (ICA)] exert their effects through multiple targets and pathways. Many of these natural products have demonstrated significant therapeutic efficacy in animal models, such as ovariectomized (OVX) mice. Our findings suggest that natural products with kidney-tonifying, anti-inflammatory, and antioxidant properties, as well as those inhibiting adipocyte differentiation, may hold promise for osteoporosis treatment. Additionally, we highlight current research gaps and propose future directions, including high-throughput screening and validation in diverse animal models, development of novel bone-targeting delivery systems, and identification of natural compounds targeting osteocytes.

1. Introduction

Bone tissue is a dynamic connective tissue composed of cells, fibers, and matrix, with its remodeling precisely governed by the balance between osteoblasts and osteoclasts [1]. Osteoblasts stimulate bone formation by producing essential matrix proteins, including type I collagen, osteonectin, osteocalcin, and alkaline phosphatase, which form the primary structural framework with hydroxyapatite. Osteoclasts, originating from monocytes, resorb bone under the influence of macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). This balance can be disrupted by various factors, such as estrogen deficiency post-menopause in females, aging, increased inflammatory factors, and oxidative stress. Upon the onset of this circumstance, osteoporosis develops (Fig. 1).

Osteoporosis is a systemic metabolic bone disease characterized by decreased overall bone mass, compromised bone microarchitecture, and increased fracture risk [2]. Osteoporosis (herein limited to primary osteoporosis) is mainly categorized into three types: type 1, postmenopausal osteoporosis, predominantly affects women beyond the age of 50; type 2, also known as senile osteoporosis, is prevalent among women aged over 65 and men over 70; and type 3, idiopathic juvenile osteoporosis [3]. Osteoporosis significantly increases the risk of fractures throughout the body, with hip and spinal fractures being the most common [4]. Fractures represent a significant public health burden, as they are a leading cause of morbidity, injury, decline in quality of life, and mortality [5]. Consequently, osteoporosis constitutes a global public health challenge. By 2025, the total burden of osteoporosis is projected to increase by 50%, with over 3 million fractures occurring annually in the United States, at an estimated cost of $ 25.3 billion [5]. Globally, 200 million people suffer from osteoporosis, resulting in 8.9 million fractures annually [6]. The prevalence rate of osteoporosis is 18.3%, with a higher incidence in women than in men (23.1% *vs* 11.7%) [7]. The cost of treating osteoporotic fractures in five European countries (France, Germany, the UK, Italy, and Spain) is € 29 billion, and 38.7 billion across all 27 EU member states. This cost is expected to rise by 25% by 2025 [8]. With the rapid increase in the aging population, the global challenge of osteoporosis will further intensify.

Currently, therapeutic approaches for osteoporosis are

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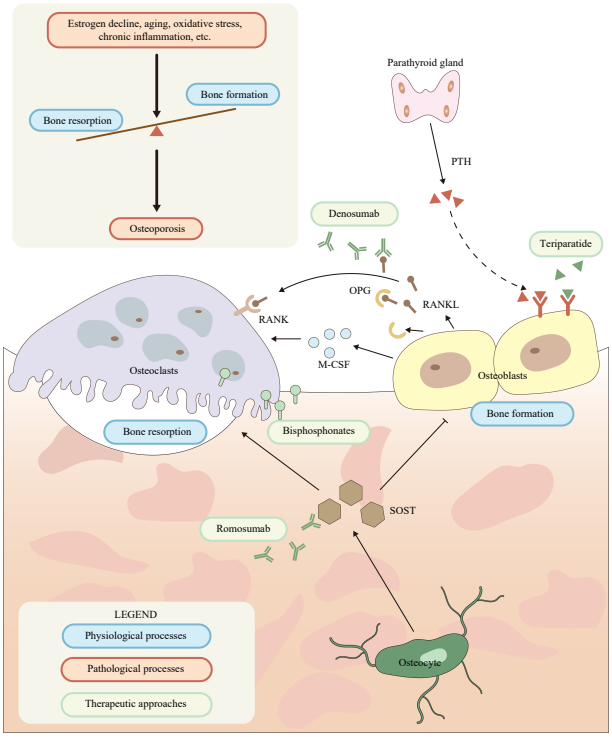

Fig. 1 Bone remodeling, osteoporosis, and therapeutic treatments currently available. Symbols: continuous lines with pointed arrowheads indicate molecule production or process upregulation; continuous lines with blunt arrowheads indicate process downregulation; dashed lines with pointed arrowheads indicate an intermittent stimulation causing process upregulation.

primarily divided into two types: attenuating bone resorption and stimulating bone formation. Fig. 1 exemplifies the main therapeutic approaches currently available and their corresponding targets. Therapeutic agents for inhibiting bone resorption encompass three main categories: bisphosphonates, denosumab, and estrogen receptor agonists. Bisphosphonates and denosumab are the most widely used anti-osteoporosis drugs, having prevented millions of fractures in the past decades [9, 10]. However, these medications may elicit adverse reactions to varying degrees. Approximately 20%–30% of patients taking oral bisphosphonates experience upper gastrointestinal symptoms, while intravenous administration may lead to fever and musculoskeletal pain. Additionally, these drugs can induce hypocalcemia in patients with severe vitamin D deficiency [11, 12]. Denosumab generally has fewer side effects, yet a notable concern is the risk of vertebral fractures upon discontinuation. In the extension phase of a Phase 3 study, 15% of women who ceased denosumab experienced subsequent vertebral fractures, with two-thirds encountering multiple fractures [13]. Estrogen receptor agonists raise concerns about breast, endometrial, and colorectal cancers, as well as coronary heart disease, stroke, and thromboembolism [14, 15]. Medications that promote bone formation mainly consist of parathyroid hormone type 1 receptor agonists (teriparatide and abaloparatide) and sclerostin antibodies (romosumab). Although the short-term effect is outstanding, the long-term use of teriparatide may require repeated injections and cause adverse effects such as nausea, headaches, and transient hypercalcemia. Romosumab is the only approved drug that simultaneously increases bone formation and decreases bone resorption, but it is currently reserved for patients at high risk of fractures, and the long-term safety profile remains unclear [16, 17]. It is increasingly recognized that only osteoanabolic approaches might restore bone microstructure and ultimately reduce fracture risk [18]. Therefore, there is an urgent need to develop novel and effective drugs that promote bone formation. Natural products, especially the main active ingredients of TCM, have long been used in China for osteoporosis prevention and treatment and are emerging as promising candidates.

2. Traditional Chinese medicines, osteoporosis, and osteoblast-mediated bone formation

Traditional Chinese medicine (TCM) formulas typically consist of two or more herbs, and their therapeutic principle hinges on the synergistic effects arising from complex herb interactions, which amplify therapeutic efficacy and mitigate potential side-effects of individual herbs [19]. In China, dozens of TCM formulas are commonly used for osteoporosis prevention and treatment, demonstrating significant clinical efficacy [20, 21]. For instance, Er Xian Decoction (EXD) is formulated with *Curculigo orchioides*, *Epimedium brevicornu*, *Phellodendron chinense*, *Morinda officinalis*, *Angelica sinensis*, and *Anemarrhena asphodeloides*. In a 12-week treatment of 35 postmenopausal osteoporotic patients, EXD markedly raises bone mineral density (BMD) and the serum levels of alkaline phosphatase (ALP), calcitonin, estradiol, and osteocalcin (OCN), while also relieving pain [21]. Similarly, Xianling-Gubao Capsules (XLGB), formulated with *Epimedium brevicornu*, *Dipsacus asper*, *Anemarrhena asphodeloides*, *Salvia miltiorrhiza*, *Rehmannia glutinosa*, and *Cullen corylifolium*, have been shown to effectively enhance femoral neck BMD and serum levels of ALP and OCN in postmenopausal osteoporotic patients when combined with calcium, according to a network meta-analysis by Luo et al. [22].

Some researchers are attempting to elucidate the potential mechanisms of TCM formulas in osteoporosis treatments. An early study by Wei et al. demonstrated that Bugu-Shengsui Decoction (BGSSD) significantly improves bone metabolism, BMD, bone morphology, and biomechanics in orchiectomized rats. Mechanistically, BGSSD promotes the proliferation and differentiation of osteoblasts through the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway [23]. Another study by Dai et al. reported that Si-Jun-Zi Decoction (SJZD) can ameliorate bone loss in diabetic mice partially *via* upregulating RUNX2 and activating the WNT/β-catenin signaling pathway [24]. Recently, researchers have shown that Jiangu Granules, another TCM formula, can alleviate postmenopausal osteoporosis by elevating the levels of osteogenic markers COL1, OCN, osterix (OSX), and RUNX2 [25]. Accumulating evidence suggests that many TCM formulas exert anti-osteoporotic effects against osteoporosis *via* activating the osteoblast-mediated bone formation. Identifying the efficacy and potential molecular mechanisms of active TCM compounds can aid in safe medication use and drug discovery. Thus, this review aims to examine the effects and molecular mechanisms of natural products, particularly TCM active ingredients, on osteoblast-mediated bone formation and explore their application prospects.

3. Osteoblast-mediated bone formation: transcription factors, bone-specific matrix proteins, and related signaling pathways

Osteoblasts originate from mesenchymal stem cells (MSCs) in the bone marrow, which can also differentiate into chondrocytes and adipocytes. The schematic of osteoblast differentiation is depicted in Fig. 2. The differentiation of MSCs into osteoblasts is primarily driven by RUNX family transcription factor 2 (RUNX2), the master regulator of osteogenesis. Inactivating mutations in RUNX2 can completely inhibit osteoblast differentiation [26]. Osterix (OSX/SP7), a downstream transcription factor of RUNX2, is crucial for osteoblast differentiation; its inactivation halts this process [27]. RUNX2 and osterix induce the differentiation of osteoblast precursor cells into preosteoblasts. During this stage, cells express type I collagen (COL1), synthesize a collagen-rich extra-

cellular matrix (ECM), and induce the expression of tissue non-specific alkaline phosphatase (ALP) to initiate bone mineralization. As preosteoblasts differentiate into mature osteoblasts, the genes *Bglap1* (bone gamma carboxyglutamate protein 1), *Bglap2*, and *Bglap3* are expressed, leading to the production and secretion of OCN. OCN is a highly abundant protein in the ECM and serves as a terminal phenotypic marker for osteogenesis. Some mature osteoblasts undergo apoptosis, while others integrate into the bone matrix and further differentiate into osteocytes [28].

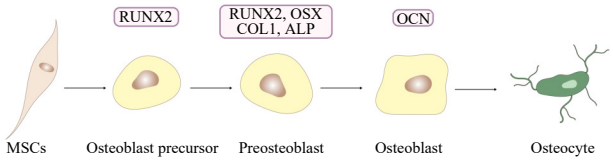

Fig. 2 The schematic of osteoblast differentiation. Important transcription factors and representative phenotypic markers are denoted at specific stages.

Bone formation is governed by transcription factors and key signaling pathways, including WNT/*β*-catenin, bone morphogenic protein (BMP), mitogen-activated protein kinase (MAPK), PI3K/AKT, oxidative stress, autophagy, and epigenetics, which collectively modulate osteoblast proliferation, differentiation, and mineralization (Fig. 3). Natural products that promote bone formation will be introduced based on the relevant pathways (Tables S1–S9).

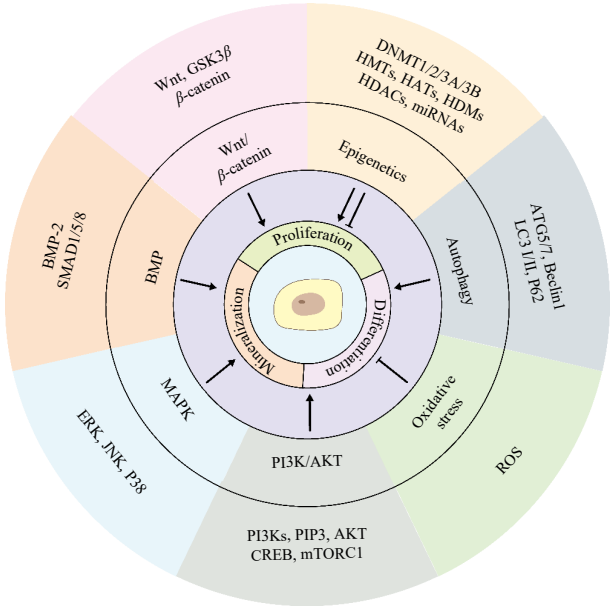

Fig. 3 The integrated schematic of the signaling pathways participating in osteoblast-mediated bone formation. The important pathways and key molecules are displayed, respectively. The proliferation, differentiation, and mineralization of osteoblasts are upregulated (pointed arrowheads), and/or downregulated (blunt arrowheads) through above pathways and key molecules, ultimately affecting bone formation.

4. Modulatory effects of natural products on transcription factors

Icariin (ICA), a flavonoid isolated from *Epimedium brevicornu*, is used as a tonic in traditional Chinese medicine. When ICA is used to treat rat calvarial osteoblasts and osteoclasts at concentrations of 0.1, 1, and 10 μmol·L^{-1}, it increases the expression of *Runx2* and *Sp7* mRNA in osteoblasts, while decreasing the expression of c-Jun *N*-terminal kinase (*JNK*) and p38 kinase (*p38*) in osteoclasts. Treating OVX rats with 40 mg·kg^{-1}·d^{-1} of ICA for 90 d significantly increases bone mass and reverses trabecular bone structural abnormalities. Subsequent transcriptome and proteome sequencing of rat femurs reveals associations with bone remodeling, energy metabolism, cytoskeleton, lipid metabolism, mitogen-activated protein kinase (MAPK) signaling, and Ca^{2+} signaling [29]. Another research by Liu and Li et al. demonstrates that ICA (0.01 μmol·L^{-1}) markedly counteracts lipopolysaccharide (LPS)-induced bone loss by suppressing the miR-34c expression and then relieving its targeting effect on RUNX2 in rat BMSCs [30].

Calycosin, formononetin, and calycosin-G, three extracts of *Astragalus membranaceus*, boost osteoblast activity and proliferation. Treating rat calvarial osteoblast precursors with concentrations ranging from 10^{-10} to 10^{-4} mol·L^{-1} of these extracts increases expression of ALP, RUNX2, and bone morphogenetic protein 2 (BMP-2) in cells, elevates COL1 and OCN levels in the culture medium, and promotes calcium nodule formation, with the most pronounced effects at 10^{-5} mol·L^{-1} [31]. Cycloastragenol (CAG), another triterpenoid saponin compound extracted from *Astragalus membranaceus*, administered at 50 μmol·L^{-1} in MC3T3-E1 cells, enhances osteogenic markers including RUNX2, ALP, OCN, osteopontin (OPN), and COL1, stimulates ALP activity, and promotes osteogenic mineralization. CAG also reverses dexamethasone-induced osteogenic inhibition in a telomerase-dependent manner, as evidenced by the inhibitory effect of a telomerase inhibitor (5 μmol·L^{-1}). In a zebrafish larval model of osteoporosis induced by 20 μmol·L^{-1} dexamethasone, CAG (25–100 μmol·L^{-1}) significantly alleviates the osteoporotic phenotype, characterized by reduced osteogenic mineralization [32].

