Research Article

Molecular docking analysis of triptoquinones from genus *Tripterygium* with iNOS and in silico ADMET prediction

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Abstract
This paper presents an investigation on the binding interaction of triptoquinones identified from genus *Tripterygium* to iNOS. In silico methods are adopted to predict ADME parameters, pharmacokinetic properties, drug-likeliness and acute toxicity of these identified compounds. A total of 20 triptoquinones are currently identified from genus *Tripterygium*. Most of these triptoquinones are found to bind to the key human iNOS residues involved in inhibitor binding. All the compounds are considered having drug-likeliness properties with no violation against Lipinski's "rule of 5" and are under safe category when administered orally. Twelve out of the 20 triptoquinones are predicted as passively crossing the blood-brain barrier. Eight of the given compounds are predicted to be pumped out by the p-glycoprotein. CYP2C19 and CYP2C9 are the significant isoforms influenced by the investigated triptoquinones from genus *Tripterygium*. As a result, triptoquinone ingredients from genus *Tripterygium* may be promising candidates for the development of drugs preventing inflammatory diseases.

Keywords Triptoquinone · *Tripterygium* · iNOS · Molecular docking · ADMET

1 Introduction

Herbs of the genus *Tripterygium* have long been used in traditional Chinese medicine (TCM) for the treatment of autoimmune and inflammatory diseases like rheumatoid arthritis (RA) [1–4]. The genus *Tripterygium* consists of three species, namely *Tripterygium hypoglaucum* (*Levl.*) *Hutch* (Kun Ming Shan Hai Tang in Chinese), *Tripterygium regelii Sprague et Takeda* (Dong Bei Lei Gong Teng in Chinese), and *Tripterygium wilfordii Hook. f.* (Lei Gong Teng in Chinese, also Thunder God Vine) [5].

Inducible nitric oxide synthase (iNOS) is one of the major mediators during inflammatory processes [6]. Nitric oxide (NO) is formed via iNOS activity mediates inflammation and has been implicated in many diseases, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), diabetes mellitus (DM), stroke, cancer, and Alzheimer's disease (AD) [7, 8]. Besides, iNOS has been found to be associated with activation of another major inflammatory mediator cyclooxygenase-2 (COX-2) [6]. Therefore, the development of iNOS inhibitors is highly desirable. Research efforts have focused on natural products for the discovery of iNOS inhibitors [9]. Niwa et al. [10] and Moritoki et al. [11] reported that the triptoquinone A, an active constituent in *Tripterygium wilfordii*, could prevent iNOS induction by LPS or IL-1β. Chen et al. [12] recently found that some of the triptoquinone constituents from *Tripterygium hypoglaucum* exhibited inhibitory activity of lipopolysaccharide (LPS)-induced NO production in macrophages. These

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triptoquinone compounds identified from genus *Tripterygium* are characterized by a *p*-benzoquinone C-ring. However, it remains unclear whether triptoquinone constituents from genus *Tripterygium* can act directly on iNOS protein.

Given the importance of iNOS in inflammatory responses and the potential role of naturally occurring triptoquinone compounds from genus *Tripterygium* against iNOS, we set up to investigate the interaction of triptoquinones from plants in the genus of *Tripterygium* with iNOS protein using molecular docking method. Physicochemical descriptors, ADME parameters, pharmacokinetic properties, and drug-like nature of molecules were computed and predicted in silico through the free web tool in SwissADME. In silico acute rat toxicity for chemical compounds was predicted by a freely accessible web tool GUSAR software based on reliable quantitative-structure activity relationships (QSAR) modeling [13].

2 Materials and methods

2.1 Discovery of triptoquinone ingredients from *Tripterygium*

Triptoquinones from plants in the genus *Tripterygium* were discovered through literature retrieval and traditional Chinese medicine systems pharmacology database (TCMSP) search. Chemical structures were either obtained from the PubChem Compound Database or drawn using the software ChemBioDraw Ultra 14.0 and saved as SDF files.

2.2 Molecular docking studies

Molecular docking analysis was performed using Schrödinger Software (Maestro, version 10.2). Briefly, the 3D coordinate of the crystal structure of human iNOSox (PDB ID: 3E7G) [14] was downloaded from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) in PDB format. The protein was prepared using the Protein Preparation Wizard panel. Water molecules were removed from the protein structure. The Receptor Grid Generation panel was then used to set up the grid generation job, which helps to show the active site of the receptor for Glide ligand docking jobs. The LigPrep panel was used for ligand preparation. Docking jobs were performed using the Glide Ligand Docking panel. Glide gscores were recorded. Ligand interactions with the protein domain were analyzed using PyMOL Molecular Graphics System Version 2.0 Schrödinger, LLC.

