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ORIGINAL ARTICLE

The effect of the most abundant bioactive compounds of the mediterranean diet on the osteoblast-mediated osteoclast differentiation signaling pathway: *in silico* analysis

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Summary

Introduction: Bones are fundamental components of the skeletal system that undergo continuous remodeling throughout life. Osteoclasts are responsible for bone resorption, while osteoblasts form new bone matrix and indirectly influence osteoclast differentiation through signaling pathways involving molecules such as M-CSF, c-Fms, GRB2, ERK, PI3K, and Akt. To maintain bone health, increasing attention is being given to nutritional interventions, including the Mediterranean diet, which is rich in bioactive compounds. The aim of this study was to perform an *in silico* analysis of potential interactions between the most prevalent bioactive compounds of the Mediterranean diet and the key proteins of the osteoclast differentiation signaling pathway.

Materials and Methods: A library comprising 115 predominant bioactive compounds from the Mediterranean diet was constructed based on literature data. Protein structures obtained via crystallography were used as targets in molecular docking simulations to predict interactions between ligands and receptors.

Results: Most ligands did not show significant interactions with the investigated proteins (ΔG > –7 kcal/mol), except in the case of Akt protein. For this protein, a larger number of ligands demonstrated ΔG values < –7 kcal/mol. The five most potent ligands (salvianolic acid, apigenin, diosmin, mangiferin and sylbin) bound to amino acid sequences forming the active site of the receptor, indicating a potential inhibitory effect.

Conclusion: The *in silico* analysis suggests that bioactive components of the Mediterranean diet possess potential to inhibit Akt protein (protein kinase B), thereby opening new perspectives for their application in modulating osteoclast activity and developing novel strategies for maintaining bone health.

Keywords: Mediterranean diet; bioactive compounds; osteoblasts; osteoclasts; protein kinase B

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INTRODUCTION

The skeletal system forms the structural framework of the body, which, in functional terms, together with the muscular system, enables movement. Bones, as the principal and most abundant anatomical components of the skeletal system, are continuously remodeled and adapt to mechanical loads that the body endures. This process depends on a delicate balance between the activities of osteoclasts and osteoblasts. In this process, osteoclasts perform bone resorption, while osteoblasts create new bone matrix and directly influence osteoclast differentiation (1–3).

One of the signaling pathways responsible for osteoclast differentiation, orchestrated by osteoblasts, begins with the binding of M-CSF (macrophage colony-stimulating factor) to the receptor with a tyrosine kinase domain, c-Fms (colony-stimulating factor 1 receptor), on the surface of osteoclast precursor cells. As a result of this activation, autophosphorylation occurs on specific tyrosine residues in the intracellular part of the receptor, after which the pathway divides into two branches (4–5). In one branch, phosphorylated c-Fms leads to the recruitment and activation of the adapter protein GRB2 (growth factor receptor-bound protein 2). Activated GRB2 further triggers a cascade of (de)phosphorylation mediated by MAP (mitogen-activated protein) kinases, ultimately activating ERK (extracellular signal-regulated kinase). Phosphorylated ERK enters the nucleus of precursor cells and regulates the expression of genes crucial for the proliferation and survival of osteoclast precursors.

In the other branch, phosphorylated c-Fms activates PI3K (phosphoinositide 3-kinase), which catalyzes the addition of a phosphate group to phosphatidylinositol-4,5-bisphosphate (PIP2), converting it into phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 enables the translocation of protein kinase B (Akt) to the membrane, where it is phosphorylated and activated by PDK1 (phosphoinositide-dependent kinase 1). Activated protein kinase B inhibits pro-apoptotic proteins such as Bax and FoxO, thereby promoting cell survival. It also leads to cytoskeletal reorganization, which is necessary for the motility of osteoclast precursors and prepares them for fusion and formation of mature osteoclasts **(Figure 1)**.

Excessive activation of these signaling pathways can lead to increased bone resorption. Increased osteoclast activity can occur in various conditions such as growth, fractures, osteoporosis, and others. In osteoporosis, an imbalance arises between bone resorption and new bone formation, resulting in reduced bone density, making bones more prone to fractures (6–7).

