چهارمین کنگره
توسعه علمی و فناوری
دانشجویان زیست
شناسی و شیمی
4th Congress of
Scientific and Technological
Development of Biology and
Chemistry Students
www.bcs.cdsts.ir

Molecular Docking Analysis of Two Natural Compounds with the 4KMY Protein: A Comparative Study of Folic Acid Receptor

Matin Ghasemipour[1], Amaneh Javid[*1]

[1]Department of Biology, Faculty of Engineering and Science ،Science and Arts University
Yazd, Iran
javid@sau.ac.ir
ORCID ID: 0000-0002-6445-6176

Abstract

Folate Receptor-β (FR-β or 4KMY) is a high-affinity folate-binding protein that is selectively expressed on M2-like tumor-associated macrophages (TAMs), where it plays a central role in maintaining the immunosuppressive characteristics of the tumor microenvironment. Due to its restricted expression pattern and functional importance, FR-β has attracted growing interest as a therapeutic target for both cancer treatment and immune regulation. Recent studies have highlighted that modulating FR-β–positive macrophages can shift the balance of the microenvironment toward an anti-tumor phenotype, further emphasizing the relevance of this receptor in oncology. In the present study, we employed a structure-based molecular docking approach to investigate the interaction of two bioactive natural compounds—Oroxylin A, derived from Scutellaria baicalensis, and Luteolin-7-O-glucoside, commonly found in Lonicera japonica, Mentha species, and Plantago major—with the binding pocket of FR-β. Their docking behavior was systematically compared with folic acid, the endogenous ligand and physiological substrate of FR-β. Docking simulations were carried out using AutoDock under physiologically relevant pH conditions, applying Gasteiger/Kollman charge assignments to ensure accurate representation of electronic distributions. Both natural compounds demonstrated strong binding affinities, with interaction energies comparable to or exceeding that of folic acid, suggesting their capability to effectively engage the FR-β active site. Additional analysis of hydrogen bonding patterns and hydrophobic interactions indicated that these flavonoids may stabilize within the receptor pocket through multiple complementary contacts. Such stability raises the possibility that these compounds could modulate macrophage behavior by altering FR-β signaling or ligand competition. Collectively, these results underscore the potential of plant-derived flavonoids as promising structural scaffolds for the development of new immunomodulatory or anticancer agents specifically targeting FR-β.

Keywords: *Folate Receptor-β (FR-β), Molecular docking, Flavonoid, Macrophage, Cancer*

Introduction

Folate receptors (FRs) are a family of high-affinity glycosylphosphatidylinositol-anchored proteins responsible for the cellular uptake of folates, including folic acid and reduced folate cofactors, which are essential for one-carbon metabolism (1). Folate-dependent pathways play central roles in amino acid biosynthesis such as the conversion of homocysteine to methionine and are required for de novo synthesis of purines and thymidylate. Consequently, proper folate transport is indispensable for DNA replication, cell proliferation, and overall cellular homeostasis. Dysregulation of folate trafficking has been strongly associated with multiple pathological conditions, including cancer and chronic inflammatory disorders. Among the folate receptor isoforms, Folate Receptor-β (FR-β or 4KMY) has attracted increasing attention because of its selective overexpression on M2-like tumor-associated macrophages (TAMs) and on activated macrophages involved in chronic inflammation (2,3). This restricted and disease-associated expression pattern, combined with the exceptionally high affinity of FR-β for folates, makes it an attractive molecular target in oncology(4). The overall architecture of the human folate receptor-α (4KMY) embedded in a model lipid bilayer is shown in ***Figure 1.***