2.3 In silico ADME profile prediction

The ADME parameters (for absorption, distribution, metabolism, and excretion) of the triptoquinones from plants in the genus *Tripterygium* were predicted using a web tool SwissADME (http://www.swissadme.ch/). A rapid appraisal of drug likeness of each compound was conducted using Bioavailability Radar method [15]. The BOILED-Egg method was used to predict simultaneously two key ADME parameters, i.e., the passive gastrointestinal absorption (HIA) and brain access (BBB) [15]. For assessment of absorption for oral drug likeness, the number of free rotatable bonds and the so-called Lipinski's "rule of 5" for the compounds were analyzed. The "rule of 5" states that drug-like compounds with good absorption or permeation are more likely to present molecular weight ≤ 500, number of H-bond acceptors ≤ 10, number of H-bond donors ≤ 5, and CLog $P \leq 5$ [16]. The SwissADME web tool also predicts pharmacokinetic properties of a given compound, including P-glycoprotein substrate and inhibition of cytochrome P450 isoenzymes (CYP) 1A2, 2C19, 2C9, 2D6, and 3A4.

2.4 Acute rat toxicity prediction

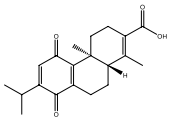
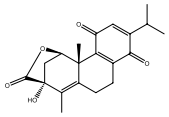
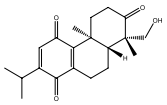
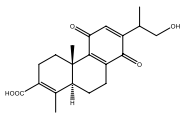
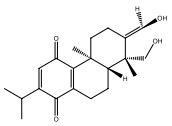
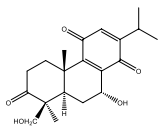
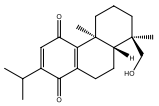
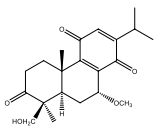
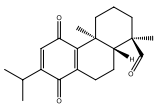
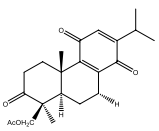
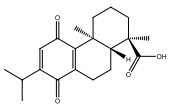
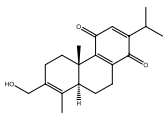
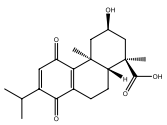
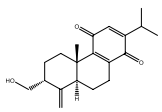
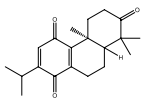
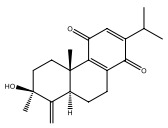
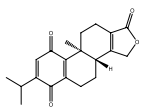
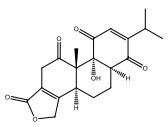
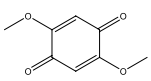
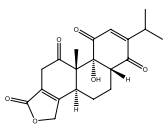
In silico prediction of LD_{50} values for rats with four types of administration (intraperitoneal, intravenous, oral, and subcutaneous) was performed using GUSAR ONLINE software (http://www.way2drug.com/gusar/acutoxpredict.html). GUSAR software was developed to create QSAR models on the basis of the appropriate training sets. The QSAR models for rat LD50 values predictions include information about ~ 10,000 chemical structures with data on acute rat's toxicity originated from SYMYX MDL Toxicity Database. The data of LD50 values were shown as mg/kg and acute rodent toxicity classification was also presented. The acute toxicity classification of the investigated triptoquinones from genus *Tripterygium* was reported in accordance with the Guidelines of Organisation for Economic Cooperation and Development (OECD) for the testing of chemicals.

3 Results and discussion

3.1 Triptoquinone constituents from genus *Tripterygium*

A total of 20 triptoquinones were currently identified from plants in the genus of *Tripterygium*, including 19 diterpene quinoides (Table 1). All these compounds were shown to possess a *p*-benzoquinone

Table 1 Chemical structures of triptoquinones in plants of the genus *Tripterygium*

No.	Chemical names	Chemical structure depiction	No.	Chemical names	Chemical structure depiction
1	**Triptoquinone A**		11	**Triregelin A**	
2	**Triptoquinone B**		12	**Triregelin B**	
3	**Triptoquinone C**		13	**Triregelin C**	
4	**Triptoquinone D**		14	**Triregelin D**	
5	**Triptoquinone E**		15	**Triregelin E**	
6	**Triptoquinone F**		16	**Hypoglicin H**	
7	**Triptoquinone G**		17	**Hypoglicin I**	
8	**Triptoquinone H**		18	**Hypoglicin J**	
9	**Triptoquinonide**		19	**Hypoglicin K**	
10	**Thermophillin**		20	**Hypoglicin L**	

ring. Compounds triptoquinone A-G were first isolated from the stems of *Tripterygium wilfordii* var. *regelii* by Shishido et al. [17]. Six years after Shishido's discovery, Fujita et al. [18] found triptoquinone H in the root bark of *Tripterygium hypoglaucum*. Triptoquinonide, also named quinone 21, was first reported as a natural