In order to stimulate bone health and correct various osteological pathological conditions, different interventions are undertaken, among which nutritional approaches are increasingly highlighted. One such intervention is the introduction of the Mediterranean diet, which has shown numerous positive effects on bone health (8–10). The Mediterranean diet is based on the traditional habits of the Mediterranean region's population and consists of a high intake of fruits, vegetables, whole grains, olive oil, nuts, legumes, and seeds (10, 11). The consumption of red meat, processed foods, and sugars is limited to a few times per month, while fish and seafood are consumed regularly. Moderate consumption of dairy products and red wine is also recommended (11). The Mediterranean diet is rich in calcium and vitamin D, which are crucial for bone mineralization and maintenance of bone density (12). Components from olive oil and fish rich in omega-3 fatty acids support the balance between osteoclasts and osteoblasts, thereby reducing bone loss (13). The Mediterranean diet also helps maintain a healthy body weight (14–16), which reduces mechanical stress on bones and further contributes to preserving bone mass. Combining this diet with physical activity and adequate sun exposure for vitamin D synthesis further improves bone health.

The aim of our study was to investigate, using an *in silico* approach, the interactions of the most abundant bioactive compounds of the Mediterranean diet with the mentioned components of the signaling pathway responsible for osteoblast-mediated osteoclast differentiation.

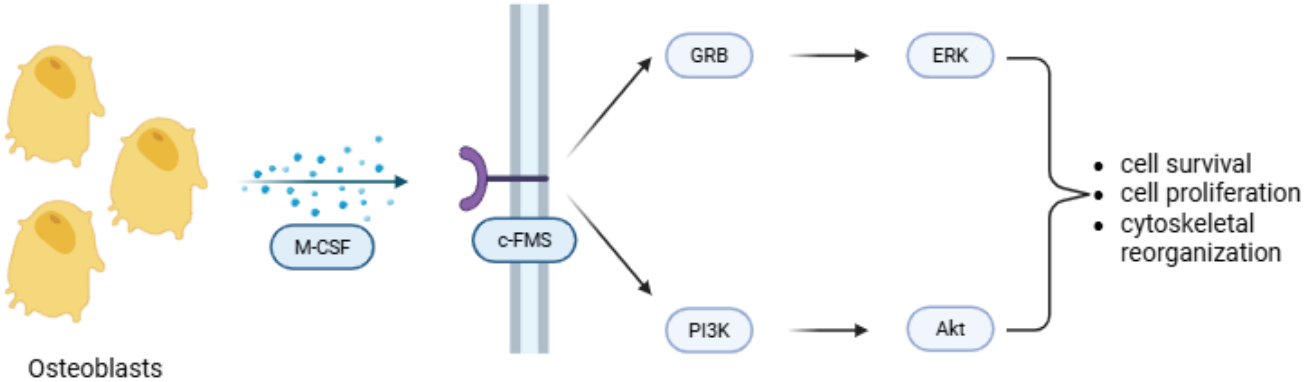

Figure 1. The signaling pathway responsible for osteoblast-mediated osteoclast differentiation.

MATERIALS AND METHODS

Ligand Library Definition

PubMed database search using the parameters "Mediterranean diet" AND "bioactive compounds" identified a total of 217 articles. Articles focusing on the chemical identification of individual molecules present in foods predominantly included in the Mediterranean diet were analyzed. Based on the data from these studies, a ligand library containing 115 bioactive compounds was established, which we further grouped according to their food sources.

Preparation for Molecular Docking

The crystallographic structures of the proteins (receptors) M-CSF, c-Fms, GRB2, ERK, PI3K, and protein kinase B (Akt) were obtained from the PDB or AlphaFold databases. The 3D structures of all ligands were retrieved from PubChem (17).

Molecular Docking

Molecular docking is a technique used to predict the orientation, affinity, and interaction of a ligand at the binding site of a receptor (protein). In this study, we employed the AutoDock Vina software, which is widely used for predicting these interactions and has found extensive application in drug discovery (18). Upon launching the software, the protein structures (in PDB format) and the ligand library (in SDF format) were imported and modified as needed. For analyses, water molecules were removed from the examined protein structures. In most cases, water molecules do not play a significant role in binding and are therefore deleted to facilitate calculations. Additionally, polar hydrogen atoms and charges were added to the receptor molecules before initiating the process, in order to "open" as many binding sites as possible. Molecular docking was performed under conditions where all ligand bonds were set as rotatable, while receptor bonds were fixed (18). After initiating the docking, the program simulates interactions between different functional groups of the receptors and ligands. Ultimately, the ten most representative interactions are displayed, along with their Gibbs free energy values and root mean square deviation (RMSD) of atomic positions. More negative Gibbs free energy values indicate a higher likelihood of spontaneous reaction and stronger binding between receptor and ligand, while RMSD values are used to evaluate the precision of the performed docking (18).

Characterization of Ligand–Receptor Interactions

To determine the nature of the interactions between the examined ligands and receptors, the PyMOL software was used for those interactions with Gibbs free energy values below –7 kcal/mol. All obtained interactions were imported into the software, and the amino acid sequences, specifically the pockets where ligands were bound were analyzed. If the amino acid sequence corresponded to the active site or the domain responsible for the protein's biological activity, the interaction was considered inhibitory.