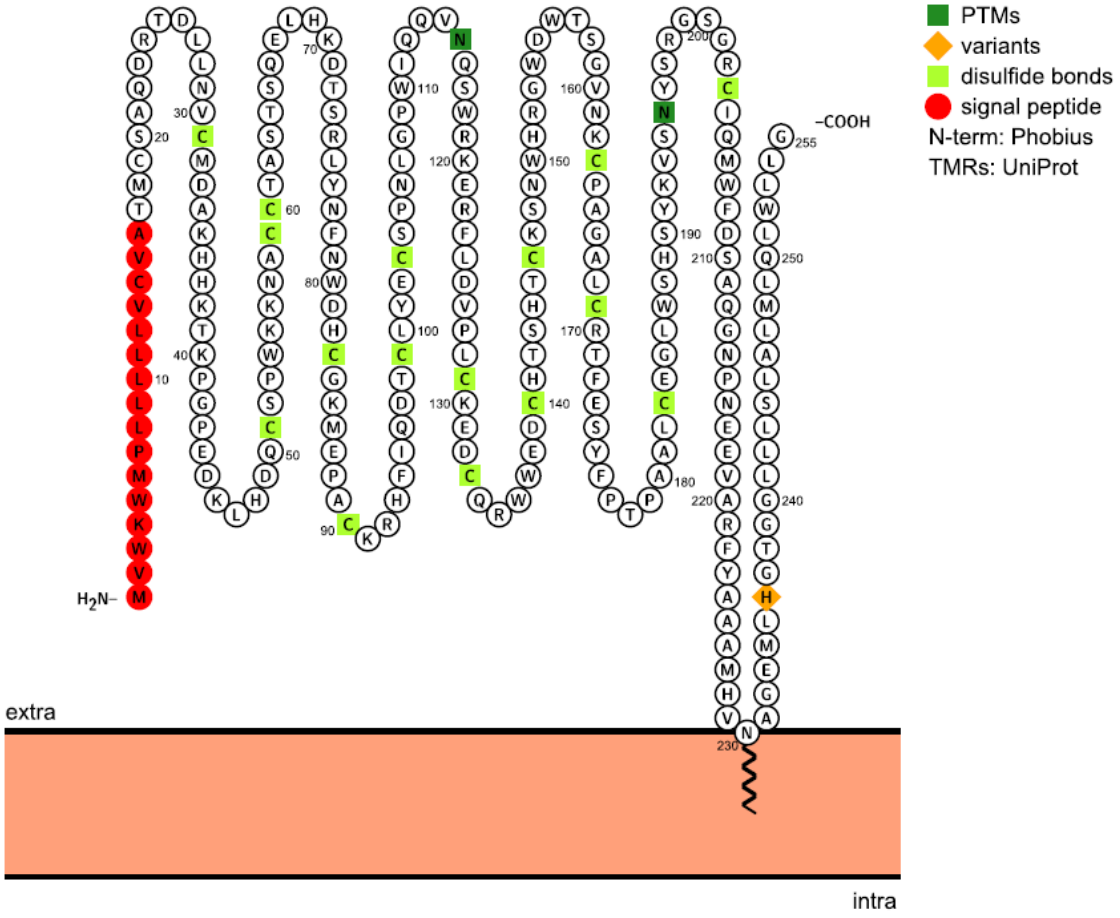

Figure 1. *Schematic representation of the human folate receptor-α (FR-α, PDB ID: 4KMY) embedded in a model lipid bilayer under physiological pH conditions. The receptor is a glycosylphosphatidylinositol-anchored membrane protein responsible for high-affinity binding and transport of folates, playing a critical role in one-carbon metabolism, cell proliferation, and cancer progression.*

FR-β–positive TAMs play a critical role in tumor progression by promoting angiogenesis, enhancing metastatic potential, and suppressing cytotoxic T-cell responses, thereby contributing to an immunosuppressive tumor

microenvironment. Consequently, identifying small molecules capable of interacting with FR-β may offer new opportunities to reprogram TAMs and enhance anti-tumor immunity (5,6). Natural compounds have emerged as promising candidates for anticancer drug discovery due to their structural diversity, favorable safety profiles, and multitarget biological activities. Among them, Oroxylin A and Luteolin-7-O-glucoside are two flavonoid-based phytochemicals extensively reported for their anti-inflammatory, anti-proliferative, and immunomodulatory effects (7–9). Oroxylin A is primarily found in species of the Scutellaria genus, particularly Scutellaria baicalensis, a medicinal plant widely used in traditional Asian medicine (10,11). This compound has been shown to induce apoptosis, inhibit NF-κB signaling, and suppress metastatic behavior in various cancer models (12,13). Luteolin-7-O-glucoside, also known as cynaroside, is abundantly present in Lonicera japonica (honeysuckle), Mentha species (mint), Plantago major, and several edible vegetables (14–16). This molecule displays potent antioxidant, anti-angiogenic, and macrophage-modulating properties, supporting its potential relevance in targeting FR-β–expressing macrophages. Given the central role of FR-β in regulating immune dynamics within the tumor microenvironment, evaluating natural compounds capable of strong interaction with this receptor may facilitate the identification of new therapeutic candidates.