Lycii Radicis Cortex (LRC, *Lycium barbarum*) and *Achyrantes japonica* (AJ) have been widely used as traditional medicine in eastern Asia. A combined extract of LRC and AJ (LRC∶AJ = 8∶2, 10 μg·mL^{-1}) remarkably enhances the expression of RUNX2, ALP, and OCN, as well as the mineralized nodule formation in MC3T3-E1 cells, while suppressing osteoclast differentiation in bone marrow macrophage cells (BMMs). Moreover, co-treating OVX mice with LRC and AJ extracts (at doses of 150 and 300 mg·kg^{-1}·d^{-1} for 12 weeks) improves the bone mineral density (BMD) and the trabecular bone structural properties, and elevates serum osteoprotegerin (OPG)/RANKL levels [33]. Kukoamine B (KB), isolated from LRC, promotes ALP activity and matrix mineralization and increases osteogenic marker expression (ALP, OSX, OCN) in MC3T3-E1 cells (20 μmol·L^{-1}). Additionally, KB treatment significantly decreases tartrate-resistant acid phosphatase (TRAP) activity and TRAP-positive osteoclasts in BMMs. *In vivo*, treatment of OVX mice with KB (5 mg·kg^{-1}·d^{-1} for 12 weeks) markedly alleviates bone loss and restores the trabecular microarchitecture [34]. Scopolin, a coumarin extracted from LRC, also exhibits the bone protective effects by promoting osteogenesis, diminishing osteoclastogenesis, as well as ameliorating the OVX-induced bone loss [35].

Loganic acid (LA), a terpenoid extracted from the root of *Gentiana lutea*, significantly enhances ALP activity and promotes the expression of *Alp*, *Bglap*, and *Sp7* in MC3T3-E1 cells at 50 μmol·L^{-1}. Additionally, treatment with LA inhibits the TRAP activity and osteoclast differentiation in BMMs. In OVX mice, treatment with LA (2, 10, and 50 mg·kg^{-1}·d^{-1}) for 12 weeks markedly reverses OVX-induced decreases in BMD and trabecular properties [36].

Cuscutae Semen polysaccharide (CSP) is a polysaccharide extracted from the seed of *Cuscuta chinensis*. In OVX rats, oral administration of CSP (400 mg·kg^{-1}·d^{-1}) for 12 weeks can reduce weight gain, increase BMD, mitigate trabecular bone loss, and modulate serum levels of key bone metabolic biomarkers. Specifically, CSP increases serum levels of insulin-like growth factor (IGF), transforming growth factor β (TGF-β), OCN, and OPG, while decreasing TRAP and *C*-terminal telopeptide of type I collagen (CTX). Mechanistically, the osteoprotective effects of CSP are mediated by elevating the osteoblast-related mRNA markers, in-

cluding *Runx2*, *Sp7*, *BMP-2*, and *Smad5*, while diminishing the expression of osteoclast markers such as proto-oncogene *c-Fos*, cathepsin K (*CTSK*), *TRAP*, and nuclear factor of activated T cells 1 (*NFATc1*) [37].

5. WNT/*β*-catenin signaling pathway and natural products

5.1. WNT/*β*-catenin signaling pathway and bone formation

The WNT signaling pathway plays a crucial role in the development and maintenance of various organs and tissues, particularly the skeletal system. When the WNT signaling pathway is inactive, cytoplasmic *β*-catenin is phosphorylated by the destruction complex (DC) and subsequently degraded *via* the ubiquitin/proteasome pathway. The DC primarily consists of three components: adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3β (GSK3β). APC and axin form a protein scaffold that facilitates the binding of GSK3β to *β*-catenin, promoting its phosphorylation. The phosphorylated *β*-catenin is then degraded through the ubiquitin/proteasome pathway mediated by *β*-transducin repeats-containing protein (*β*-TrCP). Activation of the WNT/*β*-catenin signaling pathway occurs when WNT ligands bind to the frizzled receptor and its co-receptor, low-density lipoprotein receptor-related protein 5/6 (LRP5/6), on the cell membrane. This binding event disrupts the destruction complex (DC), inhibiting the activity of GSK3β and preventing *β*-catenin phosphorylation. Consequently, *β*-catenin accumulates in the cytoplasm and translocates into the nucleus, where it binds to the T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to promote the expression of downstream genes [38]. This cascade upregulates the osteogenic transcription factor RUNX2, which in turn drives the expression of *Alp*, *Ocn*, and *Col1*. Additionally, *β*-catenin boosts *Sp7* expression, facilitating the maturation of precursor osteoblasts into fully differentiated osteoblasts [39]. It has been reported that some TCM formulas for treating osteoporosis, such as Guhong Injection, promote the proliferation and differentiation of osteoblasts by activating the WNT/*β*-catenin signaling pathway [40].

5.2. Modulatory effects of natural products on the WNT/β-catenin pathway

2,4,5-Trimethoxyldalbergiquinol (TMDQ), extracted from *Dalbergia odorifera*, exhibits osteoprotective properties by enhancing ALP activity, mineralized nodule formation, and the expression of osteogenic markers, including *Alp*, *Ocn*, *Opn*, bone sialoprotein (*Bsp*), and *Runx2*, in mouse calvarial osteoblasts at concentrations of 0.1 and 1 μmol·L^{-1}. Further investigations reveal that TMDQ stimulates osteoblast differentiation by activating the Wnt/*β*-catenin and BMP/Smad1/5/8 pathways [41].

A detailed study by Hu et al. shows that ICA exerts a significant protective effect against glucocorticoid-induced osteoporosis in both SaoS-2 cells and murine models. Mechanistically, ICA mitigates glucocorticoid-induced osteoporosis by upregulating the expression of bone enhancer DEC1 and by activating the GSK3β/*β*-catenin and PI3K/Akt signaling pathways [42].

Hymenialdisine (HMD), a kinase inhibitor derived from marine sponges, inhibits RANKL-induced osteoclastogenesis and bone resorption *via* blocking the NF-κB and MAPK pathways. Additionally, HMD stimulates osteoblast differentiation by activating ALP activity, matrix mineralization, and the expression of osteogenic markers (*Col1*, *Ocn*, *Opg*, *Runx2*) through activation of the GSK-3β/*β*-catenin/TCF/LEF pathway. Furthermore, HMD has been shown to attenuate bone loss induced by estrogen deficiency in OVX mice [43].

Hydroxychavicol (HCV), derived from *Piper betle*, significantly enhances ALP activity and mineralization in C3H10T1/2 cells at a concentration of 2000 ng·mL^{-1}, while upregulating the expression of RUNX2 and OPN. Additionally, HCV markedly improves bone mass and trabecular microarchitecture in glucocorticoid-induced osteoporosis (GIO) rats *via* the GSK-3β/*β*-catenin pathway [44].

Paeonoside (PASI), isolated from *Paeonia × suffruticosa*, enhances osteogenic mineralization and ALP activity in MC3T3-E1 cells at 30 μmol·L^{-1}. PASI upregulates the protein expression of WNT3a, *β*-catenin, p-GSK3β, RUNX2, p-SMAD1/5/8, and BMP-2. The osteogenic-promoting effects are attenuated by Noggin (a BMP-2 inhibitor) and PFK118-310 (a WNT3a inhibitor), suggesting that the osteogenic effects of PASI are largely mediated through the WNT/*β*-catenin and BMP-2/p-SMAD1/5/8 pathways [45].

Catalpol, derived from *Rehmannia glutinosa*, stimulates ALP activity and mineralization in rat BMSCs, as well as upregulates the expression of COL1 and RUNX2 *via* the Wnt/*β*-catenin signaling pathway. Further research has confirmed that catalpol accelerates bone healing in rat calvarial defect models and alleviates bone loss in OVX rats [46].

Geraniin, an ellagitannin obtained from *Phyllanthus amarus*, significantly promotes the proliferation and osteogenesis of BMSCs isolated from OVX rats. This effect is mediated by the upregulation of key genes involved in cell cycle progression and osteogenesis, including *c-myc*, *cyclin D1*, *Runx2*, *Sp7*, and components of the Wnt/*β*-catenin pathway (*β-catenin*, *Frizzled2*, *LRP6*, *TCF4*, *LEF1*), while downregulating *Axin2* [47].

Penicopeptide A (PPA), isolated from deep-sea fungi, promotes osteogenic differentiation and mineralization in BMSCs at concentrations of 10 and 20 μmol·L^{-1}, while simultaneously inhibiting adipocyte differentiation. Mechanistically, PPA directly binds to and phosphorylates protein kinase B (AKT) and GSK-3β, thereby increasing nuclear accumulation of *β*-catenin and enhancing bone formation. *In vivo* studies confirm the therapeutic potential of PPA in OVX mice [48].

Corylin, an active compound extracted from *Psoralea corylifolia*, exhibits anti-inflammatory, antioxidant, and pro-osteogenic properties. In rat osteoblasts, corylin treatment can enhance ALP activity and mineralization, and activate the transcription of key transcription factors RUNX2 and SP7. Further research have demonstrated that corylin promotes osteogenesis through the activation of the WNT/*β*-catenin and estrogen signaling pathways [49].

Albiflorin, extracted from *Paeonia lactiflora*, significantly enhances ALP activity, calcified nodule formation, and the expression of osteogenic markers (ALP, OCN, RUNX2, and OSX) in MC3T3-E1 cells *via* activating BMP-2/SMAD and WNT/*β*-catenin pathways. In a femur fracture model, albiflorin markedly accelerates bone healing and improves the morphological structure of the fracture site [50].

Agastache rugosa is a medicinal plant in Asia that exerts antioxidant, anti-inflammatory, anti-obesity, and osteoprotective properties. Administration of ethanol extract of *Agastache rugosa* (EEAR) attenuates ovariectomy-induced bone loss *via* stimulating osteoblast differentiation and improving the diversity of gut microbiota. Moreover, the therapeutic effect of EEAR is primarily mediated by the WNT, BMP, as well as TGF-β signaling pathways [51].

Bergamottin (BM), a coumarin compound derived from various citrus fruits, exhibits multiple biological activities such as anti-adipogenesis. Wang et al. note that BM significantly enhances osteoblast differentiation and mitigates OVX-induced bone loss. Mechanistically, BM activates the WNT/*β*-catenin signaling pathway, leading to a marked upregulation of *β*-catenin and the transcriptional activators of TCF4, TCF7, and RUNX2, as well as promoting the nuclear translocation of *β*-catenin [52].

6. BMP signaling pathway and natural products

6.1. BMP signaling pathway and bone formation

Bone morphogenetic proteins (BMPs), a family comprising over 20 members, are renowned for their unique capacity to induce ectopic cartilage and bone formation *in vivo*. Upon binding of BMP ligands to their receptors, the heterotetrameric serine/threonine kinase receptors are phosphorylated and activated, subsequently promoting the phosphorylation of transcription factors SMAD1/5/8 and their interaction with SMAD4. This complex then translocates into the nucleus, where it activates the downstream transcription factors such as RUNX2 and Osterix, ultimately stimulating bone formation [53]. It has been reported that some TCM formulas for treating osteoporosis, such as Duhuo-Jisheng-Tang, promote osteogenic differentiation and delay the senescence of human MSCs by activating BMP-2 and SMAD1/5/8 signaling pathways [54].

6.2. Modulatory effects of natural products on the BMP pathway

7-HYB, a phenolic compound from the seeds of *Myristica fragrans*, significantly promotes osteogenic mineralization and upregulates the expression of RUNX2, ALP, p-GSK3β, and *β*-catenin in MC3T3-E1 cells at 30 μmol·L^{-1}. Additionally, 7-HYB activates the MAPK pathway by enhancing the phosphorylation of ERK, JNK, and P38. It also activates the BMP pathway by upregulating BMP-2 expression and the phosphorylation of SMAD1/5/8. Collectively, these mechanisms synergistically enhance osteogenesis [55].

Treatment of mouse calvarial osteoblasts with *Musa × paradisiaca* flower extracts K-131 and K-122 (100 pmol·L^{-1}) results in the upregulation of osteogenic markers (RUNX2, ALP, BMP-2, and COL1), thereby enhancing osteogenic mineralization and ALP activity. In OVX and osteotomy rats, administration of the butanolic fraction (25 and 50 mg·kg^{-1}·d^{-1} for 2 weeks) markedly promotes new bone regeneration, restores trabecular bone microarchitecture, and improves bone biomechanical strength [56].

Cinnamic acid (CA), derived from *Cinnamomum verum*, exhibits anti-obesity, anti-inflammatory, anti-diabetic, and anticancer properties. Treatment of MC3T3-E1 cells with CA significantly enhances ALP activity and calcium deposition, while upregulating key osteogenic markers, including RUNX2, OCN, OPG, OSX, and COL1. CA also mitigates ovariectomy-induced bone loss in mice by improving bone mineral density and trabecular bone architecture, as well as restoring gut microbiome diversity. Moreover, the above effects are associated with the activation of the BMP/TGF-β/Smad signaling pathway [57].

LBP and LBP1C-2 are bioactive components extracted from *Lycium barbarum*. Administration of these compounds can remarkably increase bone mass, improve bone trabecular structure, enhance mechanical strength, and elevate serum levels of the bone formation marker, propeptide of type I procollagen (PINP), in both OVX and aging mice. In human mesenchymal stem cells (hMSCs), treatment with LBP and LBP1C-2 facilitates osteogenic proliferation, differentiation, and mineralization. Mechanistically, LBP1C-2 promotes bone formation by blocking the interaction between noggin and BMPs and by activating the BMP/Smad signaling pathway [58].

Circaea mollis is a traditional herbal medicine utilized by the Hani ethnic group for its anti-arthritis properties. Its ethanol extract (EECM) significantly enhances ALP activity, promotes matrix mineralization, and upregulates the expression of key osteogenic markers, including RUNX2, OSX, OPN, OPG, and COL1. Administration of EECM to OVX mice for 12 weeks results in modest improvements in bone loss and marked elevations in the levels of osteogenic markers in primary cells derived from OVX mice. Further studies confirm that the osteoprotective effects of EECM are mediated through the activation of the BMP/SMAD signaling pathway [59].

Annatto-derived tocotrienol (AnTT) promotes the differentiation and mineralization of MC3T3-E1 cells by modulating the mevalonate pathway. Specifically, AnTT downregulates the mRNA expression of HMG-CoA reductase (*HMGR*), inhibits the activation of RhoA, and boosts the protein level of BMP-2 [60].

