AUIQ Complementary Biological System
ISSN: 3007-973X
Journal homepage:
https://acbs.alayen.edu.iq

Manuscript 1038

In silico Investigation of the Anticancer Activity of Natural Isoquinoline Alkaloids Targeting the Monoamine Oxidase a Enzyme

Reham A. Al-Anssari
Anwar H. Ali
Ola Adel Al-Saad
Shaker A.N. Al-Jadaan

AUIQ COMPLEMENTARY BIOLOGICAL SYSTEM 2 (2025) 77–89

ORIGINAL STUDY

In silico Investigation of the Anticancer Activity of Natural Isoquinoline Alkaloids Targeting the Monoamine Oxidase A Enzyme

Reham A. Al-Anssari [a,*], **Anwar H. Ali** [a], **Ola Adel Al-Saad** [a], **Shaker A.N. Al-Jadaan** [b]

[a] Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Basrah, Basrah, Iraq
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq

ABSTRACT

Monoamine oxidase A (MAO-A) has recently been identified as a candidate for cancer therapy due to its roles in tumor development and growth. Conventional approaches for the discovery of MAO-A inhibitors are both costly and time-consuming. The objective of this work was to apply *in silico* methods to predict natural isoquinoline alkaloids capable of acting as potent MAO-A inhibitors, and to establish this class of compounds as potential sources of a cost-effective anticancer therapy. Twenty-one natural isoquinoline alkaloids were subjected to a multistep computer-aided drug discovery protocol. The compounds were initially screened for pharmacokinetic (ADME), toxicity, and drug-likeliness properties by computational screening tools. chemoinformatic analyses were made to predict molecular targets and subsequently, molecular docking studies was performed to evaluate binding interaction with MAO-A enzyme, focusing on docking scores and root mean square deviation (RMSD) values. The majority of the tested compounds showed good ADME characteristics and were predicted to be non-toxic, except compound 6, which showed a potentially toxic profile. Target prediction results indicated high selectivity for MAO-A of all 21 compounds. Analysis of molecular docking results showed high binding affinity of the studied compounds with root mean square deviation (RMSD) values of 0.4454–2.3 Å and binding free energy (S-score) of −4.5877 to −6.3278 kcal/mol. These findings reveal several isoquinoline alkaloids with strong MAO-A binding and favorable pharmacokinetic and safety profiles, especially compound 2. This supports their potential as anticancer leads and highlights the value of further *in vitro* and *in vivo* studies to confirm therapeutic applicability.

Keywords: Cancer, *In Silico*, Isoquinoline alkaloids, Monoamine oxidase A, Molecular docking

1. Introduction

When the control of cellular growth is lost, one of the deadliest worldwide leading causes of death arises, known as cancer. Cancer exerts its action on the human body through various pathways, therefore, the search for new drugs that target these pathways effectively continues. Recent studies pointed to the role of the monoamine oxidase A (MAO-A) enzyme as a potential therapeutic target in many cancer cell types. MAO-A belongs to a group of catalytic enzymes called Monoamine oxidase enzymes (MAOs) located at the outer membrane of mitochondria and catalyze the oxidative deamination of various biogenic amines as well as xenobiotic amines. These enzymes contain a flavin adenine dinucleotide (FAD) covalently bound to a cysteine residue through 8α-(S-cysteinyl)-riboflavin bond [1, 2]. Within human body, MAO-A enzyme is responsible for the oxidative-deamination metabolism of several neuroactive amines into their

Received 7 May 2025; revised 7 July 2025; accepted 20 July 2025.
Available online 21 August 2025

* Corresponding author.
E-mail address: reham.taha@uobasrah.edu.iq (R. A. Al-Anssari).