In this study, we performed structure-based molecular docking of selected natural compounds and compared their binding characteristics with those of folic acid, the endogenous high-affinity ligand of FR-β. The docking simulations were conducted under approximated physiological pH conditions using AutoDock with Gasteiger/Kollman charge assignments. Our objective was to identify natural molecules with favorable binding profiles that may serve as potential modulators of FR-β and tumor-associated macrophage function.

Materials & Methods

PubChem and Protein Data Bank (PDB): *Employed for acquisition of the molecular structure of Natural Compounds and FR-β protein (17,18).*

AutoDock Vina: *Utilized for the execution of molecular docking simulations (19).*

PyMOL: *Deployed for high-resolution visualization and comprehensive analysis of protein–ligand complexes, enabling detailed examination of molecular interaction interfaces (20).*

The computational workflow for evaluating the binding characteristics of the selected natural compounds toward human FR-β was carried out in four consecutive steps, beginning with protein preparation and followed by ligand optimization, molecular docking, and interaction analysis. First, the three-dimensional structure of FR-β (PDB ID: 4KMY) was retrieved from the Protein Data Bank. To ensure suitability for docking simulations, preprocessing was performed in UCSF Chimera, during which crystallographic water molecules, redundant heteroatoms, and unrelated small molecules were removed. Hydrogen atoms were added according to physiological pH (~7.4), and Kollman charges were assigned using AutoDockTools (ADT). The fully processed receptor was then converted to PDBQT format, forming the foundation for subsequent docking experiments (17).

Next, ligand acquisition and preparation were performed for the two natural compounds—Oroxylin A and Luteolin-7-O-glucoside—which were obtained from the PubChem database in SDF format. Folic acid, serving as the reference ligand, was included for comparative analysis. All ligand structures underwent energy minimization and optimization in UCSF Chimera, after which Gasteiger charges were assigned and rotatable bonds were automatically detected using ADT. The finalized ligand files were exported in PDBQT format for docking (18). Molecular docking simulations were conducted using AutoDock Vina. A grid box was defined to comprehensively encompass the folate-binding pocket of FR-β, based on the ligand position observed in the 4KMY crystal structure. Docking was performed using standard Vina parameters, and the assigned charge states—defined through Gasteiger/Kollman models—were considered reflective of approximate physiological pH. Binding affinities (kcal/mol) were obtained for each ligand, allowing comparative evaluation of their predicted interaction strengths (19). Finally, interaction profiling and visualization were performed to elucidate the molecular basis of ligand binding. LigPlot+ was used to identify and map hydrogen bonds and hydrophobic interactions between each ligand and key amino-acid residues within the FR-β pocket. Complementary three-dimensional visualizations were generated in PyMOL, enabling assessment of pose orientation,

pocket occupancy, and structural overlap with the reference ligand. These analyses provided a detailed understanding of the binding modes and interaction networks governing ligand–receptor compatibility (20).

Results

From an initial set of 100 natural compounds screened against FR-β, Oroxylin A and Luteolin-7-O-glucoside were identified as the top candidates based on their predicted binding energies and interaction profiles.

