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Computational Tools to Expedite the Identification of Potential PXR Modulators in Complex Natural Product Mixtures – A Case Study with Five Closely Related Licorice Species

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Background

- Complementary and alternative medicine (CAM) is an integral part of various traditional practices and continues to gain popularity in the US and elsewhere.
- > 80% of the worldwide population uses herbal medicines daily.
- Licorice is among the most popular medicinal plants marketed in the US to alleviate multiple ailments.
- It is most studied herbs in CAM. However, there is no appropriate recommendation of either its efficacy or safety.
- The Natural Product Drug Interaction Research has prioritized *Glycyrrhiza* as one of the high-risk herbal constituents viz., herb-drug interactions.
- Licorice extract modulates various xenobiotic receptors, which might manifest as a potential route for natural-product-induced drug interactions (NPDI); however, different mechanisms could be involved in this behavior. The induced herb-drug interaction of licorice supplements *via* Pregnane X receptor (PXR) is poorly studied.
- The broad range of test substrates highlight the diverse and crucial role of PXR in drug metabolism (efficacy, toxicity, drug interactions, and drug resistance) and its associated diseases (metabolic syndrome, cancer, and inflammation).[1-3]

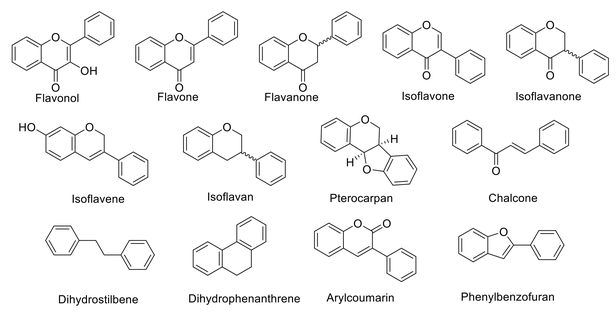

Figure 1. Representation of the main structural scaffolds identified within *Glycyrrhiza* species.

Primary focus of work

- The primary objective of this study is to rapidly dereplicate potential PXR modulators from the chemical reservoirs of five common *Glycyrrhiza* species with the help of computational tools before conducting time-consuming, expensive in vitro and in vivo methodologies to gauge the deleterious effects of licorice

Acknowledgments

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Methods and Results

- Structures of all known secondary metabolites of *Glycyrrhiza* species were collected from various references and chemical databases. The main structural scaffolds are shown in **Fig. 1**.
- A total of 518 compounds from various species of *Glycyrrhiza*: 183 (GG, *G. glabra*), 180 (GU, *G. uralensis*), 100 (GI, *G. inflata*), 33 (GE, *G. echinata*), and 22 (GL, *G. lepidota*) were retrieved and led to total 387 unique compounds.
- The 3D XYZ coordinates of two independent X-ray crystal structures of PXR (PDB: 1NRL & 1M13) were used in this study.
- The ensemble docking *via* a virtual screening workflow (VSW) module was implemented for all known secondary metabolites of *Glycyrrhiza* species with extra precision (XP).
- Best candidates were selected based on prime MM-GBSA binding free energies using ≤ −50 kcal/mol as a cutoff value (**Fig. 2**).
- Select compounds with promising, favorable ligand-PXR interactions were tested *in vitro* using a PXR reporter gene assay (**Table 1**).
- (3*R*)-Glabridin, which showed the highest PXR activation *in vitro,* was solvated with the TIP3P water model to perform MD simulations.

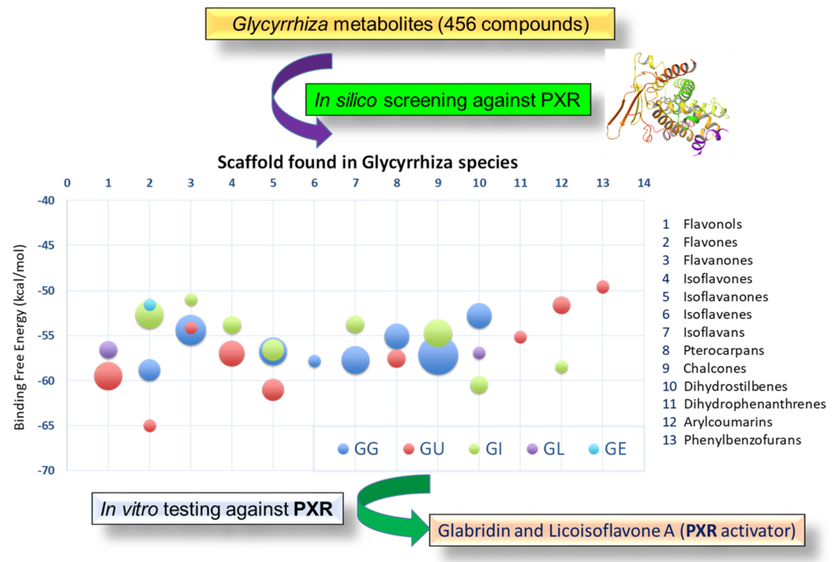

Figure 2. Bubble graph showing the PXR affinity of the compounds from *Glycyrrhiza* species.