https://doi.org/10.70176/3007-973X.1038

corresponding aldehydes and ketones with the release of reactive oxygen species (ROS); therefore, any disturbance within this enzyme level has been linked to several neurological disorders, such as depression, anxiety, mental retardation, Parkinson's and Alzheimer's diseases, as well as tumor development and progression through MAO-A-linked production of ROS and further oxidative injury of cells and DNA damage [3, 4]. Recent studies have shown that MAO-A was overexpressed within several types of cancer cells, such as prostate adenocarcinoma, non-small cell lung cancer, Hodgkin lymphoma, pancreatic adenocarcinoma, glioblastoma and breast adenocarcinoma, and this upregulation have been linked to increased invasion, metastasis and tumor growth promotion, while other studies have connected between the inhibition of MAO-A with MAO-A inhibitors (MAOIs) and reduced proliferation and increase apoptosis of prostate cancer cells [1, 3, 5–7]. These findings suggest the possibility of using MAO-A inhibitors in cancer chemotherapy. The irreversible nonselective MAO inhibitor phenelzine has demonstrated therapeutic effects for the treatment of prostate cancer in Phase II Clinical Trials [7].

Within nature, several plant secondary metabolites have shown MAO-A inhibitory activity, such as flavonoids (e.g., Luteolin, Apigenin), coumarins (e.g., Osthenol, Isopsoraien), polyphenols (e.g., Ferulic acid, Gallic acid), and alkaloids (e.g., Harmine, Harmane) [8]. Alkaloids are basic nitrogenous physiologically active compounds, with low abundance within nature, and various arrays of chemical structures, biosynthetic pathways, and pharmacological actions [8–10]. Isoquinoline alkaloids are considered to be one of the most abundant alkaloids within the plant kingdom, predominantly found within Papaveraceae, Berberidaceae, Fumariaceae, Menispermaceae, Rutaceae, Magnoliaceae, Ranunculaceae, Cactaceae, Amaryllidaceae, and Annonaceae families [9–13]. These heterocyclic aromatics exhibit an isoquinoline ring within their chemical structures and were generally derived from the amino acids tyrosine and phenylalanine [9]. Simple isoquinoline alkaloids are structurally the simplest of isoquinoline alkaloids, which have a benzene ring attached to a pyridine ring (Fig. 1).

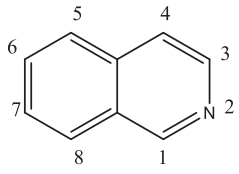

Fig. 1. Isoquinoline ring.

This research was designed to identify, using computational tools, the natural alkaloids that possess possible anticancer activity through the inhibition of MAO-A enzyme activity. 21 isoquinoline alkaloids (Fig. 2) have been selected and subjected to a computer-aided evaluation for their physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeliness, and toxicity properties as well as prediction of their molecular targets, followed by investigation of the binding mechanisms of these compounds to the MAO-A enzyme using molecular docking studies.

2. Materials and methods

In the current study, 21 simple isoquinoline alkaloids of different plant sources were collected from published research papers and sketched using Chem Draw professional 16.0 software.

2.1. Drug-likeliness and in silico ADME properties evaluation

Lipinski parameters including molecular weight (Mwt), lipophilicity, hydrogen bonds donor and acceptor and molar refractivity, as well as Pharmacokinetic parameters (Absorption, Distribution, Metabolism, and Excretion) of the studied compounds, were investigated using Swiss ADME (Swiss Institute of Bioinformatics) [14] to evaluated their potential use as drugs. Chemical structures of the selected alkaloids were sketched within the Swiss ADME server, and SMILES were generated, then bioavailability rader prediction took place.

2.2. Toxicity of compounds

The oral toxicity of selected compounds was estimated by the ProTox-II web server (Computational web server for the prediction of toxicity of small molecules) [15]. SMILES code of each compound was generated by the online application Swiss ADME then copied to the Protox-II online tool to predict different toxicity parameters. Hepatotoxicity, Mutagenicity, Carcinogenicity, Cytotoxicity, Immunotoxicity, median oral lethal doses (LD_{50} values) as well as toxicity classes of selected compounds were all evaluated.