The ligands selected for docking included two plant-derived flavonoids—Oroxylin A and Luteolin—alongside folic acid, which served as the reference ligand for FR-α due to its native high-affinity interaction. Although all three molecules share aromatic ring systems, they differ significantly in functional group distribution, which may influence their binding orientation and interaction capacity within the FR-α pocket ***(Fig. 2)****.*

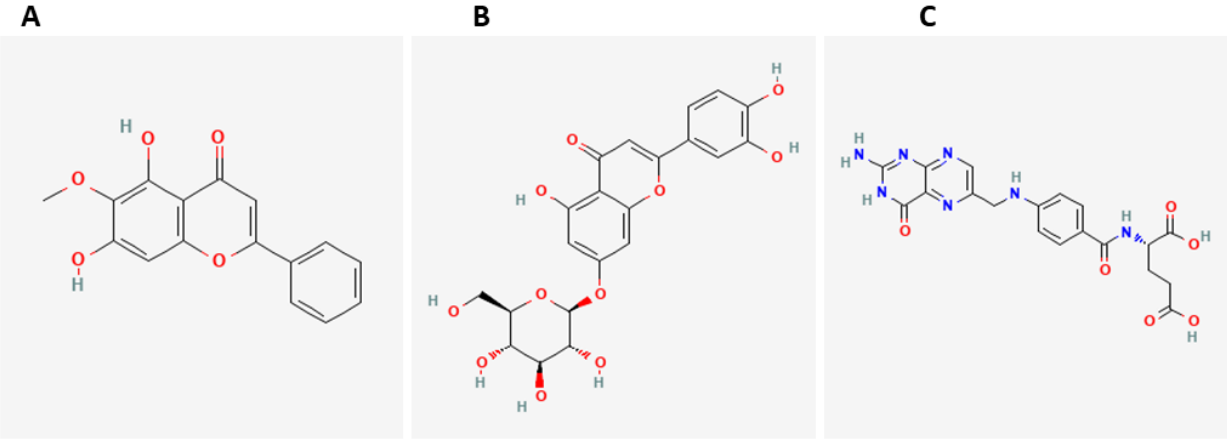

Figure 2*. Two-dimensional chemical structures of the ligands examined in this study: (A) Oroxylin A, (B) Luteolin-7-O-glucoside, and (C) Folic acid (reference compound). Oroxylin A and Luteolin are naturally occurring flavonoids found in plants such as Scutellaria baicalensis and Artemisia vulgaris, whereas folic acid represents the native high-affinity ligand of folate receptor-α.*

Molecular docking results demonstrated that the selected natural compounds exhibited favorable binding affinities toward the FR-β binding pocket, in some cases approaching or surpassing the affinity of folic acid, the endogenous reference ligand. The calculated binding energies, along with ligand identities and key interaction features, are summarized in ***Table 1****.*

Binding energys and H-bonding

Table 1.

Ligand	***Binding Affinity (kcal/mol)***	***H-bond residuse***
Oroxylin A	*−9.6*	*Ser190, Thr98, Asp97*
Luteolin-7-O-glucoside	*−9.9*	*Gly159,Trp156, Lys35, Thr98, Asn77*
Folic acid	*−9.5*	*His151, Trp154, Trp156*

Molecular docking simulations revealed that both Oroxylin A and Luteolin-7-O-glucoside exhibited strong binding affinities toward FR-β, comparable to the reference ligand, folic acid. The predicted binding energies were –9.6 kcal/mol for Oroxylin A, –9.9 kcal/mol for Luteolin-7-O-glucoside, and –9.5 kcal/mol for folic acid, indicating that the natural compounds can effectively occupy the folate-binding cavity under physiological charge conditions.

Analysis of docking poses ***(Fig. 3)*** *showed that all three ligands were positioned stably within the canonical binding pocket, with overlapping placement along the hydrophilic and aromatic regions of the cavity. Oroxylin A aligned deeply within the central groove, whereas Luteolin-7-O-glucoside formed extended interactions due to its glycosyl moiety. Interaction profiling using LigPlot+ (Figure Y) identified several key residues involved in ligand stabilization. Oroxylin A formed hydrogen bonds with Ser190, Thr98, and Asp97. Luteolin-7-O-glucoside interacted with Gly159, Trp156, Lys35, Thr98, and Asn77, forming a dense network of polar and hydrophobic contacts. The reference ligand,*

folic acid, established interactions with His151, Trp154, and Trp156, consistent with prior structural studies of folate–receptor complexes. Overall, the docking and interaction results demonstrate that the natural compounds display binding characteristics highly compatible with the structural and chemical requirements of the FR-β binding site.

