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Molecular Docking of *Glycyrrhiza glabra* Metabolites at TLR4 and MLCK: Non Classical Depression Related Targets in the Gut–Brain Axis

Rajendran Mala[1]*, Rajamohan Harseni[1]

[1]*Department of Biotechnology, Mepco Schlenk Engineering College, (autonomous), Sivakasi-626005, Tamilnadu, INDIA*

Correspondence

R. Mala

Department of Biotechnology, Mepco Schlenk Engineering College, (autonomous), Sivakasi-626005, Tamilnadu, INDIA

E-mail: maalsindia@mepcoeng.ac.in

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ABSTRACT

Introduction: Licorice (*Glycyrrhiza glabra* L.) is traditionally used for stress, mood and sleep disturbances, but the capacity of its major metabolites to modulate non-classical gut–brain-axis targets relevant to depression remains unclear. **Methods:** This in silico study investigated the anxiolytic–antidepressant potential of nine literature-reported licorice constituents. These included triterpenoid saponins, triterpenoid acids and prenylated/flavonoid scaffolds. The compounds were docked against two peripheral targets that couple inflammation and barrier dysfunction to mood disorders, namely Toll-like receptor 4 (TLR4) and myosin light-chain kinase (MLCK). Molecular docking was performed using CB-Dock2 with resatorvid (TLR4) and 5-iodonaphthalene-1-sulfonyl homopiperazine (MLCK) as reference antagonists, and drug-likeness and safety were evaluated by in silico ADMET prediction. **Results:** Several metabolites, particularly hispaglabridin A, O-(3-hydroxy-6-oxo-7-drimen-11-yl)umbelliferone and 3-oxoglycyrrhetinic acid, showed docking scores at TLR4 (–10.2 to –11.0 kcal/mol) that exceeded resatorvid, and ligands occupied the canonical antagonist pocket, supporting a potential TLR4-mediated anti-inflammatory mechanism. At MLCK, glycyrrhizin, licorice saponin and flavonoid rhamnosides formed 5-iodonaphthalene-1-sulfonyl homopiperazine like complexes in the catalytic groove. ADMET profiling revealed that bulky saponins exhibited high polarity, whereas triterpenoid acids and hispaglabridin A showed high lipophilicity and extensive plasma protein binding. **Conclusions:** Collectively, these findings suggest that *G. glabra* metabolites can, in principle, engage TLR4 and MLCK in a complementary manner, outlining a polypharmacological, gut–brain-axis-oriented hypothesis. These conclusions are preliminary and require confirmation through targeted in vitro and in vivo studies of efficacy, pharmacokinetics and safety.

Keywords: In silico ADMET prediction, Licorice root phytochemicals, Neuroinflammation, Pattern recognition receptors, Stress-related mood disorders

INTRODUCTION

Anxiety and depressive disorders are among the most prevalent neuropsychiatric conditions worldwide and are projected to remain leading contributors to global disability[1]. They are only partially controlled by current antidepressants and anxiolytics that primarily target monoaminergic neurotransmission, which are associated with delayed onset of action, incomplete remission and significant adverse effects. This maintains interest in adjunct or alternative strategies that are safer, better tolerated and mechanistically broader[2,3].

There is increasing recognition that effective interventions for mood disorders should also address stress-response pathways, neuroinflammation and signalling along the gut–brain axis[4]. Disturbances in bidirectional gut–brain communication via neural, endocrine, immune and metabolic routes have been implicated in anxiety and depression[5,6]. When the intestinal barrier becomes "leaky", bacterial products such as lipopolysaccharide (LPS) can enter the circulation and activate pattern-recognition receptors such as Toll-like receptor 4 (TLR4), triggering inflammatory cascades whose mediators may reach the brain, activate microglia and disturb hypothalamic–pituitary–adrenal (HPA) axis regulation[6,7].

Myosin light-chain kinase (MLCK) is another key component of the gut–brain interface because it regulates tight junction proteins that maintain barrier integrity in the intestinal wall and blood–brain barrier. Overactivation of MLCK increases myosin light-chain phosphorylation, opening tight junctions and facilitating translocation of LPS and circulating cytokines, which amplifies systemic inflammation, neuroinflammation and stress-hormone dysregulation. These processes are increasingly recognised as contributors to the pathophysiology of depression and stress-related disorders, and targeting TLR4 and MLCK offers an opportunity to modulate non-classical, peripheral nodes of depression by reducing inflammatory signalling and improving barrier function[8,9].