RESULTS

A total of 115 compounds were identified based on literature searches focusing on bioactive compounds from the Mediterranean diet.

The AutoDock Vina program was used for molecular docking between the ligand library and the receptors M-CSF, c-Fms, GRB2, ERK, PI3K, and protein kinase B (Akt). As an empirical threshold for higher spontaneous binding probability, interactions with Gibbs free energy values below –7 kcal/mol were considered.

The interaction between M-CSF and diosgenin showed the lowest Gibbs free energy value at –3.23 kcal/mol, while sulforaphane exhibited the highest value at –0.079 kcal/mol **(Figure 2A)**.

For c-Fms, the interaction with ursolic acid showed the lowest Gibbs free energy value at –3.9 kcal/mol, while sulforaphane had the highest value at –0.993 kcal/mol **(Figure 2B)**.

Regarding GRB2, the interaction with diosgenin had the lowest Gibbs free energy value at –3.82 kcal/mol, and sulforaphane the highest at –1.1 kcal/mol **(Figure 2C)**.

The interaction between ERK and diosgenin showed the lowest Gibbs free energy value at –3.71 kcal/mol, **whereas tannic acid had the highest value at –1 kcal/mol (Figure 2D)**.

For PI3K, diosgenin again showed the lowest Gibbs free energy value at –3.75 kcal/mol, while tannic acid had the highest at –0.95 kcal/mol **(Figure 2E)**.

The interaction between protein kinase B (Akt) and salvianolic acid showed the lowest Gibbs free energy value at –9.47 kcal/mol, while sulforaphane had the highest value at –3.36 kcal/mol **(Figure 2F)**.

Based on these results, the binding of the 5 most potent ligands was further analyzed to determine the nature of their interactions. Five top ligands were selected for additional investigation: salvianolic acid, apigenin, diosmin, mangiferin, and silybin. Salvianolic acid primarily interacted with amino acids LYS161, GLY160, PHE159, THR158, GLY157, and LYS156, which were also common binding sites for other ligands, particularly diosmin and mangiferin. This pattern indicates that regions rich in lysine and glycine residues are critical for binding stability. Apigenin partially shared binding sites with salvianolic acid, especially at LYS161, GLY160, and LYS156, but additionally bound to HIS236, PHE235,