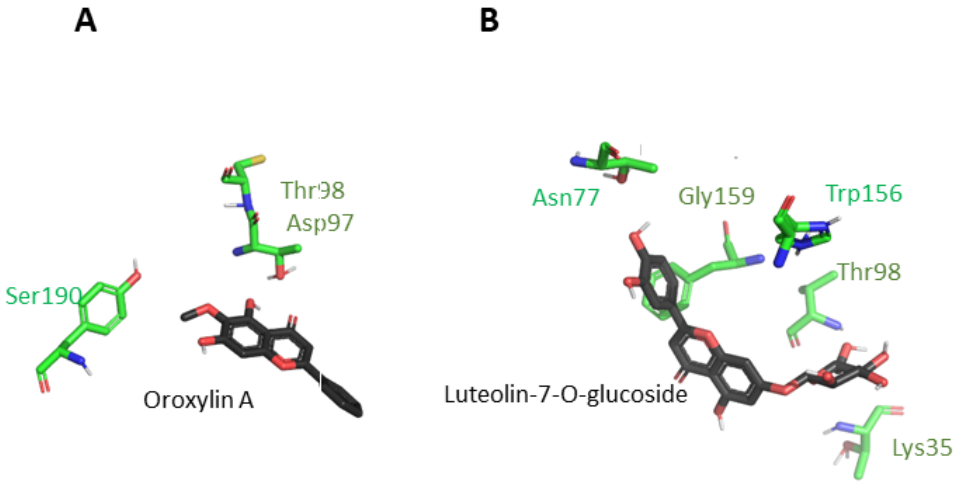

Figure 3. *Hydrogen-bonding residues interacting with the natural ligands. (A) Oroxylin A forms H-bonds with Ser190, Thr98, and Asp97. (B) Luteolin-7-O-glucoside interacts with Gly159, Trp156, Lys35, Thr98, and Asn77. Only residues directly involved in hydrogen-bond formation are shown.*

Discussion

The docking and interaction analyses provide valuable insights into the molecular basis of ligand recognition by FR-β and the potential of selected natural compounds as competitive folate mimetics (21,22). Both Oroxylin A and Luteolin-7-O-glucoside demonstrated stable accommodation within the canonical folate-binding pocket, occupying spatial regions that substantially overlap with that of folic acid. This competitive positioning also observed in the

superimposed docking poses suggests that these phytochemicals may interfere with folate recognition or transport under physiological conditions (22–24).

LigPlot+ interaction profiling further highlighted mechanistic similarities and differences among the ligands ***(Fig. 4)*** *(25,26). Oroxylin A formed hydrogen bonds with Ser190, Thr98, and Asp97, residues previously reported to participate in anchoring substrates within the FR family (27–29). Luteolin-7-O-glucoside displayed an even broader interaction network involving Gly159, Trp156, Lys35, Thr98, and Asn77, combining polar interactions with hydrophobic packing contributed by aromatic residues. Notably, Trp156 and Thr98-shared between folic acid and one or both natural compounds appear to be central determinants of ligand stabilization in the FR-β pocket (29,30). These conserved binding contacts may underlie the comparable affinities observed. The reference ligand, folic acid, engaged His151, Trp154, and Trp156, consistent with its well-established high-affinity interaction pattern. The presence of overlapping residues among all three ligands supports the hypothesis that Oroxylin A and Luteolin-7-O-glucoside could mimic endogenous folate binding. Given the overexpression of FR-β in activated macrophages and various tumor microenvironments, the observed binding features indicate possible relevance of these natural compounds in modulating folate-dependent pathways or targeting FR-β-associated cells (31–33). Overall, the structural and interaction data suggest that these plant-derived molecules possess the necessary chemical complementarity to occupy the FR-β binding cavity and may serve as promising scaffolds for anti-cancer or immunomodulatory agent development.*