Glycyrrhiza glabra L. (Fabaceae), commonly known as licorice, is widely used in Ayurveda, Traditional Chinese Medicine and other systems for respiratory, gastrointestinal, endocrine and stress-related complaints[10,11]. Its roots contain abundant triterpenoid saponins such as glycyrrhizin and glycyrrhetinic acid, together with diverse flavonoids and isoflavonoids including glabridin, glabrol, liquiritigenin, isoliquiritigenin, liquiritin and licochalcone A. These constituents contribute to anti-inflammatory, gastroprotective, antiviral, antioxidant, endocrine-modulating and immunoregulatory activities[12-14]. Experimental

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and limited clinical observations suggest a possible stress-reducing and mood-modulating role for *G.glabra*, including attenuation of anxiety-like behaviour[15,16] and barrier-related changes in rodent stress models[17], faster improvement of depressive symptoms in a pilot adjunctive trial, antidepressant-like effects of licorice flavonoids on HPA-axis and BDNF/TrkB signalling, and GABAA receptor modulation by glabridin and flavonoid-rich extracts[18-20].

Despite this evidence, it is not clear whether *G.glabra* metabolites can act in a coordinated manner on gut–brain-axis targets that link peripheral inflammation and barrier dysfunction to mood disorders, particularly TLR4 and MLCK.

Specifically, previous docking or pharmacological studies on *Glycyrrhiza glabra* have focused mainly on classical CNS targets (monoamine transporters, receptors, BDNF/TrkB etc.) or generic anti-inflammatory pathways. They have not systematically examined non-classical, peripheral depression-relevant targets along the gut–brain axis. To our knowledge, no earlier work has evaluated licorice metabolites at both TLR4 (MD-2 lipid A pocket) and MLCK (ATP-binding catalytic groove) in a unified framework. Our study therefore proposes a dual-target, gut–brain axis-oriented mechanism linking pattern-recognition signalling and barrier integrity, which we believe extends current pharmacognosy perspectives on licorice beyond monoaminergic or single-target hypotheses.

To date, no study has systematically examined whether *G.glabra*-derived metabolites interact with these targets. The main objective of the present work was therefore to examine whether well-characterised *G.glabra* metabolites can plausibly modulate these non-classical depression-relevant targets using molecular docking. Specifically, the study aimed (i) to evaluate the binding affinity and interaction patterns of nine literature-reported licorice constituents at TLR4 and MLCK, relative to selected small-molecule antagonists, and (ii) to characterise their drug-likeness and predicted safety profiles by in silico ADMET analysis, in order to generate testable hypotheses for future in vitro and in vivo studies of licorice-based strategies in stress-related mood disorders.

MATERIALS AND METHODS

Selection and preparation of ligands for docking

Phytochemicals from *G. glabra* licorice root that are reported to exhibit CNS, anti-inflammatory or gut-modulatory activities were selected as ligands for in silico studies. These included quercetin 3-(4"-O-acetylr hamnoside)-7-rhamnoside, licorice glycoside B, licorice saponin G2, 4'-Methylliquiritigenin-7-rhamnoside, glycyrrhizin, glabric acid, O-(3 -hydroxy-6-oxo-7-drimen-11-yl)umbelliferone, hispaglabridin A and 3-oxoglycyrrhetinic acid were chosen as ligands for in silico studies. The occurrence of these compounds in *G. glabra* roots has been documented in recent phytochemical reviews[12,21]. Two-dimensional structures were retrieved from the PubChem database as SDF files (https://pubchem.ncbi.nlm.nih.gov). The names of bioactive compounds selected for docking and their PubChem CIDs are summarised in Table 1. No additional local energy minimisation was performed; ligand structures were submitted to the CB-Dock server in SDF format, where standard pre-processing is carried out automatically as part of the docking workflow.

Protein target selection and preparation

Three-dimensional structures of TLR4 (PDB ID: 3FXI) and MLCK (PDB ID: 6C6M) were obtained from the Protein Data Bank (PDB; https://www.rcsb.org) as crystallographic structures. Protein preparation was performed by removing all heteroatoms, including crystallographic water molecules and non-essential co-crystallised ligands, and retaining only the amino-acid residues of the protein chains. The cleaned protein structures were saved in PDB format and used as inputs for CB-Dock docking without further modification, as CB-Dock automatically prepares the files for AutoDock Vina calculations.

Table 1. PubChem compound identifiers (CIDs) for selected *G.glabra* root phytochemicals used in molecular docking.