Table 2 Glide score and binding interaction of triptoquinones in plants of the genus *Tripterygium* with human iNOS (PDB ID: 3E7G)

Compounds	Glide gscore	Interactions
Triptoquinone A	−2.871	GLN263, GLN387, ASP382, ARG381
Triptoquinone B	−3.194	GLN263, TYR373, ARG388
Triptoquinone C	−3.713	ARG388, TYR373
Triptoquinone D	−2.715	GLU377
Triptoquinone E	−2.933	TYR347
Triptoquinone F	−3.102	GLU377, GLN263, GLN387, ARG388
Triptoquinone G	−3.077	GLU377, GLN263, GLN387, ARG388
Triptoquinone H	−2.932	GLN263, TYR347
Triptoquinonide	−2.837	GLN263, ASN354
Thermophillin	−2.337	TRP372
Triregelin A	−3.605	GLU377, GLN263, TYR373
Triregelin B	−3.298	GLU377, GLN387, ASP382, ARG381
Triregelin C	−3.455	GLN263, TYR491, ASN354
Triregelin D	−3.536	GLU377, GLN263, TRY373, HEM901
Triregelin E	−2.124	GLU377, ARG266, ARG388
Hypoglicin H	−3.479	GLN263
Hypoglicin I	−1.946	GLU377, ASP382, ARG388
Hypoglicin J	0.833	ASP382, ARG388
Hypoglicin K	−3.779	GLN263, GLN387, TYR373, ARG381
Hypoglicin L	−2.940	GLN387, ARG381

product from the heartwood of the root of *Tripterygium wilfordii* by Morota et al. [19]. Thermophillin, also named 2, 5-dimethoxybenzoquinone, was collected in the online traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP, http://lsp.nwu.edu.cn/molecule.php?qn=3171). Compounds triregelin A–E were recently isolated and identified from the stems of *Tripterygium regelii* [20] and hypoglicin H–L were from the stems of *Tripterygium hypoglaucum* [12].

3.2 Molecular docking

It has been well documented that the key human iNOS residues involved in inhibitor binding include active site residue Glu377, first-shell residues (Gln263, Tyr347, Arg266, and Arg388), second-shell residue Asn283, and third-shell residues (Phe286 and Val305) [14]. To investigate the binding modes of triptoquinones from genus *Tripterygium* with iNOS enzyme, docking of these compounds utilizing a Schrödinger software Maestro 10.2 was performed. The docking scores ranged from 0.833 to − 3.779. Among 20 triptoquinones from plants in the genus of *Tripterygium*, only compounds thermophillin