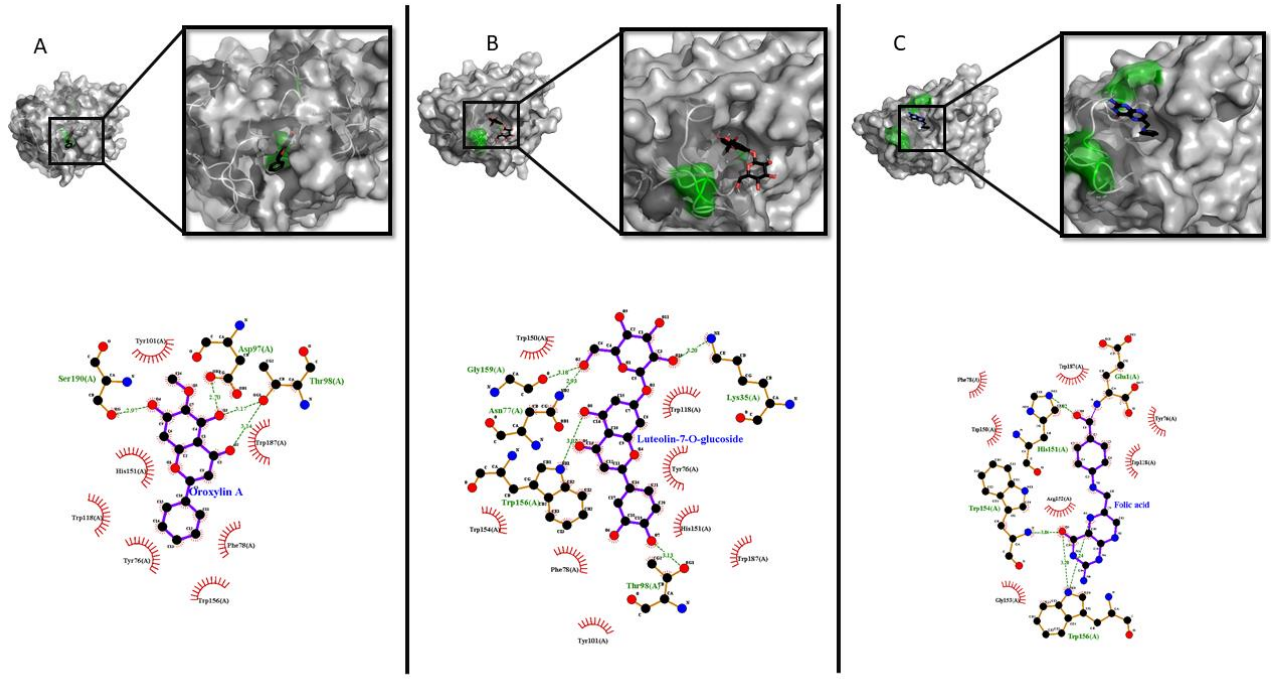

Figure 4. *Comparative interaction profiles and surface-binding orientations of the three ligands bound to FR-β. Panels (A–C) present the two-dimensional LigPlot+ diagrams for Oroxylin A, Luteolin-7-O-glucoside, and Folic acid, respectively, illustrating key hydrogen-bond interactions (green dashed lines) and hydrophobic contacts (red spoked arcs). Panels (A–C) show the corresponding surface-binding poses visualized in PyMOL, where green-highlighted surface regions represent amino-acid residues forming hydrogen bonds with each ligand. Combined, these representations reveal conserved interaction hotspots—particularly involving Thr98, Trp156, and adjacent polar residues—and demonstrate the high degree of spatial and chemical overlap between the natural compounds and the reference ligand. These shared binding features provide mechanistic support for the ability of the phytochemicals to mimic folate recognition and potentially modulate FR-β activity.*

Conclusion

In this study, structure-based molecular docking revealed that the natural flavonoids Oroxylin A and Luteolin-7-O-glucoside exhibit strong binding affinities toward Folate Receptor-β (FR-β), comparable to or exceeding the endogenous ligand, folic acid. The interaction analysis identified key hydrogen bonding and hydrophobic contacts with residues critical for ligand recognition and stabilization within the receptor binding pocket. The observed overlap between the binding sites of these natural compounds and folic acid suggests potential competitive interactions and modulation of FR-β activity.Given their natural origin, structural diversity, and previously reported anti-proliferative and immunomodulatory effects, these flavonoids represent promising candidates for further experimental validation as FR-β-targeted anticancer or immunomodulatory agents. Future studies including in vitro and in vivo assessments are warranted to evaluate their biological efficacy, selectivity, and therapeutic potential.