7. MAPK signaling pathway and natural products

7.1. MAPK signaling pathway and bone formation

Mitogen-activated protein kinases (MAPKs) are a family of evolutionarily conserved serine/threonine kinases that mediate cellular responses to various stimuli. The MAPK pathway comprises three main branches: extracellular signal-regulated kinase (ERK), JNK, and p38. In osteoblasts, the ERK pathway primarily involves ERK1 and ERK2, which are regulated by the upstream kinases MEK1 and MEK2. Further upstream, the RAS/RAF proteins or CDC42/MLK3 modulate these kinases. ERK1/2 activation is critical for early osteogenesis, where it upregulates RUNX2 expression, thereby driving the transcription of osteogenic genes. In later stages, ERK1/2 enhances the expression of RSK2 and ATF4, facilitating osteoblast maturation. The p38 MAPK pathway includes four subtypes: α (MAPK14), β (MAPK11), γ (MAPK12), and δ (MAPK13), with α and β being prominently expressed in osteoblasts. In the early stage of osteogenic differentiation, CDC42/MLK3 or TAK1 signals activate MKK3, which subsequently activates p38α, promoting the expression of key osteogenic transcription factors such as RUNX2 and OSX. Meanwhile, p38β, activated by MKK6, collaborates with p38α to support the maturation of osteoblasts in later stages. JNK1/2 is regulated by upstream MKK4/7, which in turn is regulated by various MAP3Ks. Its downstream targets include ATF2 and JunB. JNK1/2 positively regulates the expression of BSP, OCN, ATF4, and FRA1, and facilitates osteogenic mineralization [61].

7.2. Modulatory effects of natural products on the MAPK pathway

WIN-34B, an *n*-butanol fractionated mixture of *Lonicera japonica*, exhibits osteogenic and anti-osteoclastogenic properties. In mesenchymal stem cells (MSCs), WIN-34B dose-dependently enhances alkaline phosphatase (ALP) activity and osteogenic mineralization at concentrations of 1, 10, and 20 μg·mL^{-1}. In TRAP-stained BMMs, WIN-34B treatment significantly reduces the number of multinucleated cells, which indicates its inhibitory effect on osteoclastogenesis. In a co-culture system of hMSCs and BMMs, WIN-34B reverses the inflammatory factor-induced decrease in the *Opg*∶*Rankl* ratio and *Runx2* expression at concentrations of 1 and 10 mg·mL^{-1}. Furthermore, WIN-34B inhibits the expression of *IL-17*, *c-Fos*, and *TNF-α*, as well as the activation of NF-κB, IκBα, p38 MAPK, and JNK in a dose-dependent manner. These findings suggest that WIN-34B has the potential to promote osteogenesis and inhibit osteoclastogenesis in the inflammatory cytokine-induced bone marrow microenvironment [62].

Amentoflavone is a bioflavonoid isolated from TCMs, gingko and *Selaginella tamariscina*, and exerts anti-inflammatory, antioxidant, antiviral, and anticancer properties. In hBMSCs, amentoflavone treatment significantly enhances the proliferation, ALP activity, and mineralization, while upregulating the master transcription factors RUNX2 and OSX. Moreover, these osteogenic effects are mediated by the activation of the JNK and p38 MAPK sig-

naling pathways. In addition, *in vivo* studies demonstrate that amentoflavone mitigates dexamethasone (DEX)-induced inhibition of osteoblast differentiation in transgenic (sp7: egfp) zebrafish larvae [63].

Astragalin (AG), a bioactive flavonoid compound extracted from various medicinal plants, significantly enhances the ALP activity and mineralized nodule formation in MC3T3-E1 cells at concentrations of 5, 10, and 20 μmol·L^{-1}. AG also upregulates the mRNA and protein levels of osteoblastic markers such as OCN, OPN, and RUNX2, by activating the MAPK and BMP pathways. Furthermore, studies utilizing an OVX mouse model have demonstrated that AG effectively attenuates bone loss induced by estrogen deficiency [64].

Isoliquiritin (ISL), a flavonoid derived from *Glycyrrhiza glabra*, significantly enhances osteogenesis and angiogenesis in mouse BMSCs. Specifically, treatment with 25 μmol·L^{-1} ISL upregulates the expression of key osteogenic and angiogenic proteins, including COLI, RUNX2, OPN, and vascular endothelial growth factor A (VEGF-A), while also enhancing ALP activity and promoting osteoblast mineralization. The autophagy inhibitor inhibits ISL-induced osteogenesis, whereas the p38/ERK inhibitor simultaneously suppresses both autophagy and osteogenic effects elicited by ISL. These findings suggest that ISL activates the p38/ERK pathway, thereby triggering cellular autophagy and subsequently promoting osteogenesis. Additionally, treating OVX mice with ISL therapy (administered orally at doses of 10, 30, and 50 mg·kg^{-1}·d^{-1} for one month) significantly increases BMD and trabecular bone parameters, enhances the expression of osteogenic marker RUNX2, decreases the number of osteoclasts, and alleviates weight gain [65].

Treatment of MC3T3-E1 cells with yuja (*Citrus junos*) peel ethanol extract (YPEE) can promote ALP activity, mineralization, and the expression of osteogenic markers, including RUNX2, OCN, and COL1. As for the molecular mechanism, YPEE stimulates osteoblast differentiation *via* the activation of the BMP2-p38-SMAD1/5/8 pathway. Meanwhile, YPEE has been shown to attenuate the formation and number of TRAP-positive multinucleated cells in RANKL-induced RAW264.7 cells. Moreover, supplementation of YPEE significantly reduces body weight, increases uterine weight, enhances BMD, and improves the trabecular parameters in OVX rats [66].

8. PI3K/AKT signaling pathway and natural products

8.1. PI3K/AKT signaling pathway and bone formation

Phosphatidylinositide 3-kinases (PI3Ks) constitute a family of intracellular phosphoinositide kinases that function as dimeric complexes composed of catalytic and regulatory subunits. In normal cells, PI3Ks are activated by growth factors, which phosphorylate the substrate PIP2 to produce PIP3. The lipid phosphatase PTEN can dephosphorylate PIP3, thereby inhibiting the activation of the PI3K pathway. PIP3 recruits AKT/PKB and PDK1 to the cell membrane, leading to phosphorylation of AKT protein at serine 308 (S308) by PDK1, resulting in partial activation of AKT. Activated AKT subsequently promotes the phosphorylation of the transcription factor CREB, thereby facilitating the expression of downstream target genes. Additionally, AKT can indirectly enhance mTORC1 expression, which promotes osteogenic differentiation [67]. In various cells, including osteoblasts, activated AKT stimulates the expression of RUNX2, thereby initiating the expression of downstream osteogenic genes such as *Sp7* [68]. Certain TCM formulas used for treating osteoporosis, such as Bugu-Shengsui Decoction and Zhuanggu-Busui Formula, have been reported to promote bone formation *via* the PI3K/AKT signaling pathway [23,69].

8.2. Modulatory effects of natural products on PI3K/AKT pathway

Si-Wu-Tang (SWT) is a TCM formula comprised of four herbs: *Paeonia lactiflora*, *Angelica sinensis*, *Ligusticum chuanxiong*, and *Rehmannia glutinosa*. SWT extract enhances the expression of *Alp*, *Bmp-2*, and *Opn*, as well as mineralized nodule formation in MC3T3-E1 cells. The PI3K/AKT and NF-κB signaling pathways are likely involved in SWT-mediated osteoprotective effects. Moreover, treatment of OVX mice with SWT extract prevents the bone loss, elevates serum levels of the bone formation indicators ALP, BMP-2, and OPN, and decreases the bone resorption marker CTX-1 [70].

Asperosaponin VI (ASA VI), a triterpenoid saponin, is identified as the primary bioactive constituent in the dried root of *Dipsacus asper*, a traditional kidney-tonifying herbal. ASA VI (10 μmol·L^{-1}) significantly enhances the proliferation, ALP activity, and calcified nodule formation in BMSCs derived from OVX rats. Furthermore, ASA VI elevates the expression of osteogenic markers, including *Alp*, *Ocn*, *Col1*, and *Runx2*. All of these osteogenic effects are mediated through the activation of the PI3K/AKT signaling pathway [71].

Icaritin (ICT), a flavonoid isolated from both *Epimedium brevicornu* and identified among its serum metabolites after oral administration, inhibits adipogenic differentiation while promoting osteogenesis in BMSCs. In a study, ICT is co-administered with a bone-targeting delivery vehicle (lipid + ASP8 + icaritin) to OVX mice (8 mg·kg^{-1}, 3 times/week, for 6 weeks). The following results, a decrease in serum bone resorption marker CTX, an increase in serum bone formation marker P1NP, and improvements in BMD, trabecular architecture, mineral apposition rate (MAR), ratio of mineral surface area (MS/BS), bone formation rate (BFR), and ratio of osteoid surface (OS/BS), indicating icaritin prevents the OVX-induced osteoporosis in mice *via* promoting new bone formation. *In vitro*, treatment of 3T3-L1 cells with icaritin (10 and 20 μmol·L^{-1}) results in decreased expression of key adipogenic mediators, including Ccaat/enhancer-binding protein α (CEBPα), peroxisome proliferator-activated receptor γ (PPARG), fatty acid-binding protein 4 (FABP4), and lipoprotein lipase (LPL), while increased phosphorylation levels of AKT and GSK-3β, and enhanced distribution of *β*-catenin in cytoplasm and nucleus. These results suggest that ICT suppresses adipogenesis through the AKT/GSK-3β/*β*-catenin pathway, ultimately promoting osteogenic differentiation and bone formation [72].

Morroniside, an active ingredient from *Cornus officinalis*, enhances osteogenesis in MC3T3-E1 cells *via* upregulating the expression of osteogenic markers (*Ocn*, *Runx2*, *Alp*, *Sp7*) and activating the PI3K/AKT/mTOR signaling pathway. *In vivo*, treatment of OVX mice with morroniside (2 or 10 mg·kg^{-1}·d^{-1} for 12 weeks) significantly improves bone loss and trabecular structure disruption [73].

Chuanxiong (CX), also known as the rhizome of *Ligusticum chuanxiong*, is a traditional Chinese medicine utilized for the treatment of cardiovascular and cerebrovascular diseases. Treatment of H_2O_2-induced MG63 cells with CX ethanol extract (CXE) enhances both cell viability and ALP activity. The increase in osteogenic activity is due to the reduction of cellular reactive oxygen species (ROS) levels and the inhibition of osteoblast apoptosis *via* the PI3K/AKT signaling pathway. Additionally, treatment of high fat-sucrose (HFS)-fed OVX rats with CXE (600 mg·kg^{-1}·d^{-1} for 12 weeks) significantly improves the trabecular BMD and trabecular separation [74].

9. Oxidative stress-mediated pathways and natural products

9.1. Oxidative stress-mediated pathways and bone formation

Oxidative stress is characterized by an imbalance between

the excessive production of reactive oxygen species (ROS) and the relatively insufficient capacity of cellular antioxidant defense mechanisms. Free radical molecules, such as superoxide (O^{2-}), trigger a cascade of reactions that damage cellular structures, including DNA, proteins, and cell membrane lipids. Oxidative stress has been implicated in promoting osteoclastogenesis, impairing the differentiation and activity of osteoblasts, and inducing apoptosis in osteoblasts and osteocytes. The increased osteoclastogenesis associated with oxidative stress is attributed to the upregulation of RANKL and the downregulation of OPG. The detrimental effects of oxidative stress on osteogenesis are evident through the reduced expression of differentiation markers, including ALP, COL1, and RUNX2. As for osteocytes, oxidative stress is able to trigger cellular apoptosis and upregulate the Wnt inhibitors sclerostin and dickkopf-1 (DKK1), leading to the activation of osteoclastogenesis and inhibition of osteogenesis. Collectively, these events contribute to bone loss and the development of osteoporosis. Additionally, aging and estrogen deficiency have been shown to increase ROS levels, and substantial evidence supports the notion that antioxidants can mitigate bone damage caused by estrogen decline [75-77]. In recent years, some TCMs used for treating osteoporosis, such as osteoking, have been demonstrated to exert their therapeutic effects by reducing reactive oxygen species [78].

9.2. Modulatory effects of natural products on oxidative stress-mediated pathway

Jing et al. report that luteolin promotes the proliferation of dexamethasone (DXM)-treated MC3T3-E1 cells by attenuating oxidative stress and stimulates osteoblastic differentiation *via* activating the ERK/LRP-5/GSK-3β pathway in glucocorticoid-induced osteoporosis (GIO) rats [79]. In another work, Phromnoi et al. investigated the effects of luteolin and a baicalein-rich hexane fraction from *Perilla frutescens* leaf (PLH) on osteoporosis. Treating RAW264.7 cells with PLH (12.5, 25, 50 μg·mL^{-1}) effectively reduces the production of ROS and the formation of TRAP-positive multinucleated osteoclasts induced by RANKL in a dose-dependent manner. Moreover, PLH remarkably downregulates the RANKL-induced MAPK and NF-κB signaling pathways and attenuates the expression of osteoclastogenic markers NFATc1 and MMP-9. In contrast, PLH promotes osteoblast function in two osteoblastic cell lines (MG-63 and SAOS-2) by upregulating ALP activity and reversing TNF-α-mediated suppression of osteoblast proliferation and differentiation [80].

Salidroside, a phenylpropanoid glycoside extracted from *Rhodiola rosea*, possesses potent antioxidant properties. Zhang et al. demonstrated that salidroside significantly elevates the cell survival, ALP activity, calcium deposition, and the mRNA levels of *Alp*, *Col1*, and *Ocn* in H_2O_2-treated MC3T3-E1. Additionally, salidroside dramatically decreases intracellular ROS production and the levels of osteoclast differentiation inducers RANKL and IL-6, both of which are induced by H_2O_2. *In vivo*, salidroside supplementation for 15 weeks remarkably improves trabecular bone microarchitecture and BMD in the 4th lumbar vertebrae and distal femur of OVX mice, while also attenuating oxidative stress in serum [81].

Ginsenoside Rb2 (Rb2), a 20(*S*)-protopanaxadiol glycoside extracted from *Panax ginseng*, is a potent antioxidant. Huang et al. demonstrated that the anti-osteoporosis effects of Rb2 are attributed to its ability to reduce oxidative damage and suppress bone-resorbing cytokines. In MC3T3-E1 cells, Rb2 is able to enhance proliferation, ALP activity, mineralization, and the mRNA levels of *Alp*, *Col1*, *Ocn*, and *Opn* under H_2O_2-induced oxidative stress. Moreover, Rb2 effectively reduces the levels of RANKL and IL-6 and inhibits the production of ROS induced by H_2O_2. *In vivo*, Rb2 administration significantly improves trabecular bone microarchitecture and increases BMD in the lumbar vertebrae and distal femur of OVX mice [82].

Gastrodin, a glucoside isolated from the traditional Chinese herbal agent *Gastrodia elata*, exhibits potent antioxidant properties. Gastrodin significantly attenuates H_2O_2-induced dysfunction in hBMSCs, enhances their proliferation and osteogenic differentiation, and reduces lipid accumulation, adipogenic differentiation, and ROS levels. Additionally, gastrodin markedly decreases the number of TRAP-positive multinucleated osteoclasts and the expression of osteoclast-specific genes, as well as ROS levels in RAW264.7 cells. *In vivo*, gastrodin treatment dramatically reduces the activity of serum bone degradation markers, such as CTX-1 and TRAP, and the number of TRAP-positive osteoclasts, while improving trabecular bone microarchitecture and promoting bone formation in OVX mice [83].