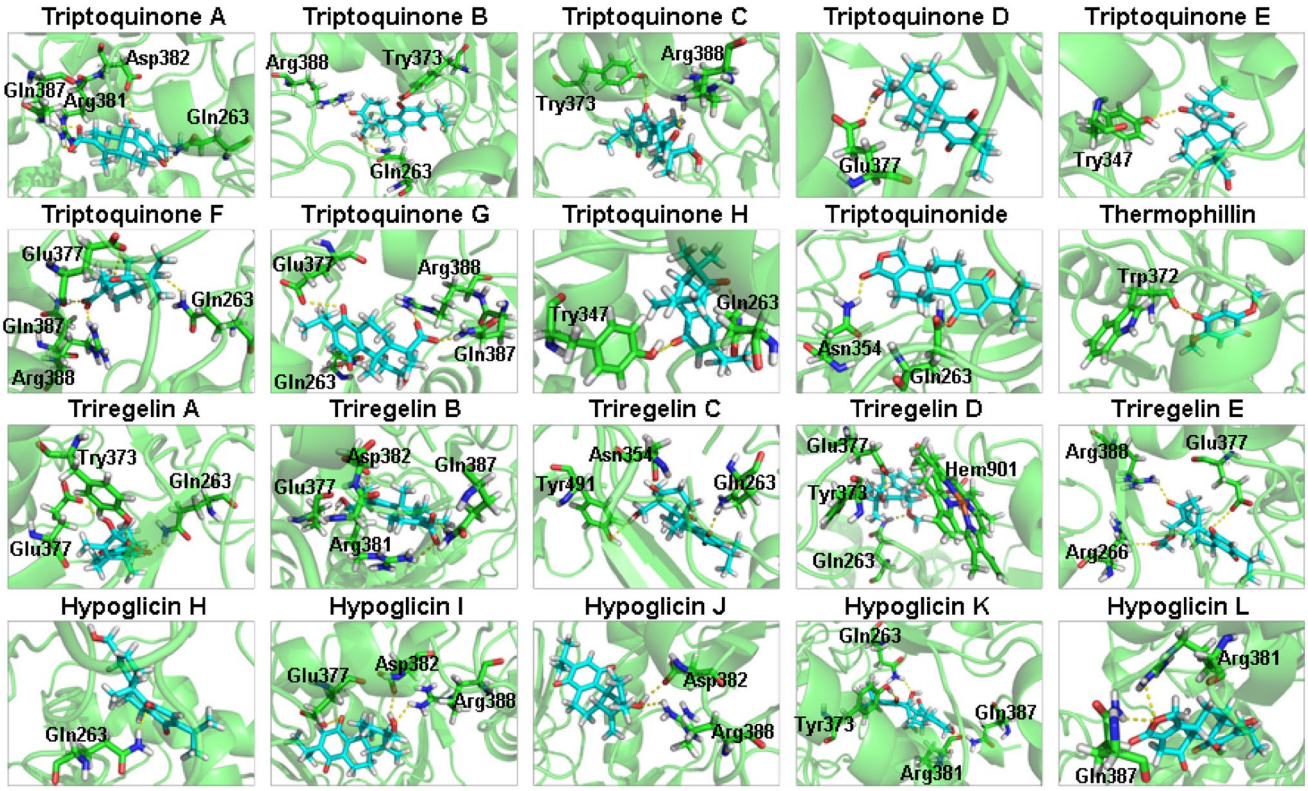


Fig. 1 3D diagrams showing the interactions between iNOS protein residues and the investigated triptoquinones in genus *Tripterygium*. The hydrogen bonds were shown as yellow dotted lines

and hypoglicin L showed no interaction with key residues involved in iNOS inhibitor binding (Table 2). Triptoquinone D, F–G, triregelin A–B, D–E, and hypoglicin I showed hydrogen bonds to Glu377, the active site residue of human iNOS (Table 2, Fig. 1). The other 10 triptoquinones showed hydrogen bonding interactions restricted to the first-shell Gln263, Tyr347, Arg266, and Arg388 residues interacting directly with the iNOS inhibitor (Table 2, Fig. 1). These findings suggested that many of these naturally occurring triptoquinones from genus *Tripterygium* could bind to human iNOS and have a potential inhibitor effect against this enzyme.

3.3 Computer-aided ADME prediction

In the present study, the SwissADME web tool, developed to support drug discovery [15], has been used for the in silico prediction of ADME parameters of triptoquinones from plants in the genus *Tripterygium*.

Bioavailability and pharmacokinetics are two important factors involved in drug development. For oral bioavailability, six important properties (i.e., lipophilicity, size, polarity, solubility, flexibility, and saturation) should be taken into account [15]. Our bioavailability radar plot showed that all the triptoquinones were in the optimal range for each physicochemical property (lipophilicity: $-0.7 < \text{XLOGP3} < 5.0$, size: $150\ \text{g/mol} < \text{MV} < 500\ \text{g/mol}$, polarity: $20\ \text{Å}^2 < \text{TPSA} < 130\ \text{Å}^2$, solubility: $0 < \text{Log S (ESOL)} < 6$, saturation: $0.25 < \text{Fraction Csp3} < 1$, and flexibility: $0 < \text{Num. of rotatable bonds} < 9$), suggesting that these triptoquinone constituents from genus *Tripterygium* were orally bioavailable (Fig. 2).

In terms of pharmacokinetic behaviors, gastrointestinal absorption and brain access are crucial to make an estimation. The Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) has been proposed as an accurate predictive model to predict gastrointestinal absorption and brain penetration of small molecules [21]. According to the readout of the BOILED-Egg model (Fig. 3), the investigated naturally occurring triptoquinones from genus *Tripterygium* were all located in the physicochemical space for highly probable HIA absorption, demonstrating that they were very likely to be passively absorbed by the gastrointestinal tract. More than half of these investigated triptoquinones were predicted to passively permeate through the blood–brain barrier (Fig. 3). The BOLIED-Egg also showed that compounds triptoquinone B–C and hypoglicin H were likely to be effluated from the central nervous system by the P-gp (Fig. 3).