2.3. Target selection

Within computer-aided drug design, searching for drug targets is an essential requirement of the work. Swiss Target Prediction free web server was used to predict the potential binding targets of the selected

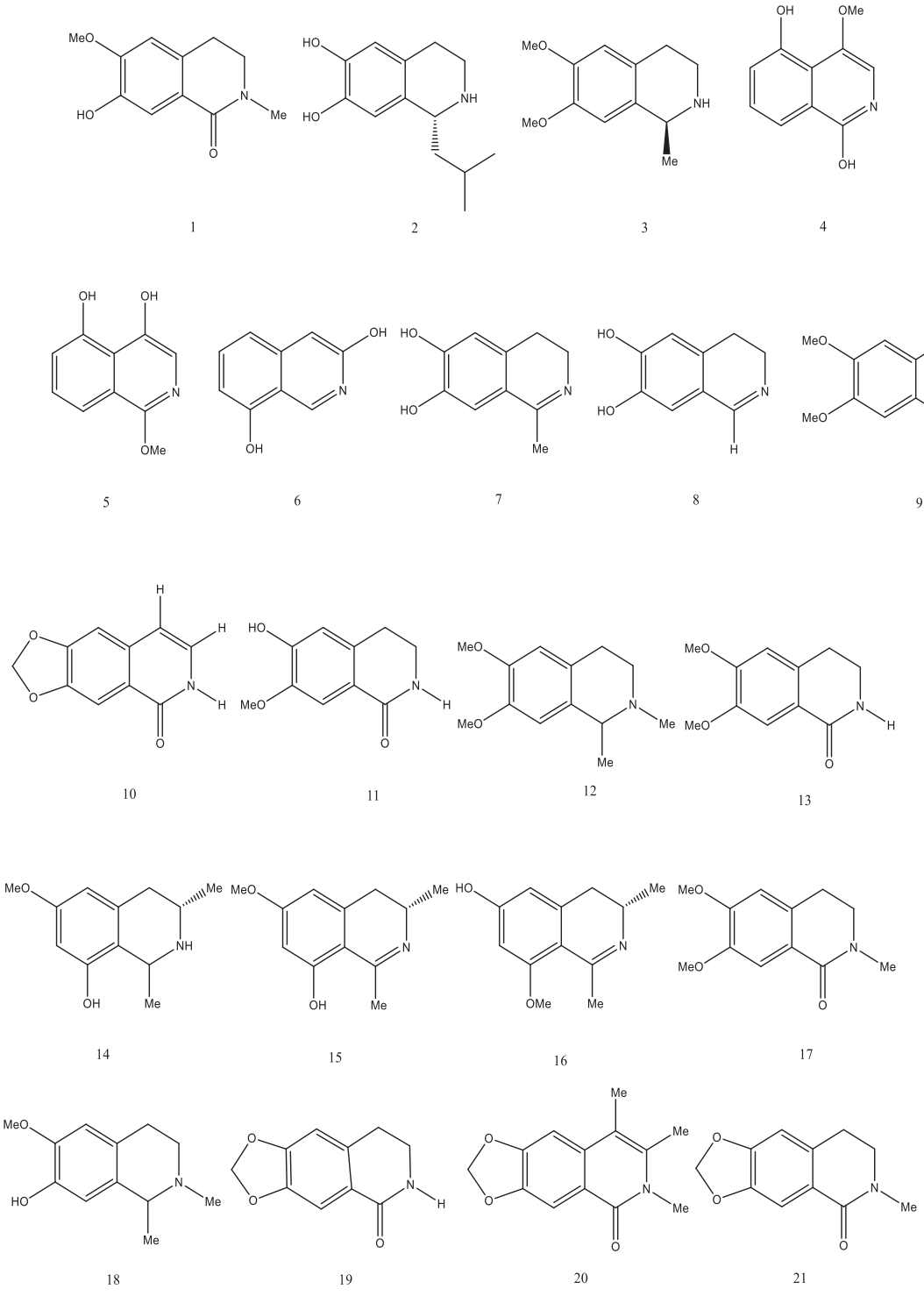

Fig. 2. Selected isoquinoline alkaloids (1. Thalifoline 2. (R)-(+)-1-Isobutyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline 3. (S)-(-)-Salsolinol 4. 1,5-dihydroxy-4-methoxyisoquinoline 5. 1-methoxy-4,5-diolisoquinoline 6. 3,8-diolisoquinoline 7. 6,7-Dihydroxy-1-methyl-3,4-dihydroisoquinolone 8. 6,7-Dihydroxy-3,4-dihydroisoquinolone 9. 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid 10. 6,7-Methylenedioxy-1(2H)-isoquinolinone 11. 7-Methoxy-1,2,3,4-tetrahydroisoquinolin-1-one 12. Carnegine 13. Corydaldine 14. Ealaine A 15. Ealaine C 16. Ealaine D 17. N-Methylcorydaldine 18. N-Methylisosalsoline 19. Noroxyhydrastinine 20. Oxohydrastinine 21. Oxyhdrastinine).

