REVIEW

New Molecules Modulating Bone Metabolism – New Perspectives in the Treatment of Osteoporosis

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Summary

In this review the authors outline traditional antiresorptive pharmaceuticals, such as bisphosphonates, monoclonal antibodies against RANKL, SERMs, as well as a drug with an anabolic effect on the skeleton, parathormone. However, there is also a focus on non-traditional strategies used in therapy for osteolytic diseases. The newest antiosteoporotic pharmaceuticals increase osteoblast differentiation *via* BMP signaling (harmine), or stimulate osteogenic differentiation of mesenchymal stem cells through Wnt/ β -catenin (icarrin, isoflavonoid caviunin, or sulfasalazine). A certain promise in the treatment of osteoporosis is shown by molecules targeting non-coding microRNAs (which are critical for osteoclastogenesis) or those stimulating osteoblast activity *via* epigenetic mechanisms. Vitamin D metabolites have specific antiosteoporotic potencies, modulating the skeleton not only *via* mineralization, but markedly also through the direct effects on the bone microstructure.

Key words

RANKL • Sclerostin • Cathepsin K • Wnt/ β -catenin • Estrogen • SERM • Bisphosphonates • Parathormone • Vitamin D • MicroRNA • Bone mineralization • Bone microarchitecture

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Introduction

Osteoporosis is a serious public health problem. In the Czech Republic around 500,000 women and 200,000 men are affected by the disease. The

osteoporotic population is endangered by increased morbidity and mortality due to fractures and their deteriorated healing, primarily in elderly, frail patients with a number of comorbidities and medicament therapies. Genetically determined both sensitivity and the side effects of therapy may complicate the choice of suitable long-term antiosteoporotic treatment in individual patients. This article reviews traditionally used antiosteoporotics and some recently discovered molecules with beneficial effects on the skeleton.

Under physiological conditions, bone is permanently remodeled to maintain its volume, microstructure and strength. When bone resorption predominates over formation, bone mass is endangered by progressive loss. The first choice of treatment in this case are usually bisphosphonates, drugs discovered 45 years ago. Bisphosphonates antagonize osteoclastogenesis through various mechanisms of action (Mac-*Way et al.* 2014, Nataka *et al.* 2015). Long-term oral or parenteral bisphosphonate therapy increases bone mineral density (BMD), bone volume, reduces bone porosity and markedly decreases fracture risk. A feature of this treatment is also its low cost. However, bisphosphonate therapy may be complicated by some serious side effects. Besides gastrointestinal intolerance, both oral and parenteral bisphosphonates may induce osteonecrosis of the jaw and/or atypical femoral fractures, partly explained by the inhibition of healing near bone cracks (Pazianas *et al.* 2016, Adler 2016). Furthermore, bisphosphonates are only marginally effective in patients with severe loss of bone mass, although effectivity of treatment may be improved by an advantageous pharmaceutical scheme, in

which bisphosphonate modified nanomaterials (conjugates with protein and polymers) directly target the bone and optimize drug delivery control (Ossipov 2015). The benefit/risk ratio in bisphosphonate therapy should be taken into account during the long-term process, which often requires more than one type of antiresorptive treatment.

Selective estrogen receptors modulators (SERMs) and their new analogs represent effective variant of antiresorptive therapy. With the exception of tamoxifen, raloxifene and lasofoxifene, a third generation molecule – bazedoxifene was synthesized. The latter drug significantly reduced the incidence of both vertebral and nonvertebral fractures in postmenopausal osteoporotic women. The pharmaceutical has been found safe in relation to endometrial and breast cancerogenesis (Komm and Chines 2012, An 2016).

Biological therapy has a good potential for increasing bone mass. The monoclonal antibody against RANKL, denosumab has positive effects on the microarchitecture of the skeleton (inhibition of erosion development and increase in cortical thickness and bone stiffness) (Bonani *et al.* 2016). Kamimura *et al.* (2017) showed inhibition of bone resorption and an increase in lumbar and hip BMD after one year of treatment with denosumab in patients unresponsive to bisphosphonate. Nevertheless, similar to bisphosphonates, a certain limitation of long-term denosumab treatment may be the reduced renewal of bone mass with subsequent osteonecrosis of the jaw and/or atypical femoral fractures (Qaisi *et al.* 2016).