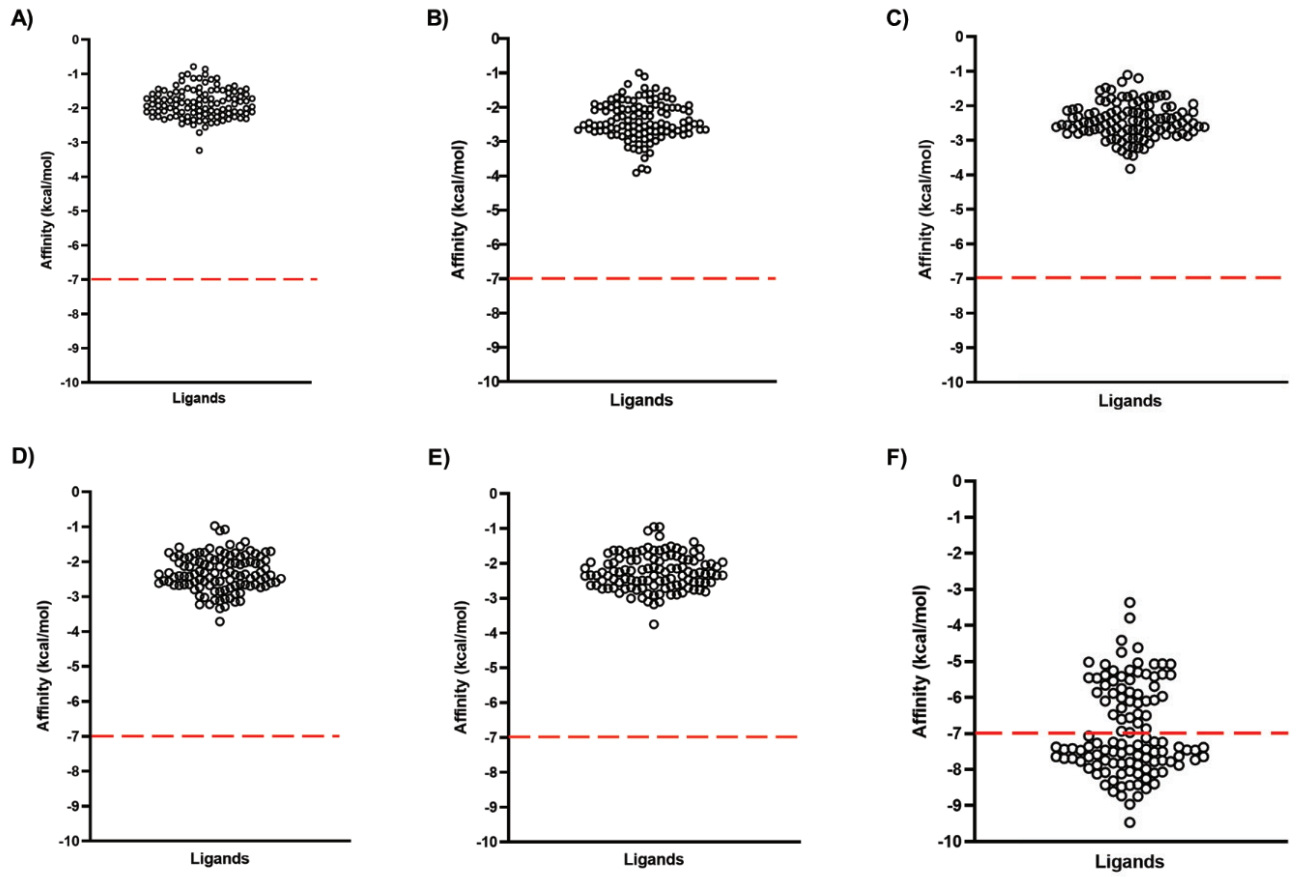

Figure 2. Gibbs free energy values for interactions between the tested ligand library and each of the investigated proteins: (A) M-CSF, (B) c-FMS, (C) GRB2, (D) ERK, (E) PI3K and (F) Akt. Each point represents an individual ligand from the Mediterranean diet-derived library evaluated by molecular docking. The red dashed line indicates the empirical threshold (ΔG = –7 kcal/mol), below which interactions are considered to have higher spontaneity and potential biological significance. Notably, only interactions with Akt (F) demonstrated multiple ligands surpassing this threshold, suggesting a potential inhibitory effect.

PHE234, and LEU233, suggesting additional interactions in a different region of the protein, near aromatic residues. Diosmin exhibited the most comprehensive spectrum of interactions, covering nearly all amino acids involved in binding with salvianolic acid and mangiferin (the GLY155–LYS161 sequence), as well as additional residues such as ASP280, ASP415, LYS416, and LYS417, indicating potentially stronger and broader binding across various functional domains of Akt. Mangiferin shared the identical binding sequence from GLY155 to LYS161 with diosmin and salvianolic acid, suggesting a similar binding mechanism in the conserved region of the protein. However, it also showed additional interactions with MET225, GLY226, and TYR227, which were not observed for salvianolic acid. Silybin significantly differed from the other ligands, primarily binding to a completely different set of amino acids (PRO50, TYR49, PRO48, LEU47, ASP46) and additionally to regions such as LEU359–ILE363 and LEU380–ILE382, which were not involved with other ligands. This suggests that silybin targets a distinct functional domain of Akt **(Table 1)**.

Finally, by filtering all amino acids interacting with these ligands, specific sequences of the Akt protein responsible for its biological activity and regulation were analyzed. The P-loop region (positions 156–162, sequence KLGGFGK) represents a conserved motif crucial for ATP binding and kinase activity regulation. Salvianolic acid, apigenin, diosmin, and mangiferin all showed binding to this region. The HRD motif (positions 194–196, sequence HRD) is important for kinase catalytic activity; however, none of the analyzed ligands exhibited binding to this motif. The DFG motif (positions 292–294, sequence DFG) plays a key role in maintaining the active conformation of the kinase. Diosmin and mangiferin interacted with this region. The activation segment (positions 307–311, sequence CGLKE) is involved in modulating the conformation required for protein activity. Only salvianolic acid bound to this region. The regulatory region (positions 174–181, sequence YAMKILKK) controls the accessibility of the activation segment and overall protein stability. Salvianolic acid also demonstrated binding to this region **(Table 2)**.

DISCUSSION

Various studies have demonstrated the positive effects of the Mediterranean diet on human health, as well as its role in preventing different diseases (19–21). Our research focused on the impact of the most abundant

Table 1. Amino acid sequences in protein kinase B (Akt) that interacted with the most potent ligands.