Drug likeness assesses the probability of a molecule to become an oral drug. The SwissADME gives access to five different rule-based filters, including Lipinski, Ghose, Veber, Egan, and Muegge filter, with diverse ranges of properties inside of which the molecule is defined as drug like [15]. The Lipinski filter is the pioneer "rule of 5" widely implemented to estimate solubility and permeability in drug discovery and development [16]. Therefore, the Lipinski filter was selected in this study. As shown in Table 3, all the triptoquinones from plants in the genus of *Tripterygium* showed acceptable number of rotatable bonds (≤ 10) with no violation of the criteria stated by the Lipinski's rule, suggesting that the identified triptoquinones from genus *Tripterygium* were drug-like molecules and thus possessed the potential to be considered oral drug candidates.

Cytochrome P450 (CYP) enzymes are a superfamily of monooxygenases, important proteins relevant to pharmacokinetics. In this study, five major CYP isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) were considered during in silico prediction. As shown in Table 3, all the compounds were non-inhibitors of CYP1A2 and CYP2D6. It was predicted that more than half of the investigated triptoquinones were CYP2C19 and CYP2C9 inhibitors, both of which are key mediators in the metabolizing of commonly prescribed drugs. For example, the CYP2C9 enzyme plays a major role in breaking down the anticlotting drug warfarin and assists in metabolizing the anti-inflammation drug ibuprofen [22]. Therefore, triptoquinone constituents of genus *Tripterygium* may help to strengthen the effect of warfarin and ibuprofen. It has been proved that more than 10 percent of commonly prescribed drugs, including the widely used antiplatelet drug clopidogrel, were processed or metabolized through CYP2C19 enzyme. This enzyme converts clopidogrel, a prodrug, to its active form, which is necessary for the drug to function in the body [23]. Thus, inhibition of CYP2C19 may reduce antiplatelet effect for clopidogrel. Triptoquinone C was the only compound among the 20 triptoquinones from genus *Tripterygium* predicted to inhibit CYP3A4. The enzyme CYP3A4, mainly located in the liver and small intestine, is responsible for the metabolism of more than 50% of medicines [24]. Thus, attention should be focused on interaction of triptoquinone C constituent in plants of genus *Tripterygium* with medicines metabolized by CYP3A4.