Ophiopogonin D (OP-D) is a steroidal saponin extracted from the radix of *Ophiopogon japonicus* and has anti-oxidative activity. OP-D significantly attenuates H_2O_2-induced dysfunction in osteogenesis, promotes the proliferation and osteogenic differentiation of MC3T3-E1, while decreasing ROS levels. Moreover, OP-D markedly decreases the number of TRAP-positive multinucleated osteoclasts, the expression of osteoclast-specific genes, and ROS levels in RAW264.7 cells. *In vivo*, OP-D treatment dramatically reduces the activity of serum bone degradation markers, such as CTX-1 and TRAP, while improving trabecular bone microarchitecture and promoting bone formation in OVX mice. Further research indicates that the anti-osteoporosis effects of OP-D are mediated by reducing ROS levels through inhibition of the FoxO3a signaling pathway and activation of the *β*-catenin signaling pathway in MC3T3-E1 cells [84].

Tanshinol, the primary bioactive constituent of *Salvia miltiorrhiza*, is recognized as a highly effective natural antioxidant. To mimic the glucocorticoid-induced osteoporosis, a dexamethasone-induced zebrafish larvae model is established. Tanshinol treatment elicits multiple beneficial effects, including the promotion of bone formation and bone mass, upregulation of osteoblast-specific gene expression (*Runx2*, *Alp*, *Ocn*, and *Sp7*), deceleration of ROS generation, and improvement of antioxidant capacity [85].

Delphinidin-3-rutinoside (D3R), derived from *Solanum melongena*, is investigated for its effects on osteoblast viability and differentiation under basal conditions as well as its capacity to protect MC3T3-E1 cells from oxidative damage induced by tert-butyl hydroperoxide (*t*-BHP). D3R (10^{-9} mol·L^{-1}) significantly enhances the proliferation of MC3T3-E1 cells and promotes osteoblast differentiation by upregulating the expression levels of *Alp*, *Ocn*, and *Col1*. Pre-treatment with D3R (10^{-9} mol·L^{-1}) markedly attenuates *t*-BHP-induced osteoblastic dysfunction and cytoskeletal disorganization by reducing intracellular ROS and preventing the decrease in glutathione to oxidized glutathione ratio (GSH/GSSG). Lastly, the protective effects of D3R are mediated through the PI3K/AKT signaling pathway [86].

Notoginsenoside R1 (NGR1), a compound with potent antioxidant properties isolated from *Panax notoginseng*, is widely used in traditional Chinese medicine. NGR1 (25 μmol·L^{-1}) significantly alleviates oxidative stress-induced mitochondrial damage and restores osteogenic differentiation in H_2O_2-treated MC3T3-E1 *via* suppressing the JNK signaling pathway [87].

Hesperidin, a flavonoid compound derived from citrus fruits, boosts bone formation in MC3T3-E1 cells at concentrations ranging from 1.25 to 10 μg·mL^{-1}. It enhances ALP activity and cell proliferation, elevates ALP and nitric oxide (NO) levels, and dose-dependently reduces TNF-α and IL-6. Additionally, hesperidin promotes bone formation in prednisolone-treated zebrafish and inhibits AAPH-induced ROS production [88].

10. Autophagy and natural products

10.1. Autophagy and bone formation

Autophagy is a cellular process by which cells degrade func-

tionally impaired or excessive organelles to provide energy under conditions of nutrient deprivation. Beyond starvation, autophagy is activated by oxidative stress, accumulation of misfolded proteins, mitochondrial damage, and related stimuli. In mammals, autophagy comprises three forms: chaperone-mediated autophagy (CMA), micro-autophagy, and macro-autophagy. This article primarily focuses on macroautophagy, the most extensively studied type [89]. The autophagy process involves the formation of autophagosomes around targeted cargo by monolayer or bilayer membranes derived from the ER and mitochondria. These autophagosomes subsequently fuse with lysosomes, organelles containing hydrolytic enzymes, to form autolysosomes, where encapsulated intracellular components and damaged organelles are degraded. Autophagy is a highly conserved process mediated by autophagy-related proteins (ATGs). For instance, the ATG13-ATG1-ATG17 complex initiates autophagy in yeast. In mammals, phosphatidylinositol 3-kinase (PtdIns3K) and BECN1 (an ATG-related protein) participate in the localization of ATG proteins on the phagophore membrane. The ATG12-ATG5-ATG16L complex and microtubule-associated protein 1A/1B-light chain 3 (LC3)-II contribute to the elongation and closure of the autophagosome. Moreover, autophagy receptors on the surface of autophagosomes, such as the ubiquitin-binding protein SQSTM1 (p62), optineurin (OPTN), mediate the selective sequestration of degradation targets. Autophagy regulates energy and chemical homeostasis in individual cells and various tissues, including bone and skeletal cells [90, 91].

Autophagy plays a critical role in regulating bone marrow stromal cells (BMSCs), osteoblasts, osteoclasts, and osteocytes within the skeletal system. During osteogenic differentiation of BMSCs, high energy demands are met through autophagy [92]. Inhibition of autophagy suppresses osteogenic differentiation and promotes the differentiation of mesenchymal stem cells into adipocytes [93]. Specific deletion of *Atg5* and *Atg7* results in autophagy defects, which impair osteoblast activity and function, and disrupt their differentiation into osteocytes [94, 95]. During osteoblast mineralization, autophagy is highly induced, with autophagosomes serving as carriers for mineralized crystals. Further research indicates that knockdown of *Atg7* and *Beclin1*, as well as deletion of *Atg5*, significantly reduces the mineralization capacity of osteoblasts [96].

10.2. Modulatory effects of natural products on autophagy

The alkaloid leonurine from *Leonurus japonicus* facilitates the proliferation and osteogenic differentiation of rat BMSCs by activating autophagy and upregulating ATG5, ATG7, and LC3II/I levels through inhibition of the PI3K/AKT/mTOR pathway [97]. Another study by Zhao et al. demonstrates that leonurine protects BMSCs against oxidative stress-induced dysfunction by activating mitophagy *via* the PI3K/AKT/mTOR pathway. Additionally, leonurine has been shown to mitigate bone loss and contribute to osteogenesis in OVX rats [98].

Osthole, a coumarin extracted from the TCM *Cnidium monnieri*, enhances osteogenic differentiation and cell proliferation in mouse BMSCs at a concentration of 10 μmol·L^{-1}. When these pretreated cells are transplanted into OVX mice, they significantly improve BMD, trabecular number, and thickness in the distal femur, and elevate serum levels of osteogenic markers ALP and P1NP. The pro-osteogenic effects of osthole may be mediated by increased autophagy [99].

Arbutin, a hydroquinone glucoside found in various plants, alleviates dexamethasone (DEX)-induced suppression of osteoblast differentiation and mineralization, as well as the downregulation of osteogenic markers, by activating autophagy in MC3T3-E1 cells. In addition, the osteoprotective and autophagy-enhancing effects of arbutin have been confirmed in a DEX-induced mouse model [100].

Ginsenoside Rg3, the major active component of *Panax ginseng*, significantly enhances osteogenic differentiation and mineralization, stimulates autophagy and AMPK signaling, and inhibits mTOR signaling in MC3T3-E1 cells. Additionally, the osteoprotective and autophagy-activating effects of ginsenoside Rg3 have been validated in an OVX rat model [101].

Monotropein, the main iridoid glycoside from the root of *Morinda officinalis*, exhibits anti-apoptotic and antioxidant activities. In H_2O_2-treated osteoblasts, monotropein significantly attenuates H_2O_2-induced ROS generation, induces autophagy, and protects osteoblasts against cytotoxicity. Specifically, monotropein inhibits osteoblast apoptosis and elevates the expression of the osteogenic marker RUNX2 and autophagy markers Beclin1 and LC3-II/LC3-I ratio. Additionally, monotropein enhances autophagy-mediated antioxidant effects *via* the AKT/mTOR pathway [102].

ICA mitigates OVX-induced bone loss by enhancing the osteogenic differentiation of BMSCs and reducing the number of TRAP-positive osteoclasts *via* activating autophagy pathway [103]. Another study by Bai et al. demonstrates that ICA (0.1 μmol·L^{-1}) not only rejuvenates declined osteogenesis in senescent BMSCs but also restrains the inflammaging of senescent macrophages, thereby alleviating bone loss in osteoporotic mice. These effects are attributed to the activation of autophagy [104].

Timosaponin BII (TBII), a steroidal saponin isolated from the rhizomes of *Anemarrhena asphodeloides*, exhibits anti-osteoporosis properties. The efficacy and mechanism of TBII in treating diabetic osteoporosis have been investigated both *in vitro* and *in vivo*. Oral administration of TBII significantly improves the tibia microarchitecture, upregulates Beclin1 expression, and downregulates phosphorylation levels of mTOR and NFκB in the proximal tibias of diabetic rats. Moreover, TBII dose-dependently reduces high glucose-induced apoptosis and oxidative stress in osteoblasts, while increasing autophagosome numbers, LC3B puncta formation, and Beclin1 expression. Further experiments confirm that the anti-osteoporotic effects of TBII are mediated by inhibition of the mTOR/NFκB pathway, thereby activating autophagy and decreasing apoptosis and oxidative stress [105].

Scoparone, a coumarin compound extracted from *Artemisia capillaris*, exhibits anticoagulant, vasorelaxant, antioxidant, and anti-inflammatory properties. Park et al. demonstrated that scoparone enhances osteogenesis by promoting osteoblast differentiation, adhesion, migration, mineralization and autophagy. These effects are mediated by the activation of the BMP-2/SMAD1/5/8 and MAPK pathways [106].

ISL, a flavonoid derived from *Glycyrrhiza glabra*, exhibits antidepressant, antioxidant and anti-inflammatory properties. A recent investigation by Su et al. shows that ISL significantly improves bone mass and trabecular microarchitecture and suppresses bone resorption in OVX mice, with no detectable toxicity. *In vitro* experiments indicate that ISL promotes the proliferation and osteogenic differentiation of BMSCs *via* activating the p38/ERK-autophagy pathway [65].

11. Epigenetics and natural products

11.1. Epigenetics and bone formation

Epigenetics refers to heritable changes in gene expression that occur without alterations in the DNA sequence, including DNA methylation, histone modifications, and post-transcriptional regulation mediated by non-coding RNAs [107].

DNA methylation involves the transfer of a methyl group from *S*-adenosyl-L-methionine to cytosine within CpG sites of DNA, forming methylcytosine. This process can inhibit gene ex-

pression by preventing the binding of key transcription factors and promoting a condensed chromatin state through the recruitment of methyl-CpG binding transcriptional repressor factors [108-109]. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), including DNMT1, DNMT2, DNMT3A, and DNMT3B [110]. In the skeletal system, DNA methylation regulates genes critical for osteogenesis and osteoclastogenesis [111]. For example, hypermethylation of the proximal promoter and first exon of the *Sost* gene, which encodes sclerostin, a WNT pathway inhibitor, reduces sclerostin expression, and activates the WNT pathway [112].

Histone modifications are primarily catalyzed by histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs), and histone deacetylases (HDACs). HMTs add methyl groups to lysine and/or arginine residues of histones, while HDMs remove them. HATs acetylate lysine residues to promote transcription, whereas HDACs deacetylate them to inhibit transcription [108, 113-114]. In the skeletal system, HDACs can deacetylate the lysine residues of non-histone proteins, including the transcription factors OSX/SP7 and RUNX2, thereby inhibiting their transcription and suppressing osteogenesis [108].

Small non-coding RNAs are functional RNA molecules transcribed from DNA that do not encode proteins. Among them, microRNAs (miRNAs) are a class of small (about 22 nucleotides) single-stranded non-coding RNAs that downregulate target gene expression by inducing mRNA degradation or translation inhibition. Accumulating evidence indicates that miRNAs play a vital role in regulating osteoblast and osteoclast differentiation, as well as bone resorption [108]. For instance, microRNA-188 regulates the age-related shift between osteogenesis and adipogenesis in BMSCs, contributing to age-related bone loss and fat accumulation in bone marrow [115].

11.2. Modulatory effects of natural products on epigenetics

Sulforaphane (SFN), an organosulfur compound biosynthesized in cruciferous vegetables after being damaged or cut, has been extensively studied for its antimicrobial and anticarcinogenic properties and is reported to potentially benefit bone healing [116-117]. An investigation by Thaler et al. demonstrates that SFN enhances osteoblast differentiation by promoting DNA demethylation [118]. Specifically, SFN accelerates DNA demethylation *via Tet1* and *Tet2*, thereby promoting the expression of osteoblastic indicators and extracellular matrix mineralization. Meanwhile, SFN downregulates the expression of RANKL in osteocytes and mouse calvarial explants and preferentially induces apoptosis in preosteoclastic cells *via* the activation of the TET1/FAS/Caspase 8 and Caspase 3/7 pathways, thereby reducing osteoclast-mediated bone resorption. This research further shows that five-week SFN treatment significantly improves the bone volume and trabecular number in both normal and OVX mice [118].

Allyl sulfide (AS), an active component extracted from *Allium sativum*, possesses antioxidant, anti-inflammatory, and cytoprotective properties. AS treatment enhances osteoblast differentiation and mineralization and increases BMD in aged mice. Additionally, AS alleviates age-related mitochondrial dysfunction in BMSCs, decreases mtDNA release, and mitigates age-related BMSC inflammation by reducing IL-1β secretion. At the epigenetic level, AS upregulates KDM6B expression, reduces H3K27me3 methylation at the *Runx2* promoter, and ultimately restores *Runx2* expression in BMSCs [119].

Resveratrol (RES), a natural polyphenol found in grapes, cranberries, peanuts, and various other plants, is a Sirtuin 1 (SIRT1) activator with beneficial effects on bone health. In obese men, the administration of RES at 1 $g \cdot d^{-1}$ significantly increases ALP activity and lumbar spine bone density [120]. Additionally, RES at 500 $mg \cdot d^{-1}$ elevates serum 25-hydroxyvitamin D levels and reduces bone loss in diabetic patients [121]. RES treatment stimulates BMP-2 expression and suppresses RANKL expression in osteoblasts, thereby promoting bone formation [122-123]. Furthermore, RES activates the osteogenic factors RUNX2 and SIRT1, enhancing bone formation [124]. In MSCs, RES treatment significantly enhances osteoblast differentiation at the expense of adipogenesis, and dramatically reduces MSC senescence and intracellular ROS level [125]. Mechanistically, RES treatment increases the binding of SIRT1 to the polycomb complex protein BMI-1, decreases BMI-1 acetylation, and promotes BMI-1 nuclear translocation, while also activating the focal adhesion kinase (FAK) signaling pathway [126]. Another research by Jiang et al. demonstrates that RES promotes osteogenesis by activating the SIRT1/FoxO1 pathway in OVX mice [127].