Any inhibition of the cytokine network appears to slow down bone loss. Zerbin *et al.* (2017) analyzed clinical studies, in which patients with rheumatoid arthritis were treated with anti-TNF antibodies (such as infliximab, adalimumab, etanercept), or IL-1 and IL-6 antagonists (abatacept or rituximab). Although some patients in this study showed significant increase in BMD, others remained unaffected. Moreover, there was no consensus regarding their effects on fracture risk.

Bone formation is known to be stimulated *via* the Wnt/ β -catenin pathway (Mosekilde *et al.* 2011). This pathway (expressed by osteocytes) is under the control of the SOST gene (located at chromosome region 17q12-q21) and inhibited by sclerostin. Mutations in SOST are known to be associated with low sclerostin level and increased bone mass. Undetectable sclerostin levels are manifested as autosomal-recessive sclerosteosis (Yavropoulou *et al.* 2014). On the other hand, bone

diseases, such as osteoporosis, bone metastases and genetically dependent low bone mass, are often associated with sclerostin overproduction.

The inconvenience of antiresorption therapy could be eliminated by using exclusively anabolic pharmaceuticals, such as intact PTH (1-84) (preotact) or PTH (1-34) (teriparatide). At the present time, teriparatide is the only currently available bone anabolic molecule working *via* the inhibition of sclerostin and other metabolic circuits, such as DKK1 and/or frizzled protein. Small pulsatile doses of exogenous PTH increase BMD, improve microarchitecture and bone strength (Mosekilde *et al.* 2011), which results in lowering the fracture risk (reviewed by Tella and Gallagher 2014, Ishitaq *et al.* 2015). Cancellous bone density increased and trabecular separation decreased in postmenopausal women with osteoporosis treated with teriparatide irrespective of previous bisphosphonate administration (Fahrleitner-Pammer *et al.* 2016). There is no consensus on teriparatide treatment in osteoporotic diabetics. However, incretin derivatives, such as GLP-1 and GIP, appear to have good prospects in the prevention or treatment of osteoporosis in diabetics. After binding to osteoblastic receptors, there is an increase in bone formation (Nuche-Berenguer *et al.* 2009, Yamada 2012).

An alternative to exogenous PTH is the endogenous molecule, the secretion of which is stimulated through the inhibition of the calcium sensing receptor (CASR) (Widler 2011). A strong anabolic effect on the skeleton was also shown in PTH fragments, and PTHrP analog (abaloparatide), which are currently under investigation (Yamaguchi 2016).

Effectivity on fracture healing is strengthened when teriparatide is combined with other antiosteoporotics. Casanova *et al.* (2016), using micro CT and quantitative histomorphometry showed that a three week administration of teriparatide together with zoledronic acid significantly increased bone volume and reduced trabecular spacing in mice with operatively induced fractures. Leder *et al.* (2016) in a randomized control trial described more significant increases in BMD at the hip and at the lumbar spine in postmenopausal women treated for two years with teriparatide and denosumab, when compared with women on single administration of these medicaments. Furthermore, better fracture repair could be obtained using a combination of teriparatide and anti-sclerostin and/or anti-cathepsin K antibodies (Tella and Gallagher 2014).

Further new molecules with antisclerostin effects

Subcutaneous administration of monoclonal antisclerostin antibodies, such as AbD09097, stimulates bone formation and increases bone mass through activation of the Wnt pathway, independently of bone remodeling (Boschert *et al.* 2016, Yavropoulou *et al.* 2014). Bone anabolic effects also have other antibodies against sclerostin, such as romosozumab or blosozumab. Both these drugs were successfully tested experimentally (reviewed by Tella and Gallagher 2014) and clinical trials in phase II are under way (Matsumoto 2015). Recently, the dose dependent stimulating effect of romosozumab on BMD at the spine was shown in 419 women with postmenopausal osteoporosis. The effect was markedly higher when compared with oral alendronate or the teriparatide (Larsson 2016). Similar effects of romosozumab on volumetric BMD at vertebral and total hip regions were observed in postmenopausal women by Genant *et al.* (2016). Additionally, a placebo controlled study on 7180 postmenopausal women showed, that romosozumab significantly decreased the risk of vertebral fractures after 12 months of treatment (Cosman *et al.* 2016).