S.No	Bioactive compound	PubChem CID
1	Quercetin 3-(4"-acetylrhamnoside)-7-rhamnoside	74978214
2	Licorice glycoside B	131751571
3	Licorice saponin G2	14891565
4	4'-Methylliquiritigenin 7-rhamnoside	131752850
5	Glycyrrhizin (glycyrrhizic acid)	14982
6	Glabric acid	46173993
7	O-(3-hydroxy-6-oxo-7-drimen-11-yl) umbelliferone	3725594
8	Hispaglabridin A	442774
9	3-Oxoglycyrrhetinic acid (3-oxo-glycyrrhetinic acid)	10114

Molecular docking using CB-Dock

Molecular docking of the selected *G.glabra* phytochemicals to TLR4 and MLCK was carried out using CB-Dock (http://cao.labshare.cn/cb-dock), an automated web-based blind docking platform that integrates CurPocket cavity detection with AutoDock Vina as the docking engine[22]. CB-Dock predicts putative binding cavities on the protein surface, generates corresponding search boxes and performs docking in an automated workflow. For each protein–ligand pair, the prepared protein structure (PDB) and ligand structure (SDF format) were uploaded to the CB-Dock server. Docking was executed with default AutoDock Vina parameters (exhaustiveness = 8, up to 10 poses per ligand). For each cavity, CB-Dock returned the Vina binding affinity score (kcal/mol) and corresponding ligand pose, and the best-scoring pose at each target was selected for further analysis. To enable comparison with established inhibitors, representative small-molecule antagonists of TLR4 (resatorvid; Compound CID 11703255) and MLCK (5-iodonaphthalene-1-sulfonyl homopiperazine Compound CID: 4216) reported in the literature were docked under the same conditions. Protein–ligand interactions in the top-ranked poses were examined using Discovery Studio Visualizer, with a focus on hydrogen bonds, hydrophobic contacts and π–π stacking with residues known to be important for ligand binding in TLR4 and MLCK.

Docking validation and limitations

Molecular docking was performed using the CB-Dock2 web server, which combines CurPocket-based cavity detection with AutoDock Vina as the docking engine and uses its default preparation and scoring workflow. No explicit re-docking of co-crystallised ligands or RMSD-based pose validation was carried out; therefore, the present docking results should be regarded as qualitative and hypothesis-generating rather than quantitatively predictive of binding affinity or inhibition potency.

For TLR4, the main cavity identified by CB-Dock corresponded to the well-characterised hydrophobic pocket within the MD-2 co-receptor that accommodates lipid A and small-molecule antagonists such as resatorvid, providing biological plausibility for the poses analysed. At MLCK, the selected poses were located within the ATP-binding/catalytic region that binds 5-iodonaphthalene-1-sulfonyl homopiperazine, consistent with a putative competitive mechanism. Future work will require re-docking of reference ligands, comparison of RMSD values across docking engines, and molecular dynamics simulations to refine these preliminary models. In silico ADMET and drug-likeness prediction ADMET and drug-likeness properties of the

selected *G.glabra* metabolites were predicted using the SwissADME web tool (https://www.swissadme.ch), which provides validated models for physicochemical descriptors, pharmacokinetics and medicinal-chemistry features of small molecules[23]. For each ligand, canonical SMILES retrieved from PubChem were submitted to SwissADME. We recorded key parameters relevant to oral exposure and developability, including molecular weight, lipophilicity (cLogP), topological polar surface area (TPSA), number of Lipinski rule-of-five violations, gastrointestinal absorption, bioavailability score and predicted blood–brain barrier permeation, together with qualitative flags for drug-likeness and structural alerts. The resulting profiles were used descriptively to compare scaffolds and to identify potential liabilities (e.g. low oral bioavailability, excessive lipophilicity or multiple rule-of-five violations), recognising that SwissADME outputs are predictive and hypothesis-generating and require experimental confirmation.

RESULTS

The docking results showed that several *G.glabra* phytochemicals exhibited stronger predicted binding to both TLR4 and MLCK than the reference control ligand which is represented in Table 2.