References

1. Puig-Kröger A, Sierra-Filardi E, Domínguez-Soto A, Samaniego R, Corcuera MT, Gómez-Aguado F, et al. Folate Receptor β Is Expressed by Tumor-Associated Macrophages and Constitutes a Marker for M2 Anti-inflammatory/Regulatory Macrophages. Cancer Research. 2009 Dec 15;69(24):9395–403.

2. Narasimhan B, Goodman JT, Vela Ramirez JE. Rational Design of Targeted Next-Generation Carriers for Drug and Vaccine Delivery. Annu Rev Biomed Eng. 2016 July 11;18(1):25–49.

3. Jiang D, Xiao R, Bai J, Wang X, Fang Y, Liu J, et al. FOLR2+ macrophages in cancer: allies or enemies. Cell Commun Signal. 2025 June 2;23(1):261.

4. Zhang F, Al-Amin MY, Utturkar S, Jiang R, Cresswell G, Alfar R, et al. Folate receptor β performs an immune checkpoint function in activated macrophages. Front Immunol. 2025 Sept 29;16:1638907.

5. Samaniego R, Domínguez-Soto Á, Ratnam M, Matsuyama T, Sánchez-Mateos P, Corbí ÁL, et al. Folate Receptor β (FRβ) Expression in Tissue-Resident and Tumor-Associated Macrophages Associates with and Depends on the Expression of PU.1. Cells. 2020 June 10;9(6):1445.

6. Goksen S, Varan G, Bilensoy E, Esendagli G. Folate Receptor β (FRβ) Expression on Myeloid Cells and the Impact of Reticuloendothelial System on Folate-Functionalized Nanoparticles' Biodistribution in Cancer. Mol Pharmaceutics. 2024 Sept 2;21(9):4688–99.

7. Zhang W, Fu X, Zhang Y, Chen X, Feng T, Xiong C, et al. Metabolome Comparison of Sichuan Dried Orange Peels (Chenpi) Aged for Different Years. Horticulturae. 2024 Apr 22;10(4):421.

8. Rai D, Aswatha Ram HN, Neeraj Patel K, Babu UV, Sharath Kumar LM, Kannan R. In vitro immuno-stimulatory and anticancer activities of Oroxylum indicum (L.) Kurz.: An evidence for substitution of aerial parts for conservation. Journal of Ayurveda and Integrative Medicine. 2022 Apr;13(2):100523.

9. *Li C, Zang C, Nie Q, Yang B, Zhang B, Duan S. Simultaneous determination of seven flavonoids, two phenolic acids and two cholesterines in Tanreqing injection by UHPLC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis. 2019 Jan;163:105–12.*

10. *Lu L, Guo Q, Zhao L. Overview of Oroxylin A: A Promising Flavonoid Compound. Phytotherapy Research. 2016 Nov;30(11):1765–74.*

11. *De Grano RVR, Vashchenko EV, Nisar M, Sung HHY, Vashchenko VV, Williams ID. Crystal structures of the flavonoid Oroxylin A and the regioisomers Negletein and Wogonin. Acta Crystallogr C Struct Chem. 2020 May 1;76(5):490–9.*

12. *Yao J, Hu R, Sun J, Lin B, Zhao L, Sha Y, et al. Oroxylin a prevents inflammation-related tumor through down-regulation of inflammatory gene expression by inhibiting NF-κB signaling. Molecular Carcinogenesis. 2014 Feb;53(2):145–58.*

13. *Karin M. NF-κB and cancer: Mechanisms and targets. Molecular Carcinogenesis. 2006 June;45(6):355–61.*

14. *Kang KB, Kang SJ, Kim MS, Lee DY, Han SI, Kim TB, et al. Chemical and genomic diversity of six Lonicera species occurring in Korea. Phytochemistry. 2018 Nov;155:126–35.*

15. *Comparative study of Mentha species growing wild in Egypt: LC-ESI-MS analysis and chemosystematic significance. J App Pharm Sci. 2018 Aug;116–22.*

16. *Peng Z. Antioxidant flavonoids from leaves of Polygonum hydropiper L. Phytochemistry. 2003 Jan;62(2):219–28.*