Curcumin, a natural polyphenol derived from the rhizomes of *Curcuma longa*, promotes bone formation and is hypothesized to modulate epigenetic mechanisms. Curcumin significantly enhances ALP activity and calcium deposition, as well as the expression levels of osteogenic markers, including *Runx2*, *Sp7*, *Col1*, *Opn*, and *Ocn*, in osteogenic-induced hBMSCs. Mechanistically, curcumin downregulates EZH2 expression, thereby increasing H3K4me3 and reducing H3K27me3 marks on the promoters of *Runx2* and *Ocn*, which in turn activates the transcription of these osteoblast-related markers [128].

Syringic acid (SA), a phenolic compound with antioxidant and anti-inflammatory properties, promotes osteogenesis by stimulating ALP activity and calcium deposition and upregulating the osteoblastic markers such as *Alp*, *Col1*, *Ocn*, and *Runx2*. Mechanistically, SA increases miR-21 expression while decreasing SMAD7 level, a negative regulator of RUNX2, ultimately enhancing osteogenic differentiation [129]. Another work by Tanaka et al. demonstrates that SA administration (100 $mg \cdot kg^{-1}$ body $weight \cdot d^{-1}$) for 10 weeks markedly attenuates bone loss and increases the ratio of osteoblasts to osteoclasts in OVX mice [130].

ICA facilitates osteogenesis through multiple molecular mechanisms and signaling pathways, including post-transcriptional regulation mediated by miRNAs. ICA remarkably enhances the osteogenic marker expression and calcium deposition in human BMSCs, and attenuates bone loss in OVX rats. Further research demonstrates that ICA ameliorates osteoporosis *via* the miR-335-5p/PTEN axis [131].

Puerarin, an isoflavone extracted from the root of *Pueraria lobata*, exhibits osteoprotective properties. Zhan et al. reported that puerarin enhances viability and osteoblast differentiation of MC3T3-E1 cells by upregulating RUNX2 expression through the downregulation of miR-204 [132].

Kaempferol, a flavonol present in various edible plants, possesses anti-inflammatory, antioxidant, and anti-osteoporotic properties. Kaempferol treatment significantly mitigates bone loss in ovariectomized rats, and boosts ALP activity, calcium deposition, and the expression of key transcription factors RUNX2 and OSX in BMSCs. Mechanistically, kaempferol enhances BMSC osteogenic differentiation and attenuates osteoporosis by diminishing miR-10a-3p levels and elevating CXCL12 expression [133].

12. Other pathways and natural products

12.1. Natural products and estrogen pathway

Estrogens play a crucial role in bone remodeling and exert physiological effects on target tissues through interactions with estrogen receptors (ERs), including estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β). These receptors are located in both the cell membrane and the nucleus and are widely distributed in bone tissue. The activation of ERs triggers down-

stream signaling pathways, such as MAPK and PI3K. Estrogens influence the generation and lifespan of osteoblasts and osteoclasts, as well as the lifespan of osteocytes [134].

An early investigation by Wang et al. showed that puerarin significantly enhances the proliferation and differentiation of human osteoblastic MG-63 cells while inhibiting cisplatin-induced apoptosis. Puerarin promotes proliferation by upregulating cell cycle regulators cyclin B1 and cyclin D1. Its anti-apoptotic effect may be associated with increased Bcl-xL levels. Further research indicates that these beneficial effects are mediated by both ERα and ERβ and partially by activating the MEK/ERK and PI3K/AKT pathways [135].

QingYan Formula, traditionally used in China for kidney tonification, comprises *Cistanche deserticola*, *Morinda officinalis*, *Achyranthes bidentata*, Pricklyash Peel, and Halitium. To investigate its anti-bone loss effects, OVX rats were orally administered with 70% ethanol extracts of QingYan Formula (QYFE). QYFE markedly improved the microstructure of trabecular and cortical bone and reduced osteoclast numbers in the femurs of OVX rats. *In vitro*, QYFE enhanced the proliferation and osteoblast differentiation of MG-63 cells while inhibiting osteoclast differentiation of RAW264.7 cells. Mechanistically, QYFE activates ER-dependent MEK/ERK and PI3K/AKT signal pathways in osteoblasts and diminishes the expressions of ER-dependent p-ERK and ER-independent p-AKT in osteoclasts [136].

Sweroside, a major active iridoid glycoside extracted from the dried root of *Dipsacus asper*, exhibits pro-osteogenic and anti-inflammatory properties. Treatment with sweroside (1 μmol·L^{-1}) significantly enhances ALP activity and mineralization in MC3T3-E1 cells. Additionally, sweroside promotes osteogenesis *via* upregulating ER-α and G protein-coupled receptor 30 (GPR30), subsequently activating the p38 signaling pathway [137].

ICA, one of the most extensively studied natural products, exerts osteoprotective effects through ER-α signaling. Administration of ICA significantly protects OVX rats from bone loss in both long bones and the lumbar spine. *In vitro* studies using BMSCs and BMMs demonstrate that ICA promotes osteogenesis and inhibits osteoclastogenesis. Furthermore, the osteoprotective effects of ICA are mediated by activating ER-α and AKT signaling pathways and inducing the interactions between IGF-IR and ERα [138].

12.2. Natural products inhibit fat cell formation and boost bone formation

Mesenchymal stem cells (MSCs) are spindle-shaped, adherent, non-hematopoietic stem cells derived from various tissues, including bone marrow, umbilical cord, and adipose tissue. These cells possess multipotent differentiation potential, enabling them to differentiate into diverse cell types such as adipocytes, chondrocytes, and osteoblasts. The differentiation of MSCs is regulated by multiple signaling pathways, including TGF-β, BMP, WNT, Hedgehog (HH), FGFs, and Notch. Numerous studies have demonstrated that inhibiting the adipogenic differentiation of BMSCs can enhance their osteogenic differentiation [139].

A combination of *Cornus officinalis* (CO) and *Ribes fasciculatum* (RF) at a ratio of 7∶3 and a concentration of 50 μg·mL^{-1} suppresses adipogenesis in 3T3-L1 cells by downregulating the adipogenic markers perilipin1 (*Plin1*) and adiponectin (*Adipoq*). At the same ratio and concentration, this combination promotes osteogenesis in MC3T3-E1 cells by upregulating the expression of osteogenic markers *Alp*, *Runx2*, and *Ocn* and increasing ALP activity. In OVX mice, dietary supplementation with CO + RF at doses of 150, and 300 mg·kg^{-1}·d^{-1} reduces hepatic steatosis and adipocyte size, inhibits the elevation of serum leptin and insulin, reverses uterine atrophy, upregulates the expression of estrogen receptor alpha, and improves bone density [140].

Green tea is recognized as a potent bone-protective agent, with epigallocatechin (EGC) and other green tea polyphenols as its main bioactive ingredients. Treatment of rat BMSCs with EGC (20 μmol·L^{-1}) dramatically enhances ALP activity and calcium deposition, while increasing the mRNA expression of bone formation markers, including *Alp*, *Runx2*, and *Opn*. Importantly, EGC exerts anti-adipogenic effects by reducing adipocyte formation and downregulating the mRNA levels of adipogenic indicators such as peroxisome proliferator-activated receptor γ (*Pparg*), Ccaat/enhancer-binding protein β (*Cebpβ*), and fatty acid-binding protein 4 (*Fabp4*) [141].

Dendrobium officinale polysaccharides (DOPs), a major ingredient from the traditional Chinese medicine *Dendrobium officinale*, exhibit significant antioxidant, anti-inflammatory, and anti-obesity properties. DOP treatment (200, 400 μg·mL^{-1}) remarkably increases osteogenic differentiation while inhibiting adipogenic differentiation in mice BMSCs. Moreover, DOPs can reverse the H_2O_2-induced shift in BMSC differentiation fate by activating Nrf2 signaling. Furthermore, DOP supplementation in aged mice markedly increases bone mass and reduces the marrow adipose tissue (MAT), accompanied by decreased oxidative stress in BMSCs [142].

13. Discussion and future prospects

13.1. Discussion

Natural products hold promise for treating osteoporosis by promoting osteoblast-mediated bone formation. In 2016, An and colleagues provided a detailed summary of natural products with anti-osteoporotic potential and their regulatory mechanisms in promoting osteoblast-mediated bone formation [143]. Their work was pivotal in advancing the screening and clinical application of anti-osteoporosis agents. Since then, significant progress has been achieved in elucidating the cellular, molecular, and pharmacological mechanisms underlying natural product-induced osteoblast-mediated bone formation. In this review, we further consolidate recent research developments on the effects and regulatory mechanisms of natural compounds in promoting osteoblast activity and bone anabolism. Additionally, we incorporate updated animal model data and highlight emerging regulatory pathways, such as autophagy and epigenetic modifications. Finally, we explore the translational potential of these findings and discuss future directions for their therapeutic application in bone-related disorders.

We conducted a comprehensive literature survey covering 65 natural products reported to promote bone formation (Tables S1–S9). These compounds were categorized into 24 structural classes, including isoflavonoids, triterpenoid saponins, coumarins, flavonoids, terpenoids, polysaccharides, polyphenols, and peptides. As shown in Fig. 4 and Tables S1–S9, these bioactive compounds primarily exert their osteogenic effects through the regulation of key transcription factors, such as RUNX2 and OSX/SP7, as well as by modulating several signaling pathways, including WNT/*β*-catenin, BMP, MAPK, and PI3K/AKT, and mechanisms related to oxidative stress, autophagy, and epigenetic regulation. In addition, certain natural products facilitate bone formation by acting on multiple targets and pathways. For instance, ICA enhances osteogenesis by activating the WNT, AKT, and autophagy pathways, upregulating miR-335-5p, downregulating miR-34c in osteoblasts, and inhibiting the adipogenic differentiation of BMSCs. Likewise, resveratrol boosts osteoblast differentiation *via* activating RUNX2 and SIRT1, while reducing adipogenesis, MSC senescence, and intracellular ROS levels. Given that osteoporosis is a complex metabolic bone disease caused by multiple factors, these natural products targeting multiple sig-

naling pathways may offer better therapeutic effects. Traditional Chinese medicine for kidney tonification has long been used to treat osteoporosis in China. Notably, many natural products, including ICA, asperosaponin VI, morroniside, luteolin, salidroside, monotropein, kaempferol, and sweroside, are key active ingredients in these kidney-tonifying herbs [20]. Based on the information collected in this review, natural products exhibiting kidney-tonifying, anti-inflammatory, antioxidant, and anti-adipogenic properties hold promise as novel therapeutic agents for osteoporosis. This review aims to serve as a reference for the screening, mechanistic investigation, and clinical translation of potential anti-osteoporotic natural compounds.

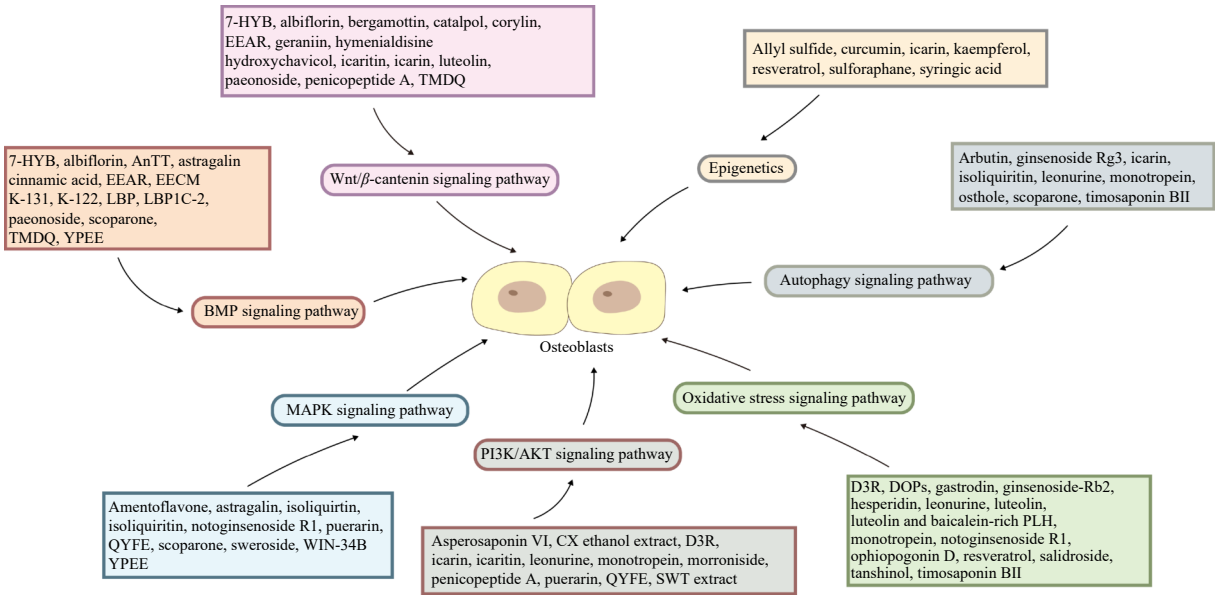

Fig. 4 The overview of natural products promoting bone formation and related signal pathways. Natrual products promote osteoblast-mediated bone formation mainly by the regulation of the following pathways, WNT/β-catenin, BMP, MAPK, PI3K/AKT, oxidative stress, autophagy, and epigenetics.

13.2. Future prospects

Despite their significant therapeutic potential, the clinical application of natural products is often hindered by challenges, such as poor absorption, rapid metabolism, low specificity, and irregular distribution [144]. The development of innovative bone-targeting delivery systems is key to overcoming these challenges. Such systems can prolong circulation time, enhance targeting efficiency, reduce dosage requirements, and improve stability and safety, thereby increasing therapeutic efficacy. Huang et al. demonstrated that Asp8-liposome, a bone-targeting delivery system, effectively delivers the osteogenic phytomolecule icaritin and significantly enhances bone formation in OVX mice [72]. In addition, some researchers are currently exploring multi-carrier, multi-drug delivery systems for osteoporosis treatment [145]. A excellent system based on mesoporous silica nanoparticles (MSNs) that co-delivering *Sost* siRNA and osteostatin (an osteogenic peptide derived from PTHrP), designed by Mora-Raimundo et al., has been used to treat osteoporotic mice [146]. Nanoparticle administration significantly upregulated osteogenic marker genes and markedly improved bone microarchitecture.