To select compounds for detailed pose visualisation and interaction analysis, we applied predefined affinity thresholds. Ligands with docking scores ≤ −10.0 kcal/mol at TLR4 and ≤ −8.0 kcal/mol at MLCK were classified as high-affinity candidates and chosen for 2D/3D binding-mode figures. Thresholds were chosen a *priori* to select ligands with predicted affinities more favourable than both the reference inhibitors and the median of the test set, allowing focused visualisation of the top-scoring tier. Top ligands at TLR4 scored ~3–4 kcal/mol more favourable than resatorvid, whereas differences at MLCK were more modest

TLR4

The 2D interaction maps for TLR4 (Figure 1) show that all docked ligands occupy a predominantly hydrophobic pocket composed mainly of Phe, Ile, Leu and Val residues, similar to the reference inhibitor resatorvid. Resatorvid is stabilised chiefly by van der Waals and π-type hydrophobic contacts with these residues and forms no clear conventional hydrogen bonds. 3-Oxoglycyrrhetinic acid displays a comparable hydrophobic interaction pattern but contacts a larger set of non-polar residues around its triterpenoid core. Licorice saponin G2 combines extensive hydrophobic contacts of the sapogenin moiety with several sugar-mediated hydrogen bonds to nearby polar and charged residues. Hispaglabridin A forms an extended network of π–π stacked, π-sigma and π-alkyl interactions between its aromatic rings and Phe/Ile/Leu side chains, together with additional van der Waals contacts, and attains the most favourable docking energy among the tested ligands at this site.

MLCK

The 2D interaction map of the control inhibitor 5-iodonaphthalene-1-sulfonyl homopiperazine at MLCK shows a single central conventional hydrogen bond between the sulfonyl oxygens and Arg A456, which serves as the main polar contact, together with π-sigma and π-alkyl interactions between its fused aromatic system and Val A463 and Ala A446, and a π–anion interaction with Glu 444. All three *G. glabra* ligands occupy the same hydrophobic pocket. The umbelliferone–drimane derivative maintains van der Waals contacts with Val/Ile and introduces multiple π-sigma and π-alkyl interactions between the coumarin ring and Phe/Tyr residues. Methyl-liquiritigenin rhamnoside exhibits π-sigma and π-alkyl contacts of its flavonoid core with Phe/Trp/Val and forms several conventional and carbon–hydrogen bonds with Tyr 464 and neighbouring Glu residues. Quercetin 3-(4''-O-acetylrhamnoside)-7-rhamnoside shows the most elaborate pattern, with multiple phenolic rings engaging in π-sigma, π–π stacked and π-alkyl interactions with Phe and Ile, alongside several hydroxyl-mediated hydrogen bonds to Glu, Tyr and Gly in the same pocket Figure 2.

Table 2. Binding Energy (AutoDock Vina docking score) of Major Flavonoid Glycosides and Triterpenoid Saponins

S.No	Compound	AutoDock Vina docking score. (kcal/mol) TLR4	AutoDock Vina docking score. (kcal/mol) MLCK
1	Quercetin 3 (4'' acetylrhamnoside) 7 rhamnoside	-9.5	-8.0
2	Licorice glycoside B	-9.8	-6.5
3	Licorice saponin G2	-10.6	-8.2
4	4'-Methylliquiritigenin 7 rhamnoside	-8.7	-8.1
5	Glycyrrhizin	-9.9	-7.7
6	Glabric acid	-9.5	-7.0
7	O-(3-hydroxy-6-oxo-7-drimen-11-yl)umbelliferone	-10.6	-10.2
8	Hispaglabridin A	-11	-7.7
9	3 Oxoglycyrrhetinic acid	-10.2	-7.1
10	Control	-7.3	-6.7

Table 3. Drug-Likeness

S.No	Compound	MW (Da)	cLogP	TPSA	HIA	F<30%	PPB (%)	BBB penetration (prob)	Ro5 violations
1	Glycyrrhizin	822.4	2.45	267	0.97	High	75	0.28	3
2	Licorice saponin	838.4	1.21	287.3	0.98	High	69	0.27	3
3	3-Oxoglycyrrhetinic acid	468.3	5.66	71.4	0.11	High	87	0.06	1 (logP)
4	Licoric acid	484.3	4.99	83.8	0.01	Low	91	0.72	0
5	Glabric acid	486.3	4.77	94.8	0.02	High	86	0.08	0
6	Hydroxy isoglabrolide	484.3	4.11	83.8	0.03	High	93	0.62	0
7	Hispaglabridin A	392.2	6.83	58.9	0.02	Very high	101	0.05	1 (logP)
8	4'-Methylliquiritigenin-7-rhamnoside	416.2	2.05	114.7	0.21	Moderate	85	0.48	0
9	Quercetin 3-(4''-acetylrhamnoside)-7-rhamnoside	318.1	2.39	80.1	0.01	Very high	88	0.55	0