Substances with anti-cathepsin effects

A recently discovered molecule with an antiosteoporotic effect is the monoclonal antibody against cathepsin K (CTSK) (Chan *et al.* 2016). Cathepsin K is a proteolytic enzyme, which degrades collagen I in the bone matrix and activates bone resorption. The cathepsin K antibody, due to the coupling between bone resorption and bone formation, inhibits both these processes, but more markedly bone resorption and only transiently bone formation. Long-term administration of cathepsin K antibodies, such as odanacatib, increased BMD and decreased the fracture rate (Boonen *et al.* 2012, Tella and Gallagher 2014). Some other cathepsin K inhibitors were evaluated, which are mostly in preclinical trials (Brömme *et al.* 2016).

Further non-traditional molecules with antiosteoporotic potential

Osteoclast formation is increased after the activation of T-cells through NF- κ B, NFATc1 or c-Fos signaling. In bone tissue culture, this process was

inhibited by β -carboline alkaloid harmine. Additionally, the alkaloid increased osteoblast differentiation *via* Runx2, osterix and bone morphogenetic peptide (BMP) (Yonezawa *et al.* 2011). Thus, harmine inhibits bone resorption and simultaneously activates bone formation. According to our knowledge, no study analyzing antiosteoporotic effectivity of the alkaloid *in vivo* has been published yet.

Certain antiosteoporotic activity was recorded in neoflavonoids, isolated chromatographically from *Dalbergia sissoo* heartwood. The flavonoids significantly stimulated calvarial osteoblast proliferation and mineralization (Kumar *et al.* 2014). Similarly, caviunin-based isoflavonoid stimulates bone formation *via* BMP2 and Wnt/ β -catenin pathways, effectively inhibits osteoclastogenesis and repairs cortical bone. In ovariectomized mice caviunin increased the mechanical strength of the vertebra and femur (Kushwaha *et al.* 2014). Similar anabolic effects on the skeleton mediated by Wnt/ β -catenin signaling have been registered experimentally in aglycone of icariin. Micro-CT analysis showed that icariine after 12-weeks of treatment increased BMD, trabecular bone number, trabecular thickness, reduced trabecular separation and increased biomechanical strength in oophorectomized rats (Chen *et al.* 2016). Some flavonoids could be positioned as potential pharmaceuticals or food supplements for fracture repair in postmenopausal osteoporosis.

Strong bioactivity in the culture of osteoblast-like cells has been shown in the three-dimensional calcium-bearing structure CaP1 (which has three molecules of water). *In vivo*, the substance increased bone mineralization without any toxicity (Shi *et al.* 2015). Bone regenerative effects were also found in synthetic diether molecules inhibiting RANKL-induced osteoclast formation (Doh *et al.* 2016), as well as in octacalcium phosphate, which increased bone mineralization *via* an irreversible transition into hydroxyapatite (Suzuki *et al.* 2008).

Furthermore, the food-derived compound sulforaphane and natural isothiocyanate promote osteoblast activity *via* epigenetic mechanisms. The molecule activates DNA demethylation increasing matrix mineralization. In mice it stimulates the expression of osteoblastic markers, such as Runx2, collagen 1A1 or ALP1, while inhibiting the nuclear factor- κ B (RANKL) in osteocytes with subsequent increases in the trabecular number (Thaler *et al.* 2016). New strategies in therapy for osteolytic diseases consist of targeting non-coding

microRNAs (miRNAs), which control gene expression in osteoclasts. Thus, miRNAs appear to be the key molecules in the regulation of bone resorption (Li *et al.* 2016). Bone homeostasis is determined by the osteogenesis/adipogenesis ratio in mesenchymal cells. Prevailing adipogenesis over osteogenesis is a principle pathological factor in accelerated bone loss. A strong modulator of osteogenic differentiation is the glutamate exchanger xCT (SLC7A11) sulfasalazine, which enhances the osteogenic potential *via* an increase in BMP2/4 expression. Sulfasalazine administered *in vivo* inhibited bone loss in hypoenestrogenic mice (Jin *et al.* 2016). Thus, sulfasalazine is a further candidate useful in the treatment of postmenopausal osteoporosis.