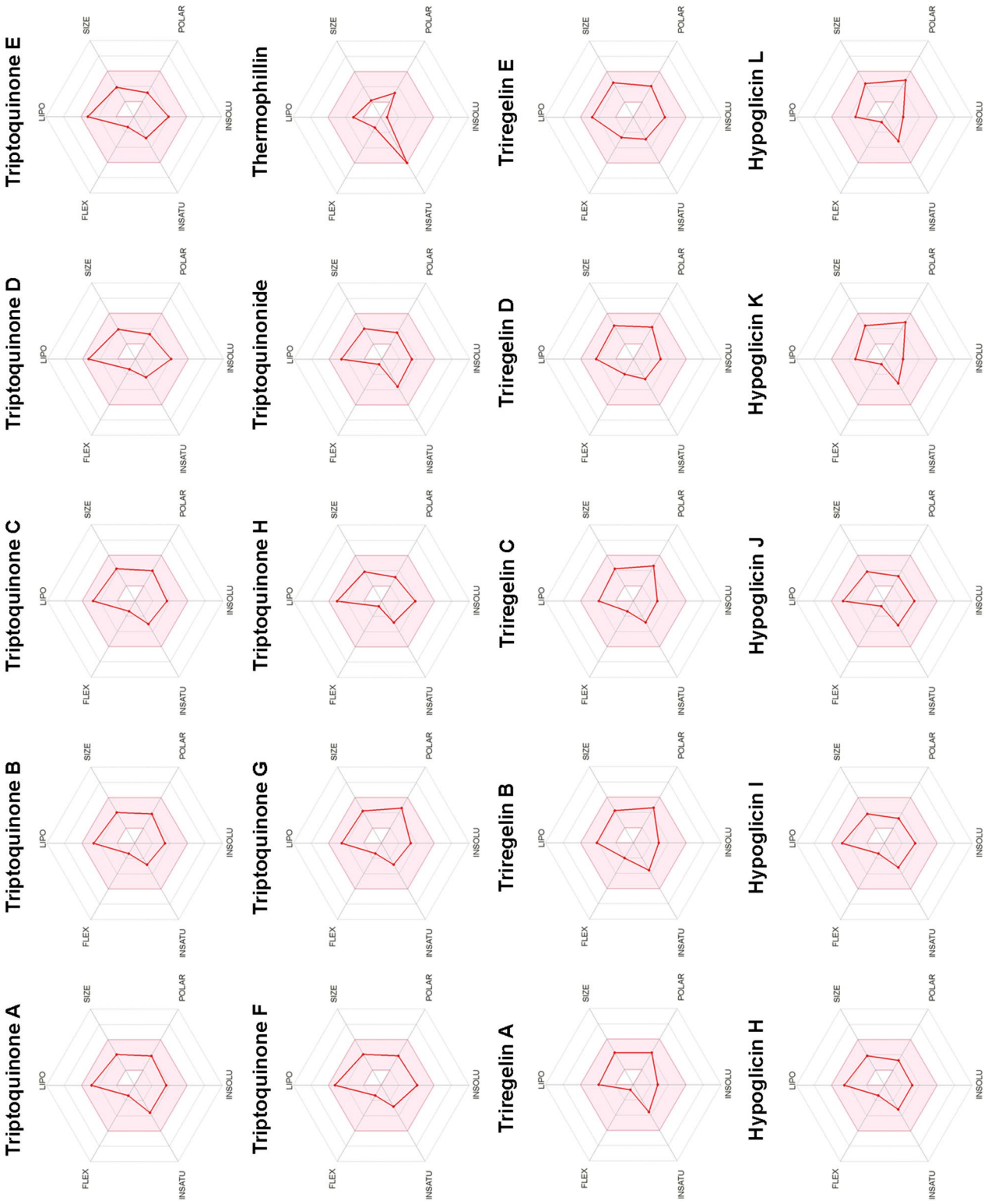


Fig. 2 Bioavailability radar plots for rapid appraisal of the drug likeness of triptoquinones from plants in the genus of *Tripterygium*