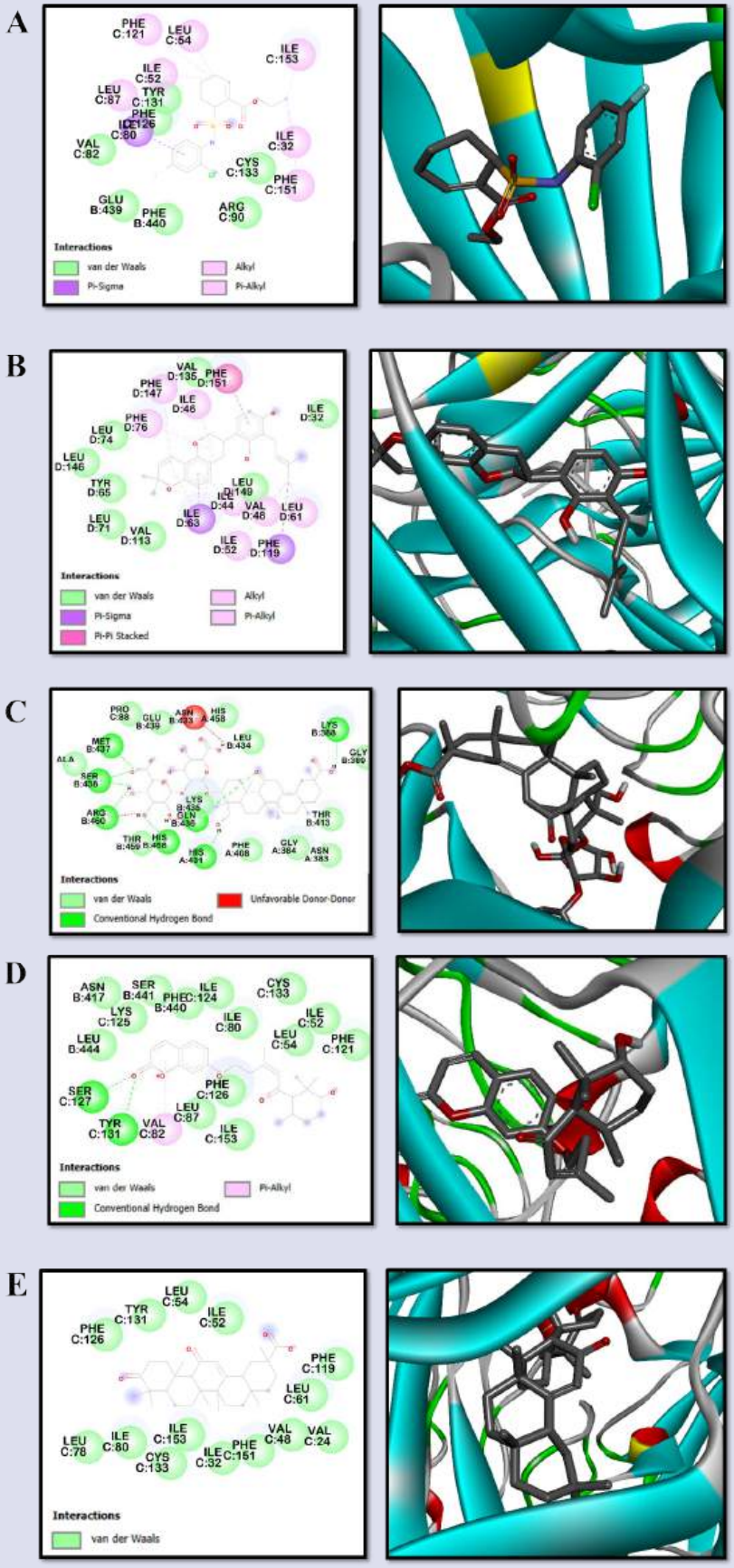

Figure 1. 2D and 3D Interaction of Bioactive Compounds with TLR4

Resatorvid (Control) B) Hispaglabridin A C) Licorice saponin G2 D) O-(3-hydroxy-6-oxo-7-drimen-11-yl)umbelliferone E) 3-Oxoglycyrrhetinic acid The illustrated poses correspond to the top-ranked docking conformations predicted by CB-Dock for each ligand at the indicated target.