Ligands	Amino acids involved in binding
Salvianolic acid	LYS161, GLY160, PHE159, THR158, GLY157, LYS156, LYS187, ASP188, GLU189, MET278, LEU277, ASN276, ILE287, LYS286, THR218, THR217, GLN216, PHE215, ALA314, LEU313, TYR312, GLU311, CYS307, GLY308, LYS177, MET176, ALA175, TYR174
Apigenin	HIS236, PHE235, PHE234, LEU233, GLU232, GLY231, PRO421, PRO420, VAL419, LEU418, LEU277, MET278, LEU279, LYS156, GLY155, LYS161, GLY160, TYR277, VAL228, GLN411, ASP412
Diosmin	GLY155, LYS156, GLY157, THR158, PHE159, GLY160, LYS161, TYR227, VAL228, ASP280, LEU279, ASP415, LYS416, LYS417, LEU418, VAL419, PRO420, ILE285, LYS286, ILE287, THR288, ASP289, PHE290, GLY291, LEU292, TYR261, ASN276, TYR251, GLY252, ALA253, GLU254, GLU275, LEU274, ILE255, VAL256, SER257, ALA258, LEU259, ASP260, LEU261
Mangiferin	GLY155, LYS156, GLY157, THR158, PHE159, GLY160, LYS161, MET225, GLV226, TYR227, LEU211, SER210, THR209, THR288, ASP289, VAL190, LEU200
Sylbin	PRO50, TYR49, PRO48, LEU47, ASP46, LEU359, MET360, GLU361, ASP362, ILE363, ASP384, LYS383, ILE382, LEU381, LEU380, GLU316, PRO315, PRO345, LEU344, ARG343, GLY342, CYS341

Abbreviations: LYS – lysine, GLY – glycine, PHE – phenylalanine, THR – threonine, ASP – aspartic acid, GLU – glutamic acid, MET – methionine, LEU – leucine, ASN – asparagine, ILE – isoleucine, GLN – glutamine, ALA – alanine, TYR – tyrosine, CYS – cysteine, HIS – histidine, PRO – proline, VAL – valine, ARG – arginine.

Table 2. Overview of the regions, sequences, and positions of amino acids that regulate and enable the biological activity of protein kinase B (Akt), and the most potent ligands binding to them.

Region	Sequence	Position	Ligands
P-loop	KLGGFGK	156 – 162	salvianolic acid, apigenin, diosmin, mangiferin
HRD motif	HRD	194 – 196	/
DFG motif	DFG	292 – 294	Diosmin, mangiferin
Activation segment	CGLKE	307 – 311	salvianolic acid
Regulatory region	YAMKILKK	174 – 181	salvianolic acid

Abbreviations: K – lysine; L – leucine; G – glycine; F – phenylalanine; H – histidine; R – arginine; D – aspartic acid; C – cysteine; E – glutamic acid; Y – tyrosine; A – alanine; M – methionine; I – isoleucine.

bioactive molecules from the Mediterranean diet on a specific osteoblast-mediated signaling pathway responsible for osteoclast differentiation, which is crucial for bone function and homeostasis. The Mediterranean diet has shown numerous benefits in preventing fractures and osteoporosis. Adherence to this dietary pattern in non-Mediterranean regions has been associated with higher bone mineral density and a reduced risk of fractures, particularly in postmenopausal women (22–23). Treatment and prevention of osteoporosis begin with a healthy lifestyle and proper nutrition (24). A prospective observational study by Quattrini et al. demonstrated that perimenopausal and postmenopausal women adhering to the Mediterranean diet had a significantly increased dietary calcium intake, essential for maintaining bone health (25).

Based on our results, the highest Gibbs free energy values of ligand–receptor interactions were observed for sulforaphane and tannic acid, while the lowest were for diosgenin and salvianolic acid. Salvianolic acid showed the lowest Gibbs free energy value when interacting with protein kinase B (Akt), at –9.47 kcal/mol. An experimental study by Cao et al. confirmed a significant role of salvianolic acid in promoting fracture healing by balancing osteoblast and osteoclast differentiation. They demonstrated that salvianolic acid dose-dependently inhibited osteoclast differentiation of bone marrow-derived macrophages, associated with NF-κB suppression. Thus, in addition to interacting significantly with protein kinase B, salvianolic acid also acts on NF-κB, thereby promoting bone fracture healing (26). Salvianolic acid also induces apoptosis and suppresses tumor growth in acute myeloid leukemia by inhibiting protein kinase B, as shown in an experimental study in mice by Pei et al. (27). Moreover, Yan et al. demonstrated that salvianolic acid alleviates inflammation and prevents pathological fibrosis by inhibiting CD36-mediated PI3K-Akt pathway activation in synovial fibroblasts isolated from human shoulder biopsies (28). Based on these studies, we can conclude that there is significant interaction between salvianolic acid and protein kinase B.