Potential bone protecting candidates are also growth factors, such as BMP, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) (Lee *et al.* 2015). Some of these mediate the action of other molecules (Jin *et al.* 2016). The beneficial effects of erythropoietin or statins on fracture healing are under investigation; however, sufficient evidence of their antiosteoporotic action *in vivo* is still lacking (Klontzas *et al.* 2016).

New aspects of the bone protecting effects of vitamin D

Vitamin D positively influences not only the mineralization of the bone matrix, but *via* genomic and non-genomic effects modulates the function of some non-skeletal systems, including muscles. D-hormone metabolites have been shown to influence bone homeostasis directly. Bioactive 25(OH) D₃, 1,25 (OH)₂D₃, as well as 24R,25 (OH)₂D₃ stimulated osteoblast growth and differentiation *in vitro* (van der Meijden *et al.* 2014). 1,25(OH)₂ D₃ administered *in vivo* for 28 consecutive days, significantly increased bone formation, reduced bone resorption and increased trabecular bone volume in mice (Oelzner *et al.* 2014). Long-term treatment with 1 α ,25[OH](2)-2 β -(3-hydroxypropyloxy)vitamin D₃ metabolite (eldecalcitol)

suppressed bone turnover, decreased the risk of bone microstructure deterioration and increased bone biomechanical strength in ovariectomized rats (Takeda *et al.* 2015). Yamasaki *et al.* (2015) found that eldecalcitol increased bone formation at the endocortical surface in female rats.

In clinical studies, significant increases in BMD in the spine of osteopenic women were found at the end of the 1st, 2nd and 3rd years of treatment with 1,25(OH)₂D₃, while no positive effects in cholecalciferol treated women were observed (Zofkova and Hill 2007). Thus, it can be said that D-hormone metabolites have unambiguously positive effects on bone mass and microstructure.

Conclusion

Although a relatively good spectrum of molecules with osteogenic potential is available at the present time, their effects (positive or even detrimental) on the skeleton may differ in individual subjects depending on their genetic predisposition. New strategies in the therapy of osteolytic diseases using drugs with satisfactory beneficial potential for the skeleton and absent or minimal side effects are necessary. Identification of candidate genes (e.g. LRP5, PSL3 and WNT1) associated with the response of bone mass and bone quality to individual pharmaceuticals will make it possible to personalize curative strategies (reviewed by Rocha-Braz and Ferraz-de-Souza 2016, Zofkova *et al.* 2015).

Conflict of Interest

There is no conflict of interest.

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References

- ADLER RA: Bisphosphonates and atypical femoral fractures. *Curr Opin Endocrinol Diabetes Obes* **23**: 430-434, 2016.
- AN KC: Selective estrogen receptor modulators. *Asian Spine J* **10**: 787-791, 2016.
- BONANI M, MEYER U, FREY D, GRAF N, BISCHOFF-FERRARI HA, WÜTHRICH RP: Effect of denosumab on peripheral compartmental bone density, microarchitecture and estimated bone strength in de novo kidney transplant recipients. *Kidney Blood Press Res* **41**: 614-622, 2016.