Table 1. ADME pharmacokinetic properties of studied isoquinoline alkaloids.

ADME properties	Ligands									
	1	2	3	4	5	6	7	8	9	10
GI absorption	High	High	High	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
P-gp substrate	No	No	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	Yes	Yes	Yes	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	Yes	No	No	No	No	No	No	No	No
CYP3A4 inhibitor.	No	No	No	No	No	No	No	No	No	No
Skin permeation (cm/s)	–6.83	–5.99	–6.38	–6.26	–5.87	–6.06	–6.72	–6.86	–8.73	–6.64
Drug-likeliness LIPINSKI	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 vilation	Yes, 0 vilation	Yes, 0 vilation	Yes, 0 vilation	Yes, 0 vilation	Yes, 0 vilation
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

Table 1. (continued) ADME pharmacokinetic properties of studied isoquinoline alkaloids.

ADME properties	Ligands										
	11	12	13	14	15	16	17	18	19	20	21
GI absorption	High	High	High	High	High	High	High	High	High	High	High
BBB permeant	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
P-gp substrate	No	No	No	No	No	No	No	No	Yes	No	Yes
CYP1A2 inhibitor	No	No	Yes	No	Yes	No	Yes	No	No	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	Yes	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	No
Skin permeation (cm/s)	–6.88	–6.13	–6.73	–6. 30	–6.37	–6.37	–6.68	–6.28	–6.72	–6.56	–6.68
Drug-likeliness LIPINSKI	Yes, o violation	Yes, o violation	Yes, o violation	Yes, 0 vilation	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 vilation	Yes, 0 vilation	Yes, 0 vilation
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

21 alkaloids. This prediction is based on identifying proteins with known ligands that are structurally highly similar to the studied molecules. SMILES of isoquinoline alkaloids generated within the Swiss ADME server were entered into the Swiss target prediction server to predict their molecular targets and Homo sapiens was chosen as a specie.

3. Molecular docking studies

3.1. Preparation of compounds

The studied compounds were sketched using MOE 2015 interface and their molecular structures were optimized for docking by adding hydrogens, assigning partial charges, and minimizing energy with the MMFF94x force field, then saved in MOE format for each compound, then an MDB database for these molecules was generated.

3.2. Protein preparation

The X-ray crystallographic structures of human MAO-A (PDB ID: 2Z5Y) [2] was downloaded from RCSB Protein Data Bank (Research Collaboratory for Structural Bioinformatics) and refined Using MOE 2015 (Molecular Operating Environment) : water molecules (except interaction water molecules HOH717 and HOH882) were removed, the FAD cofactor was kept as well as Harmine, H-bonds were added, missing side chains and residue were fixed and, partial charges were assigned and protein energy was minimized at MMFF94X forcefield, then the optimized protein was saved in MOE format.

3.3. Finding active site

MOE site finder tool was used to define the protein binding pocket by selecting the residues that are important for compound binding.

3.4. Docking analysis

The docking process was validated by extracting the MAO-A inhibitor, Harmine, from its original protein-binding site, re-docking it into the same position and restoring the co-crystalized Harmine interactions and orientation. After validation,

Table 2. Physicochemical properties of the studied isoquinoline.