OVX rats and mice, which mimic postmenopausal osteoporosis, are considered the gold standard *in vivo* models for evaluating the efficacy of anti-osteoporosis natural products and drugs [147]. Additionally, mouse models of inflammatory osteoporosis (LPS-induced bone loss), glucocorticoid-induced osteoporosis (GIOP), and age-related osteoporosis are available for assessing natural product efficacy [9, 148–149]. To the best of our knowledge, most natural products have been evaluated in OVX rodent models, with fewer tested in GIOP or aged mice, and even fewer in LPS-induced models (Tables S1–S9). Undoubtedly, the efficacy of these natural products should be further examined across diverse animal models of osteoporosis. In recent years, teleost fish, particularly zebrafish (*Danio rerio*), have emerged as crucial models in bone research, capable of replicating numerous human skeletal diseases [150–151]. These teleosts are well suitable for high-throughput screening of anti-osteoporosis drugs due to the following technical advantages, e.g., cost-effectiveness, short lifespan, high fecundity, translucency of embryonic stages throughout development, and ease of gene editing. Zebrafish-based high-throughput screening tools have proven valuable in drug discovery for cancer, cardiovascular diseases, and anti-aging therapies [152–153]. However, in anti-osteoporosis research, zebrafish models have primarily been used for validating specific compounds [85, 154]. Given the diversity of animal models, significant advancements in the screening and clinical application of anti-osteoporosis drugs are anticipated in the coming years.

Osteocytes, the most abundant bone cells (> 90% of total), have a long lifespan and mediate adaptive responses to mechanical and hormonal stimuli to maintain bone homeostasis. They also play a critical role in age-related bone deterioration [155–156]. However, their role in bone metabolism is often overlooked due to their deep embedding in the mineralized matrix, which complicates isolation. Few reports exist on anti-osteoporosis agents targeting osteocytes. Gu-Shu-Kang is a traditional Chinese medicine formula composed of *Epimedium brevicornu* and *Rehmannia glutinosa*. It not only promotes osteogenesis but also prevents apoptosis of osteocytes induced by ovariectomy [157]. With improved osteocyte isolation techniques and the widespread use of single-cell transcriptome sequencing, greater focus should be placed on identifying natural products that target osteocytes and elucidating their molecular mechanisms.

While TCMs and their active ingredients are extensively used in China for osteoporosis treatment, in the US and the EU, natural products are generally considered complementary therapies alongside conventional drug treatments. Although natural products are typically better tolerated than synthetic drugs, their bone-protective efficacy is often lower. They may be more suitable for preventing osteoporosis or managing mild cases. In addition, 'natural' does not equals to 'safe', as natural products may

have potential toxicity and can interact with prescribed drugs [158]. Moreover, clinical trials involving natural products often lack rigor, and regulatory oversight remains relatively loose. Therefore, additional high-quality studies are essential to definitively establish the clinical efficacy and safety of natural products in osteoporosis prevention and treatment.

14. Conclusion

Promoting osteoblast-mediated bone formation represents a promising strategy for natural products in osteoporosis treatment. This review comprehensively examines the effects and molecular mechanisms of 65 natural products across 24 categories on osteoblast-mediated bone formation. These compounds enhance bone formation by regulating key transcription factors (RUNX2 and Osterix) and pathways such as WNT/β-catenin, BMP, MAPK, PI3K/AKT, oxidative stress, autophagy, and epigenetics. Notably, some natural products, like ICA and resveratrol, exert their effects through multiple targets and pathways. Many of these natural products have demonstrated therapeutic efficacy in animal models, including OVX mice. Our findings suggest that natural products with kidney-tonifying, anti-inflammatory, antioxidant properties, and those inhibiting adipocyte differentiation, may be effective for osteoporosis treatment. We also clarify current research gaps and propose future directions, including high-throughput screening and validation in diverse animal models, development of novel bone-targeting delivery systems, and identification of natural drugs targeting osteocytes. Importantly, translating these findings into clinical practice remains challenging, and further high-quality studies are needed to firmly establish the clinical efficacy and safety of natural products for osteoporosis prevention and treatment.

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Supporting information

Supporting information for this work can be obtained by contacting the corresponding authors *via* E-mail.

Declaration of competing interest

The authors declare no competing conflict of interests.

References

1 Karsenty G. The complexities of skeletal biology. *Nature*. 2003;423(6937):316-318. https://doi.org/10.1038/nature01654.
2 Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet*. 2011;377(9773):1276-1287. https://doi.org/10.1016/S0140-6736(10)62349-5.
3 Amarnath SS, Kumar V, Das SL. Classification of osteoporosis. *Indian J Orthop*. 2023;57(Suppl 1):49-54. https://doi.org/10.1007/s43465-023-01058-3.
4 Compston JE, McClung MR, Leslie WD. Osteoporosis. *Lancet*. 2019;393(10169):364-376. https://doi.org/10.1016/S0140-6736(18)32112-3.
5 Papadopoulou SK, Papadimitriou K, Voulgaridou G, et al. Exercise and nutrition impact on osteoporosis and sarcopenia—the incidence of osteosarcopenia: a narrative review. *Nutrients*. 2021;13(12):4499. https://doi.org/10.3390/nu13124499.
6 Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int*. 2006;17(12):1726-1733. https://doi.org/10.1007/s00198-006-0172-4.
7 Salari N, Ghasemi H, Mohammadi L, et al. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. *J Orthop Surg Res*. 2021;16(1):609. https://doi.org/10.1186/s13018-021-02772-0.
8 Hernlund E, Svedbom A, Ivergård M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. *A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos*. 2013;8(1):136. https://doi.org/10.1007/s11657-013-0136-1.
9 Carletti A, Gavaia PJ, Cancela ML, et al. Metabolic bone disorders and the promise of marine osteoactive compounds. *Cell Mol Life Sci*. 2023;81(1):11. https://doi.org/10.1007/s00018-023-05033-x.
10 Khosla S, Shane E. A crisis in the treatment of osteoporosis. *J Bone Miner Res*. 2016;31(8):1485-1487. https://doi.org/10.1002/jbmr.2888.
11 Patel DV, Horne A, House M, et al. The incidence of acute anterior uveitis after intravenous zoledronate. *Ophthalmology*. 2013;120(4):773-776. https://doi.org/10.1016/j.ophtha.2012.10.028.
12 Robinson DE, Ali MS, Pallares N, et al. Safety of oral bisphosphonates in moderate-to-severe chronic kidney disease: a binational cohort analysis. *J Bone Miner Res*. 2021;36(5):820-832. https://doi.org/10.1002/jbmr.4235.
13 Cummings SR, Ferrari S, Eastell R, et al. Vertebral fractures after discontinuation of denosumab: a post hoc analysis of the randomized placebo-controlled freedom trial and its extension. *J Bone Miner Res*. 2018;33(2):190-198. https://doi.org/10.1002/jbmr.3337.
14 Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA*. 2007;297(13):1465-1477. https://doi.org/10.1001/jama.297.13.1465.
15 Yavropoulou MP, Makras P, Anastasilakis AD. Bazedoxifene for the treatment of osteoporosis. *Expert Opin Pharmacother*. 2019;20(10):1201-1210. https://doi.org/10.1080/14656566.2019.1615882.
16 Miller SA, St OE, Whalen KL. Romosozumab: a novel agent in the treatment for postmenopausal osteoporosis. *J Pharm Technol*. 2021;37(1):45-52. https://doi.org/10.1177/8755122520967632.
17 Tabacco G, Bilezikian JP. Osteoanabolic and dual action drugs. *Br J Clin Pharmacol*. 2019;85(6):1084-1094. https://doi.org/10.1111/bcp.13766.
18 Seeman E, Martin TJ. Antiresorptive and anabolic agents in the prevention and reversal of bone fragility. *Nat Rev Rheumatol*. 2019;15(4):225-236. https://doi.org/10.1038/s41584-019-0172-3.
19 Gao Z, Lu Y, Halmurat U, et al. Study of osteoporosis treatment principles used historically by ancient physicians in Chinese medicine. *Chin J Integr Med*. 2013;19(11):862-868. https://doi.org/10.1007/s11655-013-1328-z.
20 Duan Y, Su YT, Ren J, et al. Kidney tonifying traditional Chinese medicine: potential implications for the prevention and treatment of osteoporosis. *Front Pharmacol*. 2023;13:1063899. https://doi.org/10.3389/fphar.2022.1063899.
21 Zhang ND, Han T, Huang BK, et al. Traditional Chinese medicine formulas for the treatment of osteoporosis: implication for antiosteoporotic drug discovery. *J Ethnopharmacol*. 2016;189:61-80. https://doi.org/10.1016/j.jep.2016.05.025.
22 Luo MH, Zhao JL, Xu NJ, et al. Comparative efficacy of Xianling Gubao Capsules in improving bone mineral density in postmenopausal osteoporosis: a network meta-analysis. *Front Endocrinol (Lausanne)*. 2022;13:839885. https://doi.org/10.3389/fendo.2022.839885.
23 Wei X, Qi B, Ma R, et al. Quantitative proteomics revealed the pharmacodynamic network of Bugu Shengsui Decoction promoting osteoblast proliferation. *Front Endocrinol (Lausanne)*. 2021;12:833474. https://doi.org/10.3389/fendo.2021.833474.
24 Dai X, Liu Y, Liu T, et al. SiJunZi decoction ameliorates bone quality and redox homeostasis and regulates advanced glycation end products/receptor for advanced glycation end products and WNT/β-catenin signaling pathways in diabetic mice. *J Ethnopharmacol*. 2024;319(Pt 2):117167. https://doi.org/10.1016/j.jep.2023.117167.
25 Li C, Lin X, Lin Q, et al. Jiangu Granules ameliorate postmenopausal osteoporosis *via* rectifying bone homeostasis imbalance: a network pharmacology analysis based on multi-omics validation. *Phytomedicine*. 2024;122:155137. https://doi.org/10.1016/j.phymed.2023.155137.
26 Komori T. Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell Tissue Res*. 2010;339(1):189-195. https://doi.org/10.1007/s00441-009-0832-8.
27 Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002;108(1):17-29. https://doi.org/10.1016/s0092-8674(01)00622-5.
28 Kitase Y, Prideaux M. Targeting osteocytes *vs* osteoblasts. *Bone*. 2023;170:116724. https://doi.org/10.1016/j.bone.2023.116724.
29 Xue L, Jiang Y, Han T, et al. Comparative proteomic and metabolomic analysis reveal the antiosteoporotic molecular mechanism of icariin from *Epimedium brevicornu* Maxim. *J Ethnopharmacol*. 2016;192:370-381. https://doi.org/10.1016/j.jep.2016.07.037.
30 Liu J, Li D, Sun X, et al. Icariine restores LPS-induced bone loss by downregulating miR-34c level. *Inflammation*. 2016;39(5):1764-1770. https://doi.org/10.1007/s10753-016-0411-6.
31 Kong X, Wang F, Niu Y, et al. A comparative study on the effect of promoting the osteogenic function of osteoblasts using isoflavones from Radix Astragalus. *Phytother Res*. 2018;32(1):115-124. https://doi.org/10.1002/ptr.5955.
32 Wu J, Zeng Z, Li Y, et al. Cycloastragenol protects against glucocorticoid-induced osteogenic differentiation inhibition by activating telomerase. *Phytother Res*. 2021;35(4):2034-2044. https://doi.org/10.1002/ptr.6946.
33 Park E, Kim J, Yeo S, et al. Anti-osteoporotic effects of combined extract of Lycii Radicis Cortex and *Achyranthes japonica* in osteoblast and osteoclast cells and ovariectomized mice. *Nutrients*. 2019;11(11):2716. https://doi.org/10.3390/nu11112716.
34 Park E, Kim J, Kim M, et al. Anti-osteoporotic effects of kukoamine B isolated from Lycii Radicis Cortex extract on osteoblast and osteoclast cells