Table 1. PXR Activation by compounds from *Glycyrrhiza* Species.

Code	Compound	Fold increase in PXR activity		
		30 µM	10 µM	3 µM
GG-14	(3*R*)-Glabridin	6.53 ± 0.38	3.29 ± 0.90	1.99 ± 0.30
GU-128	Licoisoflavone A	3.88 ± 0.41	2.53 ± 0.17	1.87 ± 0.19
GG-98	Liquiritin	1.28 ± 0.07	1.07 ± 0.22	0.82 ± 0.15
GU-03	Glycycoumarin	2.46 ±0.22	1.44 ±0.05	1.19 ± 0.14
GI-19	Isoliquiritigenin*	1.34 ± 0.03	1.20 ± 0.16	0.90 ± 0.02
GU-130	Licoisoflavanone	3.29 ± 0.31	2.40 ± 0.31	1.90 ± 0.31
Positive control	Rifampicin	3.51 ± 0.42	3.20 ± 0.80	2.70 ± 0.50

*Close mimic of neoisoliquiritigenin (GG-103)

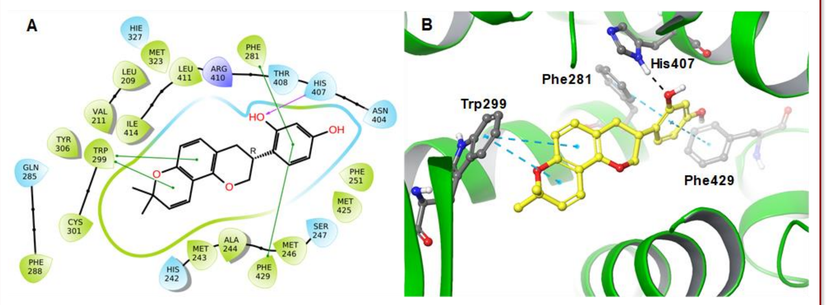

Figure 3. 2D (A) and 3D (B) interaction diagrams of glabridin (GG-14) with 1NRL PXR X-ray crystal structure.

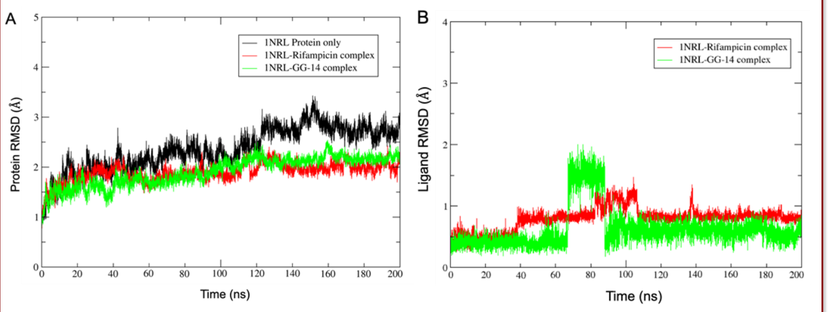

Figure 4. Root mean square deviation (RMSD) analysis of molecular dynamic (MD) simulation trajectory. The RMSD plot obtained for (A) C-α atoms of the protein PXR (PDB ID: 1NRL) with glabridin (GG-14), and rifampicin complex; and (B) ligand-heavy atoms for glabridin- and rifampicin-PXR complex (PDB ID: 1NRL), for the reference frame at time 0 ns.

Discussion and Conclusions

- An exhaustive *in silico* screening of the known components of different yet closely related *Glycyrrhiza* species for potential PXR modulation was conducted.
- The results showed that compounds from *G. glabra* have diverse and unique scaffolds and exhibit better predicted binding affinities towards PXR than other *Glycyrrhiza* species (**Fig. 3**).
- Computational results were further validated with cell-based experiments for PXR induction.
- Four of the six tested compounds showed concentration-dependent, strong PXR activation; among them, (3*R*)-glabridin (GG-14) was most active one.
- Stable and strong interactions were observed between the glabridin-PXR complex during the 200 ns simulation (**Fig. 4**).
- Glabridin showed strong π-π interactions with Phe251, Phe288, Trp299, Trp306, His407, and H-bonding with Arg410.
- Demonstrated the utility of computer-aided tools in understanding the safety and polypharmacology of traditional herbals. Implementation of such innovative tools would expedite and complement traditional pharmacognostic investigations and provide an alternative tool for the rapid dereplication of causative agents.

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