- BOONEN S, ROSENBERG E, CLAESSENS F, VANDERSCHUEREN D, PAPAPOULOS S: Inhibition of cathepsin K for treatment of osteoporosis. *Curr Osteoporos Rep* **10**: 73-79, 2012.
- BOSCHERT V, FRISCH D, BACK JW, VAN PEE K, WEIDAUER SE, MUTH EM, SCHMIEDER P, BEERBAUM M, KNAPPIK A, TIMMERMAN P, MUELLER TD: The sclerostin-neutralizing antibody AbD09097 recognizes an epitope adjacent to sclerostin's binding site for the Wnt co-receptor LRP6. *Open Biol* **6**: 160120, 2016.
- BRÖMME D, PANWAR P, TURAN S: Cathepsin K osteoporosis trials, pycnodysostosis and mouse deficiency models: commonalities and differences. *Expert Opin Drug Discov* **11**: 467-472, 2016.
- CASANOVA M, HERELLE J, THOMAS M, SOFTLEY R, SCHINDELER A, LITTLE D, SCHNEIDER P, MÜLLER R: Effect of combined treatment with zoledronic acid and parathyroid hormone on mouse bone callus structure and composition. *Bone* **92**: 70-78, 2016.
- CHAN CK, MASON A, COOPER C, DENNISON E: Novel advances in the treatment of osteoporosis. *Br Med Bull* **118**: 129-142, 2016.
- CHEN G, WANG C, WANG J, YIN S, GAO H, XIANG LU, LIU H, XIONG Y, WANG P, ZHU X, YANG LI, ZHANG R: Antiosteoporotic effect of icariin in ovariectomized rats is mediated via Wnt/ β -catenin pathway. *Exp Ther Med* **12**: 279-287, 2016.
- COSMAN F, CRITTENDEN DB, ADACHI JD, BINKLEY N, CZERWINSKI E, FERRARI S, HOFBAUER LC, LAU E, LEWIECKI EM, MIYAUCHI A, ZERBINI CA, MILMONT CE, CHEN L, MADDUX J, MEISNER PD, LIBANATI C, GRAUER A: Romosozumab treatment in postmenopausal women with osteoporosis. *N Engl J Med* **375**: 1532-1543, 2016.
- DOH KE, KANG JH, TING Z, YIM M, CHOO HY: Novel diether compounds inhibiting differentiation of osteoclasts. *Arch Pharm Res* **39**: 178-190, 2016.
- FAHRLEITNER-PAMMER, BURR D, DOBNIG H, STEPAN JJ, PETTO H, LI J, KREGGE JH, PAVO I: Improvement of cancellous bone microstructure in patients on teriparatide following alendronate pretreatment. *Bone* **89**: 16-24, 2016.
- GENANT HK, ENGELKE K, BOLOGNESE MA, MAUTALEN C, BROWN JP, RECKNOR C, GOEMAERE S, FUERST T, YANG YC, GRAUER A, LIBANATI C: Effects of romosozumab compared with teriparatide on bone density and mass at the spine and hip in postmenopausal women with low bone mass. *J Bone Miner Res* **32**: 181-187, 2016.
- ISHTIAQ S, FOGELMAN I, HAMPSON G: Treatment of post-menopausal osteoporosis: beyond bisphosphonates. *J Endocrinol Invest* **38**: 13-29, 2015.
- JIN C, ZHANG P, ZHANG M, ZHANG X, LV L, LIU H, LIU Y, ZHOU Y: Inhibition of SLC7A11 by sulfasalazine enhances osteogenic differentiation of mesenchymal stem cells by modulating BMP2/4 expression and suppresses bone loss in ovariectomized mice. *J Bone Mineral Res* **32**: 508-521, 2016.
- KAMIMURA M, NAKAMURA Y, IKEGAMI S, UCHIYAMA S, KATO H, TAGUCHI A: Significant improvement of bone mineral density and bone turnover markers by denosumab therapy in bisphosphonate-unresponsive patients. *Osteoporos Int* **28**: 559-566, 2017.
- KLONTZAS ME, KENANIDIZ EI, MACFARLANE RJ, MICHAEL T, POTOUPNIS ME, HELIOTIS M, MANTALARIS A, TSIRIDIS E: Investigational drugs for fracture healing: preclinical and clinical data. *Expert Opin Investig Drugs* **25**: 585-596, 2016.
- KOMM BS, CHINES AA: Bazedoxifene: the evolving role of third-generation selective estrogen-receptor modulators in the management of postmenopausal osteoporosis. *Ther Adv Musculoskelet Dis* **4**: 21-34, 2012.
- KUMAR P, KUSHWAHA P, KHEDGIKAR V, GAUTAM J, CHOUDHARY D, SINGH D, TRIVEDI R, MAURYA R: Neoflavonoids as potential osteogenic agents from Dalbergia sissoo heathwood. *Bioorg Med Chem Lett* **24**: 2664-2668, 2014.
- KUSHWAHA P, KHEDGIKAR V, GAUTAM J, DIXIT P, CHILLARA R, VERMA A, THAKUR R, MISHRA DP, SINGH D, MAURYA R, CHATTOPADHYAY N, MISHRA PR, TRIVEDI R: A novel therapeutic approach with Caviunin-based isoflavonoid that *en routes* bone marrow cells to bone formation via BMP2/Wnt- β -catenin signaling. *Cell Death Dis* **5**: e1422, 2014.
- LARSSON S: Anti-sclerostin – is there an indication? *Injury* **47** (Suppl 1): S31-S35, 2016.