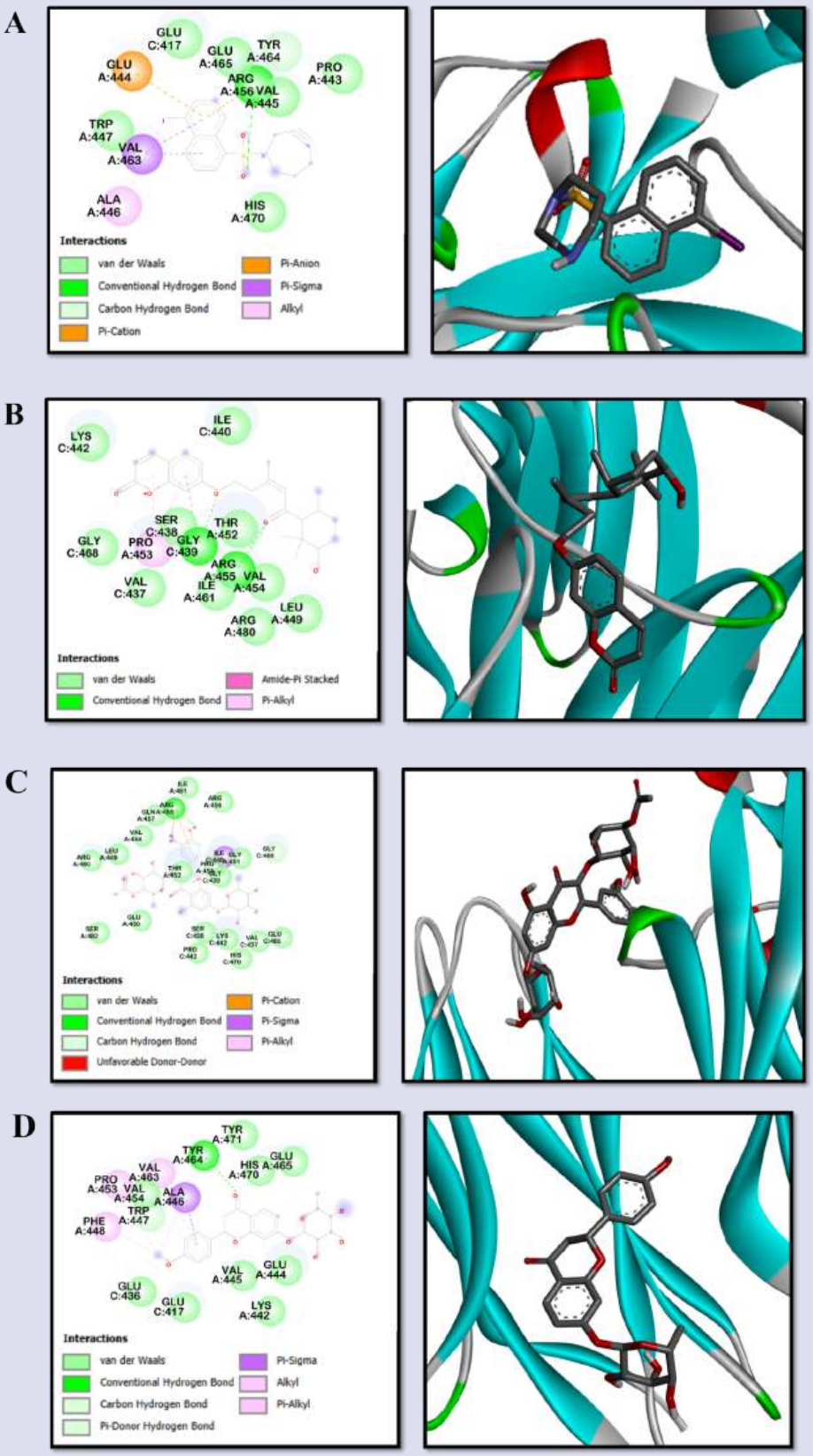

Figure 2. 2D and 3D Interaction of Bioactive Compounds with MLCK

A) 5-iodonaphthalene-1-sulfonyl homopiperazine

B) O-(3-hydroxy-6-oxo-7-drimen-11-yl)umbelliferone C) Quercetin 3 (4'' acetylrhamnoside) 7 rhamnoside D) 4'-Methylliquiritigenin 7 rhamnoside

The illustrated poses correspond to the top-ranked docking conformations predicted by CB-Dock for each ligand at the indicated target.