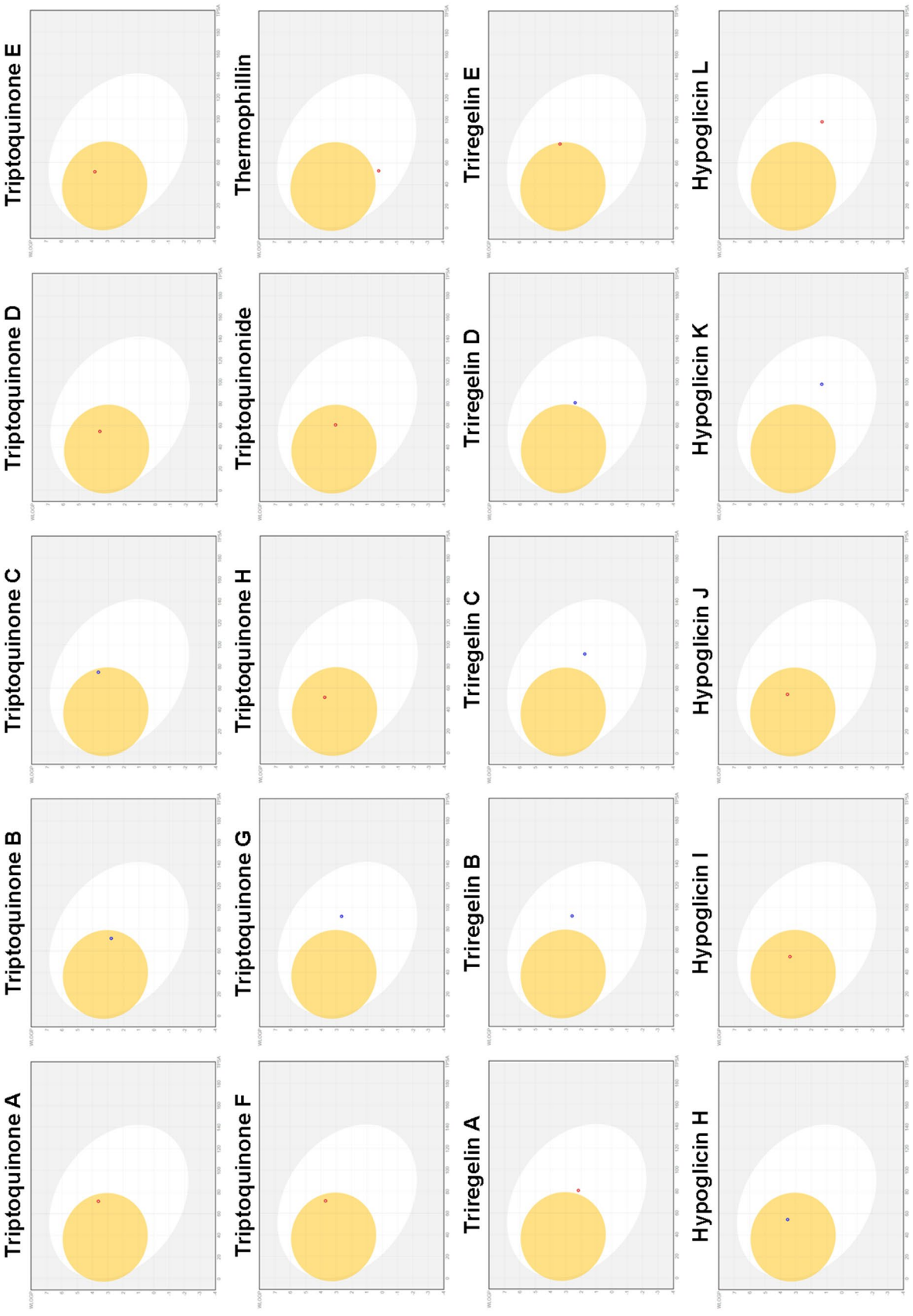


Fig. 3 WLOGP versus tPSA plots for intuitive evaluation of passive gastrointestinal absorption and brain penetration using the Brain Or Intestinal EstimateD permeation method (BOILED-Egg)

Table 3 ADME profile prediction of triptoquinones from genus *Tripterygium*

	Triptoquinone								Triptoquinonide	Thermophillin	Triregelin				
	A	B	C	D	E	F	G	H			A	B	C	D	E
Lipinski chemical properties															
Molecular weight (g/mol) (≤ 500)	328.40	330.42	344.44	316.43	314.42	330.42	346.42	314.42	314.42	168.15	342.39	344.40	346.42	360.44	372.45
Num. rotatable bonds (≤ 10)	2	2	2	2	2	2	2	1	1	2	1	3	2	3	4
Num. H-bond acceptors (≤ 10)	4	4	4	3	3	4	5	3	3	4	5	5	5	5	5
Num. H-bond donors (≤ 5)	1	1	2	1	0	1	2	0	0	0	1	2	2	1	0
Consensus log $P_{o/w}$ (≤ 5)	3.15	2.81	3.10	3.53	3.54	3.31	2.44	3.49	3.49	0.24	2.31	2.35	1.91	2.34	3.14
Absorption															
GI absorption	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No	No	No	Yes
P-gp substrate	No	Yes	Yes	No	No	No	Yes	No	No	No	No	Yes	Yes	Yes	No
Log K_p (skin permeation) (cm/s)	−6.17	−6.41	−6.36	−5.53	−5.62	−5.67	−6.72	−5.83	−5.83	−7.37	−7.39	−7.13	−7.43	−7.13	−6.65
Metabolism															
CYP1A2 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No	No	No	Yes
CYP2C9 inhibitor	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No

	Hypoglicin				
	H	I	J	K	L
Lipinski chemical properties					
Molecular weight (g/mol) (≤ 500)	314.42	314.42	314.42	358.39	358.39
Num. rotatable bonds (≤ 10)	2	2	1	1	1
Num. H-bond acceptors (≤ 10)	3	3	3	6	6
Num. H-bond donors (≤ 5)	1	1	1	1	1
Consensus log $P_{o/w}$ (≤ 5)	3.22	3.24	3.28	1.36	1.46
Absorption					
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	No	No
P-gp substrate	Yes	No	No	Yes	No
Log K_p (skin permeation) (cm/s)	−6.49	−6.14	−6.29	−8.32	−8.32
Metabolism					
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	No	No
CYP2C9 inhibitor	Yes	Yes	Yes	No	No

Table 3 (continued)

	Hypoglicin				
	H	I	J	K	L
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No

Num. number, *GI* gastrointestinal, *BBB* blood–brain barrier, *P-gp* p-glycoprotein

3.4 In silico prediction of acute toxicity

Toxicity estimation is important for drug development [13]. The free online GUSAR software has developed QSAR modeling of rat acute toxicity of compounds under investigation [13]. The values obtained for different routes of the administration of triptoquinones from plants in the genus *Tripterygium* were shown in Table 4. The LD50 values by intravenous and oral administration were found to be between 8.29–63.50 mg/kg and 397.50–2850.00 mg/kg, respectively. As can be seen in Table 4, most of the investigated triptoquinones from genus *Tripterygium* exhibited moderate toxicity (class 3) when considered intravenous route for administration. However, they turned out to be slightly toxic (class 4) or even nontoxic (class 5) when considered oral route.