and ovariectomized osteoporosis model mice. *Int J Mol Sci*. 2019;20(11):2784. https://doi.org/10.3390/ijms20112784.
35 Park E, Kim J, Jin H, et al. Scopolin attenuates osteoporotic bone loss in ovariectomized mice. *Nutrients*. 2020;12(11):3565. https://doi.org/10.3390/nu12113565.
36 Park E, Lee CG, Lim E, et al. Osteoprotective effects of loganic acid on osteoblastic and osteoclastic cells and osteoporosis-induced mice. *Int J Mol Sci*. 2021;22(1):233. https://doi.org/10.3390/ijms22010233.
37 Liu H, Zhang H, Fan H, et al. The preventive effect of Cuscutae Semen polysaccharide on bone loss in the ovariectomized rat model. *Biomed Pharmacother*. 2020;130:110613. https://doi.org/10.1016/j.biopha.2020.110613.
38 Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest*. 2006;116(5):1202-1209. https://doi.org/10.1172/JCI28551.
39 Salhotra A, Shah HN, Levi B, et al. Mechanisms of bone development and repair. *Nat Rev Mol Cell Biol*. 2020;21(11):696-711. https://doi.org/10.1038/s41580-020-00279-w.
40 Sun Z, Jin H, Zhou H, et al. Guhong Injection promotes fracture healing by activating Wnt/*β*-catenin signaling pathway *in vivo* and *in vitro*. *Biomed Pharmacother*. 2019;120:109436. https://doi.org/10.1016/j.biopha.2019.109436.
41 Yun HM, Park KR, Quang TH, et al. 2,4,5-Trimethoxyldalbergiquinol promotes osteoblastic differentiation and mineralization *via* the BMP and Wnt/*β*-catenin pathway. *Cell Death Dis*. 2015;6(7):e1819. https://doi.org/10.1038/cddis.2015.185.
42 Hu J, Mao Z, He S, et al. Icariin protects against glucocorticoid induced osteoporosis, increases the expression of the bone enhancer DEC1 and modulates the PI3K/Akt/GSK3β/*β*-catenin integrated signaling pathway. *Biochem Pharmacol*. 2017;136:109-121. https://doi.org/10.1016/j.bcp.2017.04.010.
43 Wang Q, Chen D, Jin H, et al. Hymenialdisine: a marine natural product that acts on both osteoblasts and osteoclasts and prevents estrogen-dependent bone loss in mice. *J Bone Miner Res*. 2020;35(8):1582-1596. https://doi.org/10.1002/jbmr.4025.
44 Mishra R, Das N, Varshney R, et al. Betel leaf extract and its major component hydroxychavicol promote osteogenesis and alleviate glucocorticoid-induced osteoporosis in rats. *Food Funct*. 2021;12(14):6603-6625. https://doi.org/10.1039/D0FO02619K.
45 Park KR, Lee JY, Cho M, et al. Biological mechanisms of paeonoside in the differentiation of pre-osteoblasts and the formation of mineralized nodules. *Int J Mol Sci*. 2021;22(13):6899. https://doi.org/10.3390/ijms22136899.
46 Zhu Y, Wang Y, Jia Y, et al. Catalpol promotes the osteogenic differentiation of bone marrow mesenchymal stem cells *via* the Wnt/*β*-catenin pathway. *Stem Cell Res Ther*. 2019;10(1):37. https://doi.org/10.1186/s13287-019-1143-y.
47 Li K, Zhang X, He B, et al. Geraniin promotes osteoblast proliferation and differentiation *via* the activation of Wnt/*β*-catenin pathway. *Biomed Pharmacother*. 2018;99:319-324. https://doi.org/10.1016/j.biopha.2018.01.040.
48 Xie CL, Yue YT, Xu JP, et al. Penicopeptide A (PPA) from the deep-sea-derived fungus promotes osteoblast-mediated bone formation and alleviates ovariectomy-induced bone loss by activating the AKT/GSK-3β/*β*-catenin signaling pathway. *Pharmacol Res*. 2023;197:106968. https://doi.org/10.1016/j.phrs.2023.106968.
49 Yu AX, Xu ML, Yao P, et al. Corylin, a flavonoid derived from Psoralea Fructus, induces osteoblastic differentiation *via* estrogen and Wnt/*β*-catenin signaling pathways. *FASEB J*. 2020;34(3):4311-4328. https://doi.org/10.1096/fj.201902319RRR.
50 Kim JH, Kim M, Hong S, et al. Albiflorin promotes osteoblast differentiation and healing of rat femoral fractures through enhancing BMP-2/Smad and Wnt/*β*-catenin signaling. *Front Pharmacol*. 2021;12:690113. https://doi.org/10.3389/fphar.2021.690113.
51 Hong S, Cha KH, Kwon DY, et al. *Agastache rugosa* ethanol extract suppresses bone loss *via* induction of osteoblast differentiation with alteration of gut microbiota. *Phytomedicine*. 2021;84:153517. https://doi.org/10.1016/j.phymed.2021.153517.
52 Wang X, Tian Y, Liang X, et al. Bergamottin promotes osteoblast differentiation and bone formation *via* activating the Wnt/*β*-catenin signaling pathway. *Food Funct*. 2022;13(5):2913-2924. https://doi.org/10.1039/d1fo02755g.
53 Lowery JW, Rosen V. The BMP pathway and its inhibitors in the skeleton. *Physiol Rev*. 2018;98(4):2431-2452. https://doi.org/10.1152/physrev.00028.2017.
54 Wang JY, Chen WM, Wen CS, et al. Du-Huo-Ji-Sheng-Tang and its active component *Ligusticum chuanxiong* promote osteogenic differentiation and decrease the aging process of human mesenchymal stem cells. *J Ethnopharmacol*. 2017;198:64-72. https://doi.org/10.1016/j.jep.2016.12.011.
55 Park KR, Kwon YJ, Park JE, et al. 7-HYB, a phenolic compound isolated from *Myristica fragrans* Houtt increases cell migration, osteoblast differentiation, and mineralization through BMP2 and *β*-catenin signaling. *Int J Mol Sci*. 2020;21(21):8059. https://doi.org/10.3390/ijms21218059.
56 Rai R, Kumar S, Singh KB, et al. Extract and fraction of *Musa paradisiaca* flower have osteogenic effect and prevent ovariectomy induced osteopenia. *Phytomedicine*. 2021;93:153750. https://doi.org/10.1016/j.phymed.2021.153750.
57 Hong S, Cha KH, Park JH, et al. Cinnamic acid suppresses bone loss *via* induction of osteoblast differentiation with alteration of gut microbiota. *J Nutr Biochem*. 2022;101:108900. https://doi.org/10.1016/j.jnutbio.2021.108900.
58 Sun C, Chen X, Yang S, et al. LBP1C-2 from *Lycium barbarum* alleviated age-related bone loss by targeting BMPRIA/BMPRII/Noggin. *Carbohydr Polym*. 2023;310:120725. https://doi.org/10.1016/j.carbpol.2023.120725.
59 Park JH, Son YJ, Lee CH, et al. *Circaea mollis* Siebold & Zucc. alleviates postmenopausal osteoporosis in a mouse model *via* the BMP-2/4/Runx2 pathway. *BMC Complement Med Ther*. 2020;20(1):123. https://doi.org/10.1186/s12906-020-02914-7.
60 Wan HW, Chin KY, Abd GN, et al. Annatto-derived tocotrienol promotes mineralization of MC3T3-E1 cells by enhancing BMP-2 protein expression *via* inhibiting RhoA activation and HMG-CoA reductase gene expression. *Drug Des Devel Ther*. 2020;14:969-976. https://doi.org/10.2147/DDDT.S224941.
61 Greenblatt MB, Shim JH, Glimcher LH. Mitogen-activated protein kinase pathways in osteoblasts. *Annu Rev Cell Dev Biol*. 2013;29:63-79. https://doi.org/10.1146/annurev-cellbio-101512-122347.
62 Seo BK, Ryu HK, Park YC, et al. Dual effect of WIN-34B on osteogenesis and osteoclastogenesis in cytokine-induced mesenchymal stem cells and bone marrow cells. *J Ethnopharmacol*. 2016;193:227-236. https://doi.org/10.1016/j.jep.2016.07.022.
63 Zha X, Xu Z, Liu Y, et al. Amentoflavone enhances osteogenesis of human mesenchymal stem cells through JNK and p38 MAPK pathways. *J Nat Med*. 2016;70(3):634-644. https://doi.org/10.1007/s11418-016-0993-1.
64 Liu L, Wang D, Qin Y, et al. Astragalin promotes osteoblastic differentiation in MC3T3-E1 cells and bone formation *in vivo*. *Front Endocrinol (Lausanne)*. 2019;10:228. https://doi.org/10.3389/fendo.2019.00228.
65 Su Z, Chen D, Huang J, et al. Isoliquiritin treatment of osteoporosis by promoting osteogenic differentiation and autophagy of bone marrow mesenchymal stem cells. *Phytother Res*. 2024;38(1):214-230. https://doi.org/10.1002/ptr.8032.
66 Jeon EJ, Lee DH, Kim YJ, et al. Effects of yuja peel extract and its flavanones on osteopenia in ovariectomized rats and osteoblast differentiation. *Mol Nutr Food Res*. 2016;60(12):2587-2601. https://doi.org/10.1002/mnfr.201600257.
67 Sun K, Luo J, Guo J, et al. The PI3K/AKT/mTOR signaling pathway in osteoarthritis: a narrative review. *Osteoarthr Cartil*. 2020;28(4):400-409. https://doi.org/10.1016/j.joca.2020.02.027.
68 Cohen-Solal KA, Boregowda RK, Lasfar A. RUNX2 and the PI3K/AKT axis reciprocal activation as a driving force for tumor progression. *Mol Cancer*. 2015;14:137. https://doi.org/10.1186/s12943-015-0404-3.
69 Zhang H, Zhou C, Zhang Z, et al. Integration of network pharmacology and experimental validation to explore the pharmacological mechanisms of Zhuanggu Busui Formula against osteoporosis. *Front Endocrinol (Lausanne)*. 2021;12:841668. https://doi.org/10.3389/fendo.2021.841668.
70 Wu CM, Chen PC, Li TM, et al. Si-Wu-Tang extract stimulates bone formation through PI3K/Akt/NF-*κ*B signaling pathways in osteoblasts. *BMC Complement Altern Med*. 2013;13:277. https://doi.org/10.1186/1472-6882-13-277.
71 Ke K, Li Q, Yang X, et al. Asperosaponin VI promotes bone marrow stromal cell osteogenic differentiation through the PI3K/AKT signaling pathway in an osteoporosis model. *Sci Rep*. 2016;6:35233. https://doi.org/10.1038/srep35233.
72 Huang L, Wang X, Cao H, et al. A bone-targeting delivery system carrying osteogenic phytomolecule icaritin prevents osteoporosis in mice. *Biomaterials*. 2018;182:58-71. https://doi.org/10.1016/j.biomaterials.2018.07.046.
73 Liu H, Li X, Lin J, et al. Morroniside promotes the osteogenesis by activating PI3K/Akt/mTOR signaling. *Biosci Biotechnol Biochem*. 2021;85(2):332-339. https://doi.org/10.1093/bbb/zbaa010.
74 Dong XL, Yu WX, Li CM, et al. Chuanxiong (rhizome of *Ligusticum chuanxiong*) protects ovariectomized hyperlipidemic rats from bone loss. *Am J Chin Med*. 2020;48(2):463-485. https://doi.org/10.1142/S0192415X2050024X.
75 Fontani F, Marcucci G, Iantomasi T, et al. Glutathione, *N*-acetylcysteine and lipoic acid down-regulate starvation-induced apoptosis, RANKL/OPG ratio and sclerostin in osteocytes: involvement of JNK and ERK1/2 signalling. *Calcif Tissue Int*. 2015;96(4):335-346. https://doi.org/10.1007/s00223-015-9961-0.
76 Kimball JS, Johnson JP, Carlson DA. Oxidative stress and osteoporosis. *J Bone Joint Surg Am*. 2021;103(15):1451-1461. https://doi.org/10.2106/JBJS.20.00989.
77 Motoyama T, Okamoto K, Kukita I, et al. Possible role of increased oxidant stress in multiple organ failure after systemic inflammatory response syndrome. *Crit Care Med*. 2003;31(4):1048-1052. https://doi.org/10.1097/01.CCM.0000055371.27268.36.
78 Qin D, Zhang H, Zhang H, et al. Anti-osteoporosis effects of osteoking *via* reducing reactive oxygen species. *J Ethnopharmacol*. 2019;244:112045. https://doi.org/10.1016/j.jep.2019.112045.
79 Jing Z, Wang C, Yang Q, et al. Luteolin attenuates glucocorticoid-induced osteoporosis by regulating ERK/Lrp-5/GSK-3β signaling pathway *in vivo* and *in vitro*. *J Cell Physiol*. 2019;234(4):4472-4490. https://doi.org/10.1002/jcp.27252.
80 Phromnoi K, Yodkeeree S, Pintha K, et al. Anti-osteoporosis effect of *Perilla frutescens* leaf hexane fraction through regulating osteoclast and osteoblast differentiation. *Molecules*. 2022;27(3):824. https://doi.org/10.3390/molecules27030824.
81 Zhang JK, Yang L, Meng GL, et al. Protection by salidroside against bone loss *via* inhibition of oxidative stress and bone-resorbing mediators. *PLoS One*. 2013;8(2):e57251. https://doi.org/10.1371/journal.pone.0057251.
82 Huang Q, Gao B, Jie Q, et al. Ginsenoside-Rb2 displays anti-osteoporosis effects through reducing oxidative damage and bone-resorbing cytokines during osteogenesis. *Bone*. 2014;66:306-314. https://doi.org/10.1016/j.bone.2014.06.010.
83 Huang Q, Shi J, Gao B, et al. Gastrodin: an ancient Chinese herbal medicine