Table 4. In-silico toxicity

S.No	Compound	hERG	Ames	Glycyrrhizin	DILI (prob)	Respiratory toxicity	Overall acute oral toxicity
1	Glycyrhizin	0.01	0.09	0.17	0.01	0.94	Low
2	Licorice saponin	0.03	0.09	0.21	0.01	0.95	Low
3	3-Oxoglycyrrhetinic acid	0.01	0.03	0.31	0.01	0.98	Low
4	Licoric acid	0.06	0.04	0.23	0.03	0.98	Low
5	Glabric acid	0.02	0.02	0.32	0.01	0.98	Low
6	Hydroxy isoglabrolide	0.01	0.06	0.26	0.02	0.97	Low
7	Hispaglabridin A	0.27	0.05	0.94	0.05	0.84	Low
8	4'-Methylliquiritigenin-7-rhamnoside	0.06	0.67	0.13	0.9	0.23	Low
9	Quercetin 3-(4''-acetylrhamnoside)-7-rhamnoside	0.04	0.32	0.57	0.93	0.95	Low

ADMET

The nine *G. glabra* metabolites (Table 3) showed molecular weights from 318.1 to 838.4 Da and cLogP values between 1.21 and 6.83. Saponins (glycyrrhizin and Licorice saponin G2) were the largest and most polar compounds, with TPSA (topological polar surface area) values of 267–287 Å², three Lipinski rule-of-five violations each, high predicted human intestinal absorption and high probabilities of oral bioavailability below 30%. Triterpenoid acids (oxyglycyrrhetinic, licoric and glabric acids and hydroxy-isoglabrolide) had intermediate molecular weights (468.3–486.3 Da), cLogP values of 4.1–5.7, low-to-moderate predicted HIA and predominantly high probabilities of F<30%, with 0–1 Lipinski violation. Hispaglabridin A (392.2 Da) displayed the highest cLogP (6.83) and an estimated plasma protein binding of approximately 100%. The flavonoids methyl-liquiritigenin and quercetin rhamnoside were lower in molecular weight and moderately lipophilic (cLogP 2.05–2.39), had acceptable TPSA, no rule-of-five violations and low to very high predicted probabilities of F<30%

In silico safety predictions (Table 4) indicated low overall acute oral toxicity for all nine compounds and low probabilities of hERG channel inhibition (≤0.06 for most ligands). Ames mutagenicity probabilities were 0.02–0.09 for most metabolites, while methyl-liquiritigenin and quercetin rhamnoside showed higher values of 0.67 and 0.32, respectively. Predicted human hepatotoxicity and DILI risks were low for the saponins and triterpenoid acids, but higher for hispaglabridin A, methyl-liquiritigenin and quercetin rhamnoside, with H-HT values up to 0.94 and DILI probabilities up to 0.93. Respiratory-toxicity probabilities were 0.94–0.98 for the triterpenoid acids and quercetin rhamnoside and 0.23 for methyl-liquiritigenin.

DISCUSSION

Docking indicates that all selected *G.glabra* ligands occupy the MD-2 hydrophobic cavity of the TLR4/MD-2 complex in the same region as Resatorvid, engaging key lipid A-binding residues (Phe, Leu, Ile and Val.) This pattern supports the hypothesis that licorice metabolites could interfere with LPS recognition at MD-2 and thereby modulate downstream NF-κB/MAPK-dependent inflammatory signalling and associated neuroinflammatory mechanisms relevant to depression. However, this remains to be demonstrated experimentally. Similarities between the binding modes of some triterpenoids and resatorvid further suggest that TLR4–MD-2 may be a plausible peripheral target for licorice-derived scaffolds in gut–brain axis modulation.

At MLCK, 5-iodonaphthalene-1-sulfonyl homopiperazine and the licorice ligands dock within the ATP-binding/catalytic region, consistent with a putative competitive mechanism at the kinase active site. MLCK activation contributes to tight-junction opening and barrier dysfunction in intestinal and brain microvascular endothelium. Inhibition at this site could, in principle, help stabilise gut and blood–brain barrier integrity and reduce inflammatory inputs into mood-related circuits. Existing experimental and clinical data linking MLCK-dependent barrier changes, endotoxin-related biomarkers and depressive symptomatology provide a biological context. However, the inhibitory potential of *G.glabra* metabolites on MLCK activity remains to be confirmed.

Molecular docking provides a useful first step for exploring plausible protein–ligand interactions, but it has important limitations and should be viewed as hypothesis-generating rather than confirmatory. Scoring functions only approximate binding free energy and often perform unreliably when comparing chemically diverse, flexible natural products, so docking energies cannot be interpreted as direct measures of biological potency. In addition, standard docking workflows treat the protein largely as rigid and neglect explicit solvent, metabolism and pharmacokinetics, which may be especially critical for bulky saponins and glycosides. Consequently, the present results are intended to prioritise *Glycyrrhiza glabra* metabolites and interaction patterns for future biophysical and pharmacological validation, not to demonstrate actual target inhibition or therapeutic efficacy.