- LEDER BZ, TSAI JN, NEER RM, UIHLEIN AV, WALLACE PM, BURNETT-BOWIE SA: Response to therapy with teriparatide, denosumab, or both in postmenopausal women in the DATA (denosumab and teriparatide administration) Study Randomized Controlled Trial. *J Clin Densitom* **19**: 346-351, 2016.
- LEE ZH, KIM HJ, RYOO HM: A novel osteogenic activity of suberoylanilide hydroxamic acid is synergized by BMP-2. *J Bone Metab* **22**: 51-56, 2015.
- LI H, ZHAI Z, QU X, XU J, QIN A, DAI K: MicroRNAs as potential targets for treatment of osteoclast-related diseases. *Curr Drug Targets* in press 2016.
- MAC-WAY F, TROMBETTI A, NOEL C, LAFAGE-PROUST MH: Giant osteoclasts in patients under bisphosphonates. *BMC Clin Pathol* **14**: 31, 2014.
- MATSUMOTO T: Pharmacology of bone anabolic agents. *Nihon Rinsho* **73**: 1639-1644, 2015.
- MOSEKILDE L, TORRING O, REJNMARK L: Emerging anabolic treatments in osteoporosis. *Curr Drug Saf* **6**: 62-74, 2011.
- NAGAOKA Y, KAJIYA H, OZEKI S, IKEBE T, OKABE K: Mevalonates restore zoledronic acid-induced osteoclastogenesis inhibition. *J Dent Res* **94**: 594-601, 2015.
- NUCHE-BERENGUER B, MORENO P, ESBRI T, DAPIA S, CAREIRO JR, CANCELAS J, HARO-MORA JJ, VILLANUEVA-PENACRILLO ML: Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states. *Calcif Tissue Int* **84**: 453-461, 2009.
- OELZNER P, PETROW PK, WOLF G, BRÄUER R: 1,25-dihydroxyvitamin D3 prevents bone loss of the secondary spongiosa in arthritic rats by an increase of bone formation and mineralization and inhibition of bone resorption. *BMC Musculoskelet Disord* **15**: 345, 2014.
- OSSIPOV DA: Bisphosphonate-modified biomaterials for drug delivery and bone tissue engineering. *Expert Opin Drug Deliv* **12**: 1443-1458, 2015.
- PAZIANAS M, ABRAHAMSEN B: Osteoporosis treatment: bisphosphonates reign to continue for a few more years, at least? *Ann N Y Acad Sci* **1376**: 5-13, 2016.
- QAISI M, HARGETT J, LOEB M, BROWN J, CALOSS R: Denosumab related osteonecrosis of the jaw with spontaneous necrosis of the soft palate: report of a life threatening case. *Case Rep Dent* **2016**: 5070187, 2016.
- ROCHA-BRAZ MG, FERRAZ-DE-SOUZA B: Genetics of osteoporosis: searching for candidate genes for bone fragility. *Arch Endocrinol Metab* **60**: 391-401, 2016.
- SHI FN, ALMEIDA JC, HELGUERO LA, FERNANDES MH, KNOWLES JC, ROCHA J: Calcium phosphonate frameworks for treating bone tissue disorders. *Inorg Chem* **54**: 9929-9935, 2015.
- SUZUKI O, IMAIZUMI H, KAMAKURA S, KATAGIRI T: Bone regeneration by synthetic octacalcium phosphate and its role in biological mineralization. *Curr Med Chem* **15**: 305-313, 2008.
- TAKEDA S, SMITH SY, TAMURA T, SAITO H, TAKAHASHI F, SAMADFAM R, HAILE S, DOYLE N, ENDO K: Long-term treatment with eldecacitol (1 α , 25-dihydroxy-2 β -(3-hydroxypropyloxy) vitamin D3) suppresses bone turnover and leads to prevention of bone loss and bone fragility in ovariectomized rats. *Calcif Tissue Int* **96**: 45-55, 2015.
- TELLA SH, GALLAGHER JC: Biological agents in management of osteoporosis. *Eur J Clin Pharmacol* **70**: 1291-1301, 2014.
- THALER R, MAURIZI A, ROSCHGER P, STURMLECHNER I, KHANI F, SPITZER S, RUMPLER M, ZWERINA J, KARLIC H, DUDAKOVIC A, KLAUSHOFFER K, TETI A, RUCCI N, VARGA F, VAN WIJNEN AJ: Anabolic and antiresorptive modulation of bone homeostasis by the epigenetic modulator sulforaphane, a naturally occurring isothiocyanate. *J Biol Chem* **291**: 6754-6771, 2016.
- VAN DER MEIJDEN K, LIPS P, VAN DRIEL M, HEIJBOER AC, SCHULTEN EA, DEN HEIJER M, BRAVENBOER N: Primary human osteoblasts in response to 25-hydroxyvitamin D3, 1,25-dihydroxyvitamin D3 and 24R,25-dihydroxyvitamin D3. *PLoS One* **9**: e110283, 2014.
- WIDLER L: Catalytics: antagonists of the calcium-sensing receptor for the treatment of osteoporosis. *Future Med Chem* **3**: 535-547, 2011.
- YAMADA Y: Diabetes mellitus and osteoporosis. Incretin as a coordinator of glucose and bone metabolism. *Clin Calcium* **22**: 1353-1358, 2012.
- YAMAGUCHI M: The importance of teriparatide in the treatment of osteoporosis. *Clin Calcium* **26**: 905-913, 2016.