Although sulforaphane did not show significant interactions with any protein in our study, an experimental study by Kim et al. demonstrated its role in inhibiting osteoclastogenesis by acting on NF-κB. They found that sulforaphane selectively inhibits NF-κB. Likewise, their study did not confirm significant interaction with M-CSF and c-Fms proteins, as was also the case in our study, suggesting that the inhibitory effect on osteoclastogenesis is not mediated through interaction with these proteins (29).

Tannic acid also did not show significant interactions in our study; however, despite the absence of significant nutritional effects, it plays an important role in orthopedic surgery, as shown in the study by Sun et al. Tannic acid is a natural polyphenolic compound, and polyphenol-based materials play a major role in orthopedic transplantation. This compound can form strong interactions with metals, creating stable coatings on their surfaces, thereby improving the physical and chemical properties of bone implant surfaces and increasing implantation success rates (30).

Additionally, diosgenin did not show significant interactions with most of the examined receptor in our study, yet it is considered to play a significant role in bone formation. This was confirmed by an experimental study by Alper et al., which investigated the therapeutic effects of diosgenin on alveolar bone loss and apoptosis in diabetic rats with experimental periodontitis. The study revealed that diosgenin significantly improved bone formation and contributed to periodontal healing by increasing the expression of BMP-2 (bone morphogenetic protein 2), alkaline phosphatase, osteocalcin, and type I collagen, while reducing apoptosis and RANKL expression (31).

The most significant ligand interactions were observed with protein kinase B. Besides salvianolic acid, the most potent ligands binding to the active site of protein kinase B included apigenin, diosmin, and mangiferin. The inhibitory effect of apigenin on protein kinase B was confirmed in a study by Yang et al., who demonstrated that apigenin induces apoptosis in hepatocellular carcinoma cells by inhibiting the PI3K/AKT/mTOR signaling pathway (32). Mangiferin also has a significant impact on protein kinase B, as evidenced in a study by Shi et al., which investigated the inhibitory effect of mangiferin on cell migration and angiogenesis through PI3K/AKT/mTOR signaling (33).

CONCLUSION

Our results did not demonstrate significant interactions between the investigated ligands and receptors, except in the case of protein kinase B (Akt). During the interaction of protein kinase B with the five most potent ligands examined further, it was determined that salvianolic acid, apigenin, diosmin, and mangiferin bind to amino acid sequences corresponding to the active site of the receptor, and this interaction is therefore considered inhibitory.

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References

1. Kim JM, Lin C, Stavre Z, Greenblatt MB, Shim JH. Osteoblast-Osteoclast Communication and Bone Homeostasis. Cells. 2020;9(9):2073. doi: 10.3390/cells9092073. PMID: 32927921
2. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int. 2015;2015:421746. doi: 10.1155/2015/421746. PMID: 26247020
3. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. J Clin Pathol. 2008;61(5):577-87. doi: 10.1136/jcp.2007.048868. PMID: 18441154.
4. Kalbasi Anaraki P, Patecki M, Tkachuk S, Kiyan Y, Haller H, Dumler I. Urokinase receptor mediates osteoclastogenesis via M-CSF release from osteoblasts and the c-Fms/PI3K/Akt/NF-κB pathway in osteoclasts. J Bone Miner Res. 2015;30(2):379-88. doi: 10.1002/jbmr.2350. PMID: 25196912.
5. Bradley EW, Ruan MM, Vrable A, Oursler MJ. Pathway crosstalk between Ras/Raf and PI3K in promotion of M-CSF-induced MEK/ERK-mediated osteoclast survival. J Cell Biochem. 2008;104(4):1439-51. doi: 10.1002/jcb.21719. PMID: 18275061
6. Armas LA, Recker RR. Pathophysiology of osteoporosis: new mechanistic insights. Endocrinol Metab Clin North Am. 2012;41(3):475-86. doi: 10.1016/j.ecl.2012.04.006. PMID: 22877425.
7. Lane N. Epidemiology, etiology, and diagnosis of osteoporosis. Am J Obstet Gynecol 2006; 194(2 Suppl):S3-11. doi: 10.1016/j.ajog.2005.08.047. PMID: 16448873.
8. Romero Pérez A, Rivas Velasco A. Adherence to Mediterranean diet and bone health. Nutr Hosp. 2014;29(5):989-96. doi: 10.3305/nh.2014.29.5.7332. PMID: 24951976.
9. Silva T, Oppermann K, Reis F, Spritzer P. Nutrition in menopausal women: a narrative review. Nutrients 2021; 13(7):2149. doi: 10.3390/nu13072149. PMID: 34208693.
10. Dominguez L, Di Bella G, Veronese N, Barbagallo M. Impact of Mediterranean diet on chronic non-communicable diseases and longevity. Nutrients 2021; 13(6):2028. doi: 10.3390/nu13062028. PMID: 34070034.
11. Morris L, Bhatnagar D. The Mediterranean diet. Curr Opin Lipidol. 2016;27(1):89-91. doi: 10.1097/MOL.0000000000000266. PMID: 26655296.
12. Khazai N, Judd SE, Tangpricha V. Calcium and vitamin D: skeletal and extraskeletal health. Curr Rheumatol Rep. 2008;10(2):110-7. doi: 10.1007/s11926-008-0020-y. PMID: 18460265.
13. Benova A, Ferencakova M, Bardova K, Funda J, Prochazka J, Spoutil F et al. Omega-3 PUFAs prevent bone impairment and bone marrow adiposity in mouse model of obesity. Commun Biol. 2023;6(1):1043. doi: 10.1038/s42003-023-05407-8. PMID: 37833362
14. Dominguez L, Veronese N, Di Bella G, Cusumano C, Parisi A, et al. Mediterranean diet in the management and prevention of obesity. Exp Gerontol. 2023;174:112121. doi: 10.1016/j.exger.2023.112121. PMID: 37119800.