17. *Kellenberger E, Muller P, Schalon C, Bret G, Foata N, Rognan D. sc-PDB: an Annotated Database of Druggable Binding Sites from the Protein Data Bank. J Chem Inf Model. 2006 Mar 1;46(2):717–27.*

18. *Xie XQS. Exploiting PubChem for virtual screening. Expert Opinion on Drug Discovery. 2010 Dec;5(12):1205–20.*

19. *Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010 Jan 30;31(2):455–61.*

20. *Makarewicz T, Kaźmierkiewicz R. Molecular Dynamics Simulation by GROMACS Using GUI Plugin for PyMOL. J Chem Inf Model. 2013 May 24;53(5):1229–34.*

21. *Della-Longa S, Arcovito A. Structural and functional insights on folate receptor α (FRα) by homology modeling, ligand docking and molecular dynamics. Journal of Molecular Graphics and Modelling. 2013 July;44:197–207.*

22. *Carron PM, Crowley A, O'Shea D, McCann M, Howe O, Hunt M, et al. Targeting the Folate Receptor: Improving Efficacy in Inorganic Medicinal Chemistry. CMC. 2018 July 4;25(23):2675–708.*

23. *Jurczyk M, Jelonek K, Musiał-Kulik M, Beberok A, Wrześniok D, Kasperczyk J. Single- versus Dual-Targeted Nanoparticles with Folic Acid and Biotin for Anticancer Drug Delivery. Pharmaceutics. 2021 Mar 3;13(3):326.*

24. *Xia W, Low PS. Folate-Targeted Therapies for Cancer. J Med Chem. 2010 Oct 14;53(19):6811–24.*

25. *Barzegar A, Naghizadeh E, Zakariazadeh M, Azamat J. Molecular Dynamics Simulation Study of the HIV-1 Protease Inhibit ion Using Fullerene and New Fullerene Derivatives of Carbon Nanostructures. MRMC. 2017 Apr 11;17(7):633–47.*

26. *Medina-Franco JL, Méndez-Lucio O, Martinez-Mayorga K. The Interplay Between Molecular Modeling and Chemoinformatics to Characterize Protein–Ligand and Protein–Protein Interactions Landscapes for Drug Discovery. In: Advances in Protein Chemistry and Structural Biology [Internet]. Elsevier; 2014 [cited 2025 Nov 21]. p. 1–37. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1876162314000029*

27. *Holm J, Bruun SW, Hansen SI. The complex interplay between ligand binding and conformational structure of the folate binding protein (folate receptor): Biological perspectives. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. 2015 Oct;1854(10):1249–59.*

28. Ahmadi M, Ritter CA, Von Woedtke T, Bekeschus S, Wende K. Package delivered: folate receptor-mediated transporters in cancer therapy and diagnosis. Chem Sci. 2024;15(6):1966–2006.

29. Moon DO. Curcumin in Cancer and Inflammation: An In-Depth Exploration of Molecular Interactions, Therapeutic Potentials, and the Role in Disease Management. IJMS. 2024 Mar 2;25(5):2911.

30. Reddy JA, Low PS. Folate-Mediated Targeting of Therapeutic and Imaging Agents to Cancers. Crit Rev Ther Drug Carrier Syst. 1998;15(6):41.

31. Moharil P, Wan Z, Pardeshi A, Li J, Huang H, Luo Z, et al. Engineering a folic acid-decorated ultrasmall gemcitabine nanocarrier for breast cancer therapy: Dual targeting of tumor cells and tumor-associated macrophages. Acta Pharmaceutica Sinica B. 2022 Mar;12(3):1148–62.

32. Chandrupatla DMSH, Molthoff CFM, Lammertsma AA, Van Der Laken CJ, Jansen G. The folate receptor β as a macrophage-mediated imaging and therapeutic target in rheumatoid arthritis. Drug Deliv and Transl Res. 2019 Feb;9(1):366–78.

33. Varghese B, Vlashi E, Xia W, Ayala Lopez W, Paulos CM, Reddy J, et al. Folate Receptor-β in Activated Macrophages: Ligand Binding and Receptor Recycling Kinetics. Mol Pharmaceutics. 2014 Oct 6;11(10):3609–16.