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- YAMASAKI Y, NAGIRA K, OSAKI M, NAGASHIMA H, HAGINO H: Effects of eldecacitol on cortical bone response to mechanical loading in rats. *BMC Musculoskelet Disord* **16**: 158, 2015.
- YAVROPOULOU MP, XYGONAKIS C, LOLOU M, KARADIMOU F, YOVOS JG: The sclerostin story: from human genetics to the development of novel anabolic treatment for osteoporosis. *Hormones (Athens)* **13**: 323-337, 2014.
- YONEZAWA T, LEE JW, HIBINO A, ASAI M, HOJO H, CHA BY, TERUYA T, NAGAI K, CHUNG UI, YAGASAKI K, WOO JT: Harmine promotes osteoblast differentiation through bone morphogenetic protein signaling. *Biochem Biophys Res Commun* **409**: 260-265, 2011.
- ZERBINI CA, CLARK P, MENDEZ-SANCHEZ L, PEREIRA RM, MESSINA OD, UÑA CR, ADACHI JD, LEMS WF, COOPER C, LANE NE, IOF CHRONIC INFLAMMATION AND BONE STRUCTURE (CIBS) WORKING GROUP: Biologic therapies and bone loss in rheumatoid arthritis. *Osteoporos Int* **28**: 429-446, 2017.
- ZOFKOVA I, HILL M: Long-term 1,25(OH)₂ vitamin D therapy increases bone mineral density in osteopenic women. Comparison with the effect of plain vitamin D. *Aging Clin Exp Res* **19**: 472-477, 2007.
- ZOFKOVA I, NEMCIKOVA P, KUKLIK M: Polymorphisms associated with low bone mass and high risk of atraumatic fracture. *Physiol Res* **64**: 621-631, 2015.
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