15. Di Rosa C, Lattanzi G, Spiezia C, Piccirilli S, Beato I, et al. Mediterranean Diet versus Very Low-Calorie Ketogenic Diet: Effects of Reaching 5% Body Weight Loss on Body Composition in Subjects with Overweight and with Obesity-A Cohort Study. Int J Environ Res Public Health. 2022;19(20):13040. doi: 10.3390/ijerph192013040. PMID: 36292376.
16. Estruch R, Ros E. The role of the Mediterranean diet on weight loss and obesity-related diseases. Rev Endocr Metab Disord. 2020;21(3):315-327. doi: 10.1007/s11154-020-09579-0. PMID: 32829455.
17. Lehrer S, Rheinstein PH. Ivermectin Docks to the SARS-CoV-2 Spike Receptor-binding Domain Attached to ACE2. In Vivo. 2020;34(5):3023-3026. doi: 10.21873/invivo.12134. PMID: 32871846.
18. Yu R, Chen L, Lan R, Shen R, Li P. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. Int J Antimicrob Agents. 2020;56(2):106012. doi: 10.1016/j.ijantimicag.2020.106012. PMID: 32389723.
19. Guasch-Ferré M, Willett W. The Mediterranean diet and health: a comprehensive overview. J Intern Med. 2021;290(3):549-566. doi: 10.1111/joim.13333. PMID: 34327780.
20. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. BMJ. 2008;337:a1344. doi: 10.1136/bmj.a1344. PMID: 18786971.
21. Muscogiuri G, Verde L, Sulu C, Katsiki N, Hassapidou M, Frias-Toral E, Cucalón G et al. Mediterranean Diet and Obesity-related Disorders: What is the Evidence? Curr Obes Rep. 2022;11(4):287-304. doi: 10.1007/s13679-022-00481-1. PMID: 36178601.
22. Malmir H, Saneei P, Larijani B, Esmaillzadeh A. Adherence to Mediterranean diet in relation to bone mineral density and risk of fracture: a systematic review and meta-analysis of observational studies. Eur J Nutr. 2018;57(6):2147-2160. doi: 10.1007/s00394-017-1490-3. PMID: 28638994.
23. Jennings A, Mulligan A, Khaw KT, Luben R, Welch A. A Mediterranean diet is positively associated with bone and muscle health in a non-Mediterranean region in 25,450 men and women from EPIC-Norfolk. Nutrients. 2020;12(4):1154. doi: 10.3390/nu12041154. PMID: 32260457.
24. Rizzoli R, Biver E, Brennan-Speranza TC. Nutritional intake and bone health. Lancet Diabetes Endocrinol. 2021;9(9):606-621. doi: 10.1016/S2213-8587(21)00119-4. PMID: 34242583.
25. Quattrini S, Pampaloni B, Gronchi G, Giusti F, Brandi ML. The Mediterranean diet in osteoporosis prevention: an insight in a peri- and post-menopausal population. Nutrients. 2021;13(2):531. doi: 10.3390/nu13020531. PMID: 33572654.
26. Cao B, Wu X, Zhou C, Chen H, Xue D, Pan Z. Salvianolic acid A promotes bone-fracture healing via balancing osteoblast and osteoclast differentiation. FASEB J. 2025;39(2):e70364. doi: 10.1096/fj.202402515R. PMID: 39878631.
27. Pei R, Si T, Lu Y, Zhou JX, Jiang L. Salvianolic acid A, a novel PI3K/Akt inhibitor, induces cell apoptosis and suppresses tumor growth in acute myeloid leukemia. Leuk Lymphoma. 2018;59(8):1959-1967. doi: 10.1080/10428194.2017.1399314. PMID: 29164984.
28. Yan Y, Zhou M, Meng K, Zhou C, Jia X, Li X et al. Salvianolic acid B attenuates inflammation and prevent pathologic fibrosis by inhibiting CD36-mediated activation of the PI3K-Akt signaling pathway in frozen shoulder. Front Pharmacol. 2023;14:1230174. doi: 10.3389/fphar.2023.1230174. PMID: 37593175.
29. Kim SJ, Kang SY, Shin HH, Choi HS. Sulforaphane inhibits osteoclastogenesis by inhibiting nuclear factor-kappaB. Mol Cells. 2005;20(3):364-70. PMID: 16404151.
30. Sun Y, Qu Y, Zhao J. The application of tannic acid in orthopedics. J Orthop Translat. 2022;32:87-94. doi: 10.3389/fmats.2021.801369
31. Kızıldağa A, Alpanb AL, Özdedec M, Aydınd T, Özmene Ö. Therapeutic effects of diosgenin on alveolar bone loss and apoptosis in diabetic rats with experimental periodontitis. Iran J Basic Med Sci. 2023;26(7):785-790. doi: 10.22038/IJBMS.2023.68801.14995. PMID: 37396943.
32. Yang J, Pi C, Wang G. Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells. Biomed Pharmacother. 2018;103:699-707. doi: 10.1016/j.biopha.2018.04.072. PMID: 29680738.
33. Shi J, Lv H, Tang C, Li Y, Huang J, Zhang H. Mangiferin inhibits cell migration and angiogenesis via PI3K/AKT/mTOR signaling in high glucose- and hypoxia-induced RRCECs. Mol Med Rep. 2021;23(6):473. doi: 10.3892/mmr.2021.12112. PMID: 33899114.