4 Conclusion

In this study, we performed molecular docking, pharmacokinetic, and toxicity prediction of triptoquinones from plants in the genus of *Tripterygium*. The outcomes of this study concerning the interactions of these compounds with the iNOS were important for the discovery and development of novel drugs specific to iNOS enzyme. All the given triptoquinones were estimated as drug like for oral bioavailable, and most of them could cross the BBB. CYP2C19 and CYP2C9 were the main affected human cytochromes by the investigated triptoquinones, and consequently, their use may influence other medication. The investigated triptoquinones were of low toxicity with oral route of administration. These studies thus provide in silico evidence for understanding of triptoquinone constituents from genus *Tripterygium* as medicinal chemicals. Further, detailed experimental testing is indicated to confirm the role, safety, and efficacy of these naturally occurring triptoquinones found in plant species from the *Tripterygium* genus.

Table 4 Acute rat toxicity of triptoquinones from *Tripterygium* by GUSAR software and toxicity classification by OECD project

Compounds	LD_{50} (mg/kg)			
	i.p.	i.v.	oral	s.c.
Triptoquinone A	402.50 (Class 4) in AD	48.67 (Class 4) in AD	2257.00 (Class 5) in AD	914.20 (Class 4) in AD
Triptoquinone B	745.40 (Class 5) in AD	24.65 (Class 3) in AD	2826.00 (Class 5) in AD	862.60 (Class 4) out of AD
Triptoquinone C	783.40 (Class 5) in AD	14.65 (Class 3) in AD	2850.00 (Class 5) in AD	103.90 (Class 3) in AD
Triptoquinone D	666.00 (Class 5) in AD	23.14 (Class 3) in AD	2843.00 (Class 5) in AD	414.10 (Class 4) in AD
Triptoquinone E	925.20 (Class 5) in AD	17.23 (Class 3) in AD	2607.00 (Class 5) in AD	1140.00 (Class 5) in AD
Triptoquinone F	933.30 (Class 5) in AD	33.96 (Class 3) in AD	2618.00 (Class 5) out of AD	1163.00 (Class 5) in AD
Triptoquinone G	1105.00 (Class 5) in AD	24.00 (Class 3) in AD	1743.00 (Class 4) in AD	397.70 (Class 4) out of AD
Triptoquinone H	781.30 (Class 5) in AD	25.40 (Class 3) in AD	1547.00 (Class 4) in AD	1982.00 (Class 4) in AD
Triptoquinonide	605.20 (Class 5) in AD	11.18 (Class 3) in AD	1204.00 (Class 5) in AD	834.40 (Class 4) in AD
Thermophillin	456.40 (Class 4) in AD	63.50 (Class 4) in AD	2604.00 (Class 5) in AD	1572.00 (Class 5) in AD
Triregelin A	353.90 (Class 4) in AD	27.20 (Class 3) in AD	625.90 (Class 4) in AD	379.50 (Class 4) in AD
Triregelin B	679.70 (Class 5) in AD	106.70 (Class 4) in AD	2103.00 (Class 5) in AD	797.50 (Class 4) in AD
Triregelin C	878.30 (Class 5) in AD	9.90 (Class 3) in AD	550.70 (Class 4) in AD	101.30 (Class 3) in AD
Triregelin D	413.90 (Class 4) in AD	12.09 (Class 3) in AD	987.30 (Class 4) in AD	87.91 (Class 3) in AD
Triregelin E	764.90 (Class 5) in AD	8.82 (Class 3) in AD	2011.00 (Class 5) in AD	1114.00 (Class 5) in AD
Hypoglicin H	732.30 (Class 5) in AD	28.40 (Class 3) in AD	2832.00 (Class 5) in AD	785.90 (Class 4) out of AD
Hypoglicin I	667.90 (Class 5) in AD	11.42 (Class 3) in AD	1574.00 (Class 4) in AD	227.90 (Class 4) in AD
Hypoglicin J	1170.00 (Class 5) in AD	14.23 (Class 3) in AD	817.90 (Class 4) in AD	1443.00 (Class 5) in AD
Hypoglicin K	298.60 (Class 4) in AD	8.29 (Class 3) in AD	397.50 (Class 4) in AD	63.54 (Class 3) in AD
Hypoglicin L	298.60 (Class 4) in AD	8.29 (Class 3) in AD	397.50 (Class 4) in AD	63.54 (Class 3) in AD

i.p. intraperitoneal, *i.v.* intravenous, *s.c.* subcutaneous, *AD* applicability domain; in AD, compound falls in applicability domain of models; out of AD, compound is out of applicability domain of models

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights No animals were directly involved in the present study.

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