as a source for anti-osteoporosis agents *via* reducing reactive oxygen species. *Bone*. 2015;73:132-144. https://doi.org/10.1016/j.bone.2014.12.059.
84 Huang Q, Gao B, Wang L, et al. Ophiopogonin D: a new herbal agent against osteoporosis. *Bone*. 2015;74:18-28. https://doi.org/10.1016/j.bone.2015.01.002.
85 Luo S, Yang Y, Chen J, et al. Tanshinol stimulates bone formation and attenuates dexamethasone-induced inhibition of osteogenesis in larval zebrafish. *J Orthop Translat*. 2016;4:35-45. https://doi.org/10.1016/j.jot.2015.07.002.
86 Casati L, Pagani F, Fibiani M, et al. Potential of delphinidin-3-rutinoside extracted from *Solanum melongena* L. as promoter of osteoblastic MC3T3-E1 function and antagonist of oxidative damage. *Eur J Nutr*. 2019;58(3):1019-1032. https://doi.org/10.1007/s00394-018-1618-0.
87 Li X, Lin H, Zhang X, et al. Notoginsenoside R1 attenuates oxidative stress-induced osteoblast dysfunction through JNK signalling pathway. *J Cell Mol Med*. 2021;25(24):11278-11289. https://doi.org/10.1111/jcmm.17054.
88 Hu HY, Zhang ZZ, Jiang XY, et al. Hesperidin anti-osteoporosis by regulating estrogen signaling pathways. *Molecules*. 2023;28(19):6987. https://doi.org/10.3390/molecules28196987.
89 Li Z, Li D, Su H, et al. Autophagy: an important target for natural products in the treatment of bone metabolic diseases. *Front Pharmacol*. 2022;13:999017. https://doi.org/10.3389/fphar.2022.999017.
90 Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol*. 2018;19(6):349-364. https://doi.org/10.1038/s41580-018-0003-4.
91 Wang J, Zhang Y, Cao J, et al. The role of autophagy in bone metabolism and clinical significance. *Autophagy*. 2023;19(9):2409-2427. https://doi.org/10.1080/15548627.2023.2186112.
92 Ceccariglia S, Cargnoni A, Silini AR, et al. Autophagy: a potential key contributor to the therapeutic action of mesenchymal stem cells. *Autophagy*. 2020;16(1):28-37. https://doi.org/10.1080/15548627.2019.1630223.
93 Ma Y, Qi M, An Y, et al. Autophagy controls mesenchymal stem cell properties and senescence during bone aging. *Aging Cell*. 2018;17(1):e12709. https://doi.org/10.1111/acel.12709.
94 Li H, Li D, Ma Z, et al. Defective autophagy in osteoblasts induces endoplasmic reticulum stress and causes remarkable bone loss. *Autophagy*. 2018;14(10):1726-1741. https://doi.org/10.1080/15548627.2018.1483807.
95 Weng YM, Ke CR, Kong JZ, et al. The significant role of ATG5 in the maintenance of normal functions of Mc3T3-E1 osteoblast. *Eur Rev Med Pharmacol Sci*. 2018;22(5):1224-1232. https://doi.org/10.26355/eurrev_201803_14462.
96 Nollet M, Santucci-Darmanin S, Breuil V, et al. Autophagy in osteoblasts is involved in mineralization and bone homeostasis. *Autophagy*. 2014;10(11):1965-1977. https://doi.org/10.4161/auto.36182.
97 Zhao B, Peng Q, Poon E, et al. Leonurine promotes the osteoblast differentiation of rat BMSCs by activation of autophagy *via* the PI3K/Akt/mTOR pathway. *Front Bioeng Biotechnol*. 2021;9:615191. https://doi.org/10.3389/fbioe.2021.615191.
98 Zhao B, Peng Q, Wang D, et al. Leonurine protects bone mesenchymal stem cells from oxidative stress by activating mitophagy through PI3K/Akt/mTOR pathway. *Cells*. 2022;11(11):1724. https://doi.org/10.3390/cells11111724.
99 Zheng X, Yu Y, Shao B, et al. Osthole improves therapy for osteoporosis through increasing autophagy of mesenchymal stem cells. *Exp Anim*. 2019;68(4):453-463. https://doi.org/10.1538/expanim.18-0178.
100 Zhang Y, Li M, Liu Z, et al. Arbutin ameliorates glucocorticoid-induced osteoporosis through activating autophagy in osteoblasts. *Exp Biol Med (Maywood)*. 2021;246(14):1650-1659. https://doi.org/10.1177/15353702211002136.
101 Zhang X, Huang F, Chen X, et al. Ginsenoside Rg3 attenuates ovariectomy-induced osteoporosis *via* AMPK/mTOR signaling pathway. *Drug Dev Res*. 2020;81(7):875-884. https://doi.org/10.1002/ddr.21705.
102 Shi Y, Liu XY, Jiang YP, et al. Monotropein attenuates oxidative stress *via* Akt/mTOR-mediated autophagy in osteoblast cells. *Biomed Pharmacother*. 2020;121:109566. https://doi.org/10.1016/j.biopha.2019.109566.
103 Liang X, Hou Z, Xie Y, et al. Icariin promotes osteogenic differentiation of bone marrow stromal cells and prevents bone loss in OVX mice *via* activating autophagy. *J Cell Biochem*. 2019;120(8):13121-13132. https://doi.org/10.1002/jcb.28585.
104 Bai L, Liu Y, Zhang X, et al. Osteoporosis remission *via* an anti-inflammaging effect by icariin activated autophagy. *Biomaterials*. 2023;297:122125. https://doi.org/10.1016/j.biomaterials.2023.122125.
105 Wang N, Xu P, Wu R, et al. Timosaponin BII improved osteoporosis caused by hyperglycemia through promoting autophagy of osteoblasts *via* suppressing the mTOR/NFκB signaling pathway. *Free Radic Biol Med*. 2021;171:112-123. https://doi.org/10.1016/j.freeradbiomed.2021.05.014.
106 Park KR, Kim B, Lee JY, et al. Effects of scoparone on differentiation, adhesion, migration, autophagy and mineralization through the osteogenic signalling pathways. *J Cell Mol Med*. 2022;26(16):4520-4529. https://doi.org/10.1111/jcmm.17476.
107 Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. *Nature*. 2019;571(7766):489-499. https://doi.org/10.1038/s41586-019-1411-0.
108 Ghayor C, Weber F. Epigenetic regulation of bone remodeling and its impacts in osteoporosis. *Int J Mol Sci*. 2016;17(9):1446. https://doi.org/10.3390/ijms17091446.
109 Reppe S, Datta H, Gautvik K. The influence of DNA methylation on bone cells. *Curr Genomics*. 2015;16(6):384-392. https://doi.org/10.2174/1389202916666150817202913.
110 Martin SL, Hardy TM, Tollefsbol TO. Medicinal chemistry of the epigenetic diet and caloric restriction. *Curr Med Chem*. 2013;20(32):4050-4059. https://doi.org/10.2174/09298673113209990189.
111 Husain A, Jeffries MA. Epigenetics and bone remodeling. *Curr Osteoporos Rep*. 2017;15(5):450-458. https://doi.org/10.1007/s11914-017-0391-y.
112 Visconti VV, Cariati I, Fittipaldi S, et al. DNA methylation signatures of bone metabolism in osteoporosis and osteoarthritis aging-related diseases: an updated review. *Int J Mol Sci*. 2021;22(8):4244. https://doi.org/10.3390/ijms22084244.
113 Altaf M, Saksouk N, Côté J. Histone modifications in response to DNA damage. *Mutat Res*. 2007;618(1-2):81-90. https://doi.org/10.1016/j.mrfmmm.2006.09.009.
114 Widmayer P, Xu Y, Zhu B. Combinatorial optimization and applications. *Theor Comput Sci*. 2014;556:1. https://doi.org/10.1016/j.tcs.2014.09.047.
115 Li CJ, Cheng P, Liang MK, et al. microRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation. *J Clin Invest*. 2015;125(4):1509-1522. https://doi.org/10.1172/JCI77716.
116 Nabeshima T, Tsukamoto M, Wang KY, et al. Delayed cortical bone healing due to impaired nuclear Nrf2 translocation in COPD mice. *Bone*. 2023;173:116804. https://doi.org/10.1016/j.bone.2023.116804.
117 Raut N, Wicks SM, Lawal TO, et al. Epigenetic regulation of bone remodeling by natural compounds. *Pharmacol Res*. 2019;147:104350. https://doi.org/10.1016/j.phrs.2019.104350.
118 Thaler R, Maurizi A, Roschger P, et al. Anabolic and antiresorptive modulation of bone homeostasis by the epigenetic modulator sulforaphane, a naturally occurring isothiocyanate. *J Biol Chem*. 2016;291(13):6754-6771. https://doi.org/10.1074/jbc.M115.678235.
119 Behera J, Ison J, Rai H, et al. Allyl sulfide promotes osteoblast differentiation and bone density *via* reducing mitochondrial DNA release mediated Kdm6b/H3K27me3 epigenetic mechanism. *Biochem Biophys Res Commun*. 2021;543:87-94. https://doi.org/10.1016/j.bbrc.2021.01.016.
120 Ornstrup MJ, Harsløf T, Kjær TN, et al. Resveratrol increases bone mineral density and bone alkaline phosphatase in obese men: a randomized placebo-controlled trial. *J Clin Endocrinol Metab*. 2014;99(12):4720-4729. https://doi.org/10.1210/jc.2014-2799.
121 Bo S, Gambino R, Ponzo V, et al. Effects of resveratrol on bone health in type 2 diabetic patients. *A double-blind randomized-controlled trial. Nutr Diabetes*. 2018;8(1):51. https://doi.org/10.1038/s41387-018-0059-4.
122 Bäckesjö CM, Li Y, Lindgren U, et al. Activation of Sirt1 decreases adipocyte formation during osteoblast differentiation of mesenchymal stem cells. *J Bone Miner Res*. 2006;21(7):993-1002. https://doi.org/10.1359/jbmr.060415.
123 Danks L, Komatsu N, Guerrini MM, et al. RANKL expressed on synovial fibroblasts is primarily responsible for bone erosions during joint inflammation. *Ann Rheum Dis*. 2016;75(6):1187-1195. https://doi.org/10.1136/annrheumdis-2014-207137.
124 Shakibaei M, Buhrmann C, Mobasheri A. Resveratrol-mediated SIRT-1 interactions with p300 modulate receptor activator of NF-κB ligand (RANKL) activation of NF-κB signaling and inhibit osteoclastogenesis in bone-derived cells. *J Biol Chem*. 2011;286(13):11492-11505. https://doi.org/10.1074/jbc.M110.198713.
125 Ali D, Chen L, Kowal JM, et al. Resveratrol inhibits adipocyte differentiation and cellular senescence of human bone marrow stromal stem cells. *Bone*. 2020;133:115252. https://doi.org/10.1016/j.bone.2020.115252.
126 Wang H, Hu Z, Wu J, et al. Sirt1 promotes osteogenic differentiation and increases alveolar bone mass *via* Bmi1 activation in mice. *J Bone Miner Res*. 2019;34(6):1169-1181. https://doi.org/10.1002/jbmr.3677.
127 Jiang Y, Luo W, Wang B, et al. Resveratrol promotes osteogenesis *via* activating SIRT1/FoxO1 pathway in osteoporosis mice. *Life Sci*. 2020;246:117422. https://doi.org/10.1016/j.lfs.2020.117422.
128 Ghorbaninejad M, Khademi-Shirvan M, Hosseini S, et al. Effective role of curcumin on expression regulation of EZH2 histone methyltransferase as a dynamic epigenetic factor in osteogenic differentiation of human mesenchymal stem cells. *Biochim Biophys Acta Gene Regul Mech*. 2023;1866(1):194903. https://doi.org/10.1016/j.bbagrm.2022.194903.
129 Arumugam B, Balagangadharan K, Selvamurugan N. Syringic acid, a phenolic acid, promotes osteoblast differentiation by stimulation of Runx2 expression and targeting of Smad7 by miR-21 in mouse mesenchymal stem cells. *J Cell Commun Signal*. 2018;12(3):561-573. https://doi.org/10.1007/s12079-018-0449-3.
130 Tanaka T, Kawaguchi N, Zaima N, et al. Antiosteoporotic activity of a syringic acid diet in ovariectomized mice. *J Nat Med*. 2017;71(4):632-641. https://doi.org/10.1007/s11418-017-1105-6.
131 Teng JW, Bian SS, Kong P, et al. Icariin triggers osteogenic differentiation of bone marrow stem cells by up-regulating miR-335-5p. *Exp Cell Res*. 2022;414(2):113085. https://doi.org/10.1016/j.yexcr.2022.113085.
132 Zhan XQ, Zeng XW, Zhang YY, et al. Puerarin promotes the viability and differentiation of MC3T3-E1 cells by miR-204-regulated Runx2 upregulation. *Mol Med Rep*. 2017;16(5):6262-6268. https://doi.org/10.3892/mmr.2017.7386.
133 Liu H, Yi X, Tu S, et al. Kaempferol promotes BMSC osteogenic differentiation and improves osteoporosis by downregulating miR-10a-3p and upregulating CXCL12. *Mol Cell Endocrinol*. 2021;520:111074. https://doi.org/10.1016/j.mce.2020.111074.
134 Almeida M, Laurent MR, Dubois V, et al. Estrogens and androgens in skeletal physiology and pathophysiology. *Physiol Rev*. 2017;97(1):135-187. https://doi.org/10.1152/physrev.00033.2015.
135 Wang Y, Wang WL, Xie WL, et al. Puerarin stimulates proliferation and differentiation and protects against cell death in human osteoblastic MG-63 cells *via* ER-dependent MEK/ERK and PI3K/Akt activation. *Phytomedicine*. 2013;20(10):787-796. https://doi.org/10.1016/j.phymed.2013.03.005.
136 Zhao Y, Xu Y, Zheng H, et al. QingYan Formula extracts protect against postmenopausal osteoporosis in ovariectomized rat model *via* active ER-

dependent MEK/ERK and PI3K/Akt signal pathways. *J Ethnopharmacol*. 2021;268:113644. https://doi.org/10.1016/j.jep.2020.113644.
137 Wu QC, Tang XY, Dai ZQ, et al. Sweroside promotes osteoblastic differentiation and mineralization *via* interaction of membrane estrogen receptor-α and GPR30 mediated p38 signalling pathway on MC3T3-E1 cells. *Phytomedicine*. 2020;68:153146. https://doi.org/10.1016/j.phymed.2019.153146.
138 Zhou L, Poon CC, Wong KY, et al. Icariin ameliorates estrogen-deficiency induced bone loss by enhancing IGF-I signaling *via* its crosstalk with non-genomic ERα signaling. *Phytomedicine*. 2021;82:153413. https://doi.org/10.1016/j.phymed.2020.153413.
139 Chen Q, Shou P, Zheng C, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? *Cell Death Differ*. 2016;23(7):1128-1139. https://doi.org/10.1038/cdd.2015.168.
140 Park E, Lim E, Yeo S, et al. Anti-menopausal effects of *Cornus officinalis* and *Ribes fasciculatum* extract *in vitro* and *in vivo*. *Nutrients*. 2020;12(2):369. https://doi.org/10.3390/nu12020369.
141 Ko CH, Siu WS, Wong HL, et al. Pro-bone and antifat effects of green tea and its polyphenol, epigallocatechin, in rat mesenchymal stem cells *in vitro*. *J Agric Food Chem*. 2011;59(18):9870-9876. https://doi.org/10.1021/jf202015t.
142 Peng H, Yang M, Guo Q, et al. *Dendrobium officinale* polysaccharides regulate age-related lineage commitment between osteogenic and adipogenic differentiation. *Cell Prolif*. 2019;52(4):e12624. https://doi.org/10.1111/cpr.12624.
143 An J, Yang H, Zhang Q, et al. Natural products for treatment of osteoporosis: the effects and mechanisms on promoting osteoblast-mediated bone formation. *Life Sci*. 2016;147:46-58. https://doi.org/10.1016/j.lfs.2016.01.024.
144 Yao H, Liu J, Xu S, et al. The structural modification of natural products for novel drug discovery. *Expert Opin Drug Discov*. 2017;12(2):121-140. https://doi.org/10.1080/17460441.2016.1272757.
145 Wani TU, Khan RS, Rather AH, et al. Local dual delivery therapeutic strategies: using biomaterials for advanced bone tissue regeneration. *J Control Release*. 2021;339:143-155. https://doi.org/10.1016/j.jconrel.2021.09.029.
146 Mora-Raimundo P, Lozano D, Benito M, et al. Osteoporosis remission and new bone formation with mesoporous silica nanoparticles. *Adv Sci (Weinh)*. 2021;8(16):e2101107. https://doi.org/10.1002/advs.202101107.
147 Yousefzadeh N, Kashfi K, Jeddi S, et al. Ovariectomized rat model of osteoporosis: a practical guide. *EXCLI J*. 2020;19:89-107. https://doi.org/10.17179/excli2019-1990.
148 Sophocleous A, Idris AI. Rodent models of osteoporosis. *Bonekey Rep*. 2014;3:614. https://doi.org/10.1038/bonekey.2014.109.
149 Xavier A, Toumi H, Lespessailles E. Animal model for glucocorticoid induced osteoporosis: a systematic review from 2011 to 2021. *Int J Mol Sci*. 2021;23(1):377. https://doi.org/10.3390/ijms23010377.
150 Dietrich K, Fiedler IA, Kurzyukova A, et al. Skeletal biology and disease modeling in zebrafish. *J Bone Miner Res*. 2021;36(3):436-458. https://doi.org/10.1002/jbmr.4256.
151 Lleras-Forero L, Winkler C, Schulte-Merker S. Zebrafish and medaka as models for biomedical research of bone diseases. *Dev Biol*. 2020;457(2):191-205. https://doi.org/10.1016/j.ydbio.2019.07.009.
152 Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nat Rev Drug Discov*. 2021;20(8):611-628. https://doi.org/10.1038/s41573-021-00210-8.
153 Ma J, Chen Y, Song J, et al. Establishment and application of a zebrafish model of Werner syndrome identifies sapanisertib as a potential antiaging drug. *Proc Natl Acad Sci U S A*. 2025;122(5):e2413719122. https://doi.org/10.1073/pnas.2413719122.
154 Tan L, Tu Y, Miao Z, et al. Glycyrol alleviates osteoporosis through dual modulation on osteoclastogenesis and osteogenesis by targeting Syk signaling pathway. *Phytomedicine*. 2025;138:156429. https://doi.org/10.1016/j.phymed.2025.156429.
155 Cui J, Shibata Y, Zhu T, et al. Osteocytes in bone aging: advances, challenges, and future perspectives. *Ageing Res Rev*. 2022;77:101608. https://doi.org/10.1016/j.arr.2022.101608.
156 Metzger TA, Kreipke TC, Vaughan TJ, et al. The *in situ* mechanics of trabecular bone marrow: the potential for mechanobiological response. *J Biomech Eng*. 2015;137(1):28985. https://doi.org/10.1115/1.4028985.
157 Chai S, Wan L, Wang JL, et al. Gushukang inhibits osteocyte apoptosis and enhances BMP-2/Smads signaling pathway in ovariectomized rats. *Phytomedicine*. 2019;64:153063. https://doi.org/10.1016/j.phymed.2019.153063.
158 Izzo AA, Hoon-Kim S, Radhakrishnan R, et al. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytother Res*. 2016;30(5):691-700. https://doi.org/10.1002/ptr.5591.