UTICAJ NAJZASTUPLJENIJIH BIOAKTIVNIH JEDINJENJA MEDITERANSKE ISHRANE NA SIGNALNI PUT OSTEOBLASTIMA POSREDOVANE DIFERENCIJACIJE OSTEOKLASTA: *IN SILICO* ANALIZA

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Sažetak

Uvod: Kosti su osnovni elementi skeletnog sistema koji se neprekidno remodeluju. Osteoklasti vrše resorpciju kosti, dok osteoblasti formiraju novi koštani matriks i posredno utiču na diferencijaciju osteoklasta putem signalnih puteva u kojima učestvuju signalni molekuli: M-CSF, c-Fms, GRB2, ERK, PI3K i Akt. U cilju očuvanja zdravlja kostiju danas se sve značajnija pažnja posvećuje nutritivnim intervencijama, poput mediteranske dijete koja je bogata bioaktivnim jedinjenjima. Cilj ovog rada je *in silico* ispitivanje potencijalnih interakcija najzastupljenijih bioaktivnih jedinjenja mediteranske dijete sa navedenim proteinima signalnog puta diferencijacije osteoklasta.

Materijali i metode: Formirana je biblioteka od 115 najzastupljenijih bioaktivnih jedinjenja mediteranske dijete identifikovanih iz literaturnih podataka. Strukture proteina dobijene kristalografijom korišćene su kao targeti u simulacijama molekularnog dokinga radi ispitivanja predikcije interakcija između liganada i receptora.

Rezultati: Većina liganada nije ostvarila značajne interakcije sa ispitivanim proteinima ($\Delta G > -7$ kCal/mol), osim u slučaju Akt proteina. Za ovaj protein identifikovan je veći broj liganada sa $\Delta G < -7$ kCal/mol. Najpotentnijih 5 liganada (salvianolna kiselina, apigenin, diosmin, mangiferin i silbin) vezivali su se za aminokiselinske sekvence koje čine aktivno mesto receptora, ukazujući na mogući inhibicioni efekat.

Zaključak: *In silico* analiza ukazuje da bioaktivne komponente mediteranske dijete poseduju potencijal za inhibiciju Akt proteina (protein kinaze B), čime se otvara perspektiva za njihovu primenu u modulaciji osteoklastne aktivnosti i razvoju novih strategija za očuvanje zdravlja kostiju.

Ključne reči: mediteranska ishrana; bioaktivna jedinjenja; osteoblasti; osteoklasti; protein kinaza B

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