Physicochemical properties	Ligands									
	1	2	3	4	5	6	7	8	9	10
Molecular weight	207.73	221.30	207.27	191.18	191.18	161.16	177.20	163.17	237.25	189.17
H bond acceptor	3	3	3	4	4	3	3	3	5	3
H bond donor	1	3	1	2	2	2	2	2	2	1
Fraction C sp3	0.36	0.54	0.50	0.10	0.10	0.0	0.30	0.22	0.42	0.10
Rotatable bonds	1	2	2	1	1	0	0	0	3	0
TPSA (A^2)	49.77	52.49	30.49	62.58	62.58	53.35	52.82	52.82	67.79	51.32
Molar refractivity	59.62	69.06	63.57	52.28	52.28	45.79	54.93	50.12	65.35	50.63
Log P o/w avg	1.20	2.05	1.86	1.45	1.54	1.38	1.40	1.03	0.07	1.57
Log S (ali)	–1.67	–3.08	–1.92	–2.63	–3.20	–2.64	–1.63	–1.30	0.46	–1.82

Table 2. (continued) Physicochemical properties of the studied isoquinoline alkaloids.

Physicochemical properties	Ligands										
	11	12	13	14	15	16	17	18	19	20	21
Molecular weight	193.2	221.30	207.23	207.27	205.25	205.25	221.25	207.27	191.18	231.25	205.21
H bond acceptor	3	3	3	3	3	3	3	3	3	3	3
H bond donor	2	0	1	2	1	1	0	1	1	0	0
Fraction C sp3	0.30	0.54	0.36	0.50	0.42	0.42	0.42	0.50	0.30	0.31	0.36
Rotatable bonds	1	2	2	1	1	1	2	1	0	0	0
TPSA (A^2)	58.56	21.70	47.56	41.49	41.82	41.82	38.77	32.70	47.56	40.46	38.77
Molar refractivity	54.72	68.48	59.19	63.91	64.21	64.21	64.09	64.01	52.27	65.47	57.17
Log Po/w avg	1.01	2.12	1.34	1.75	2.06	2.02	1.56	1.78	1.23	2.02	1.45
Log S (ali)	–1.66	–2.23	–1.77	–2.27	–2.15	–2.15	–1.78	–2.12	–1.64	–2.08	–1.64

docking of the selected alkaloids against MAO-A enzyme utilizing Triangle Matcher placement method and London dG scoring function took place.

4. Results and discussion

4.1. Drug-likeliness and in silico ADME properties evaluation

An *in silico* computational study of selected isoquinoline alkaloids was performed in order to detect their physicochemical properties and evaluate their drug likeliness, in addition to studying their pharmacokinetic properties (such as gastrointestinal absorption 'GI absorption', blood-brain barrier 'BBB' permeation, skin permeation and several CYP enzymes inhibition) and bioavailability score using SWISS ADME, a free web tool that predicts and evaluates pharmacokinetics and drug likeness of molecules based on several models [14]. Results show that all selected isoquinoline alkaloids demonstrated no violation, and were all in agreement with the role of five RO5 of LIPINSKI (hydrogen bond donors < 5; hydrogen bond acceptors < 10; molecular weight < 500 Dalton; calculated log P < 5) (Table 1), in addition, their topological polar surface area (TPSA) (which predict the passive transport through membrane properties of a drug) were all < 140 A^2 (Table 2), and possess optimum physicochemical properties ranges represented by the pink area within bioavailability rader (Figs. 3 and 4) which indicate that these compounds possess the required cell membrane permeability properties and drug-likeliness

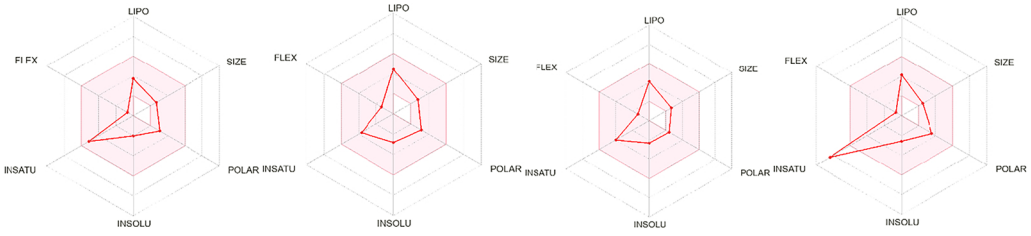

Fig. 3. Bioavailability radar plots of compounds 1–4.