Docking scores are interpreted here as indicators of binding plausibility and interaction patterns, not as direct measures of biological potency. Large, highly polar saponins may achieve favorable docking energies due to extensive surface contacts, but their systemic bioavailability is limited, making intact target engagement unlikely. Thus, the comparative docking scores should be viewed as supportive of potential binding modes rather than evidence of therapeutic efficacy. The emphasis is placed on the hydrophobic and hydrogen-bond interactions that suggest plausible engagement with TLR4 and MLCK."

The ADMET profiling underscores that any translational development would have to account for substantial differences in physicochemical and safety-prediction profiles across scaffolds. Bulky, highly polar saponins show multiple rule-of-five violations and low predicted oral bioavailability. Triterpenoid acids and hispaglabridin A are highly lipophilic with extensive predicted plasma protein binding. Smaller flavonoids, although formally "drug-like", may still show low predicted oral exposure. Safety models suggest a generally acceptable acute profile but highlight higher mutagenicity and hepatotoxicity/DILI probabilities for methyl-liquiritigenin, quercetin rhamnoside and hispaglabridin A, and elevated respiratory-toxicity signals for triterpenoid acids. This indicates that individual constituents will require careful selection, dosing and formulation if pursued further. These ADMET findings should be regarded as preliminary and as prioritisation tools rather than as substitutes for experimental pharmacokinetic and toxicological evaluation.

The ADMET predictions highlight important pharmacognostic considerations. Glycosides and saponins are subject to intestinal metabolism and microbial transformation, which may generate smaller metabolites with improved bioavailability and activity. Therefore, gut–brain axis modulation may occur via local intestinal or immune mechanisms, rather than direct CNS penetration. The high

plasma protein binding observed for lipophilic triterpenoid acids and hispaglabridin A suggests limited free drug availability, which could influence pharmacological outcomes. These findings underscore the need to consider metabolic conversion, local intestinal effects, and pharmacokinetic constraints when evaluating licorice metabolites for gut–brain axis modulation.

Together, the docking and ADMET data support a cautious, hypothesis-driven view of licorice as a source of metabolites that might modulate peripheral targets such as TLR4 and MLCK, HPA-axis-related and immune pathways, rather than as evidence that licorice itself is an established antidepressant or anxiolytic nutraceutical. The work highlights non-classical, gut–brain axis-related mechanisms that warrant further investigation but does not establish efficacy or safety in clinical settings. Robust in vitro assays of TLR4 signalling, MLCK activity and barrier function, followed by in vivo studies integrating behaviour, pharmacokinetics and organ-specific toxicity, are essential next steps before any translational claims can be made.

It is important to clarify that this study does not demonstrate therapeutic effectiveness. No in vitro, in vivo, or behavioral assays were performed, and the findings are limited to in silico docking and ADMET predictions. The results serve solely to propose mechanistic hypotheses for future experimental validation. Any references to antidepressant or anxiolytic potential are intended as hypothesis-driven suggestions, not as evidence of clinical efficacy. This distinction ensures that the work is positioned as an initial mechanistic exploration rather than a therapeutic claim.

CONCLUSION

This work is an initial, mechanistic in silico exploration rather than evidence of clinical benefit. In silico study suggests that selected *G. glabra* metabolites can bind with high predicted affinity to the MD-2 lipid A pocket of TLR4 and the ATP-binding region of MLCK, in modes comparable to reference inhibitors resatorvid and 5-iodonaphthalene-1-sulfonyl homopiperazine. Such interactions provide a plausible peripheral mechanism for dampening LPS-driven TLR4 signalling and MLCK-mediated barrier disruption, thereby attenuating inflammatory inputs along the gut–brain axis that are implicated in mood disorders. ADMET predictions indicate a reasonable acute safety margin but reveal scaffold-specific liabilities in oral bioavailability, lipophilicity and potential hepatic, respiratory or genotoxic risks, supporting the need for careful constituent selection rather than indiscriminate extract use.

Future work should focus on validating TLR4 and MLCK inhibition in vitro, characterising pharmacokinetics of the most promising ligands, and testing their effects on behaviour, barrier integrity and inflammatory markers in relevant animal models, alongside targeted toxicological evaluation to define safe exposure ranges.

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