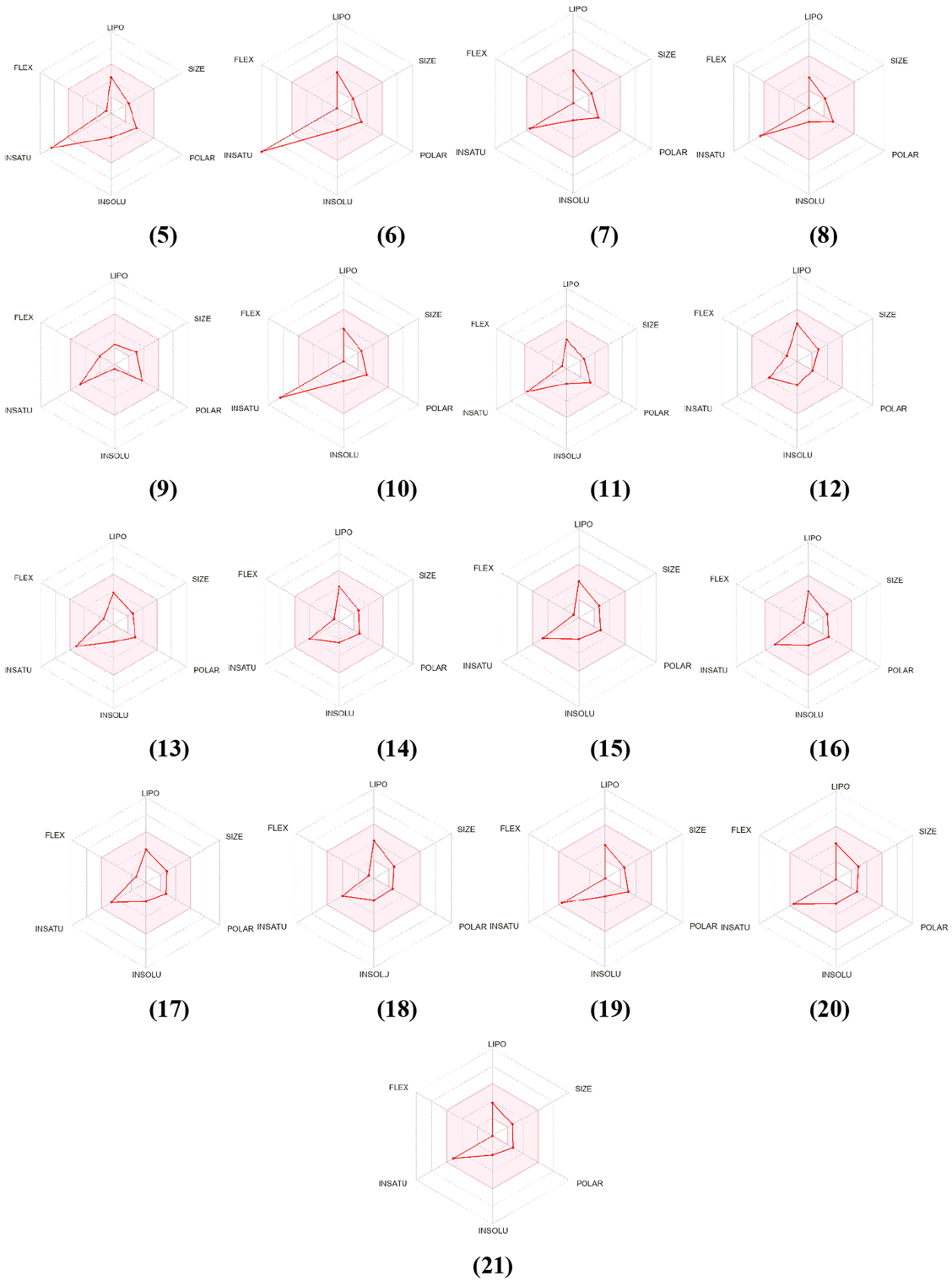

Fig. 4. Bioavailability radar plots of compounds 5–21.

behavior [5, 14]. Gastrointestinal absorption (GIA) and blood–brain barrier (BBB) permeation was estimated by Brain Or IntestinaL Estimated permeation method (BOILED-egg), a prediction method that is based on the polarity and lipophilicity of studied compounds (Fig. 5) [16]. Gastrointestinal absorption was high for all the studied compounds, and this corresponds with their high bioavailability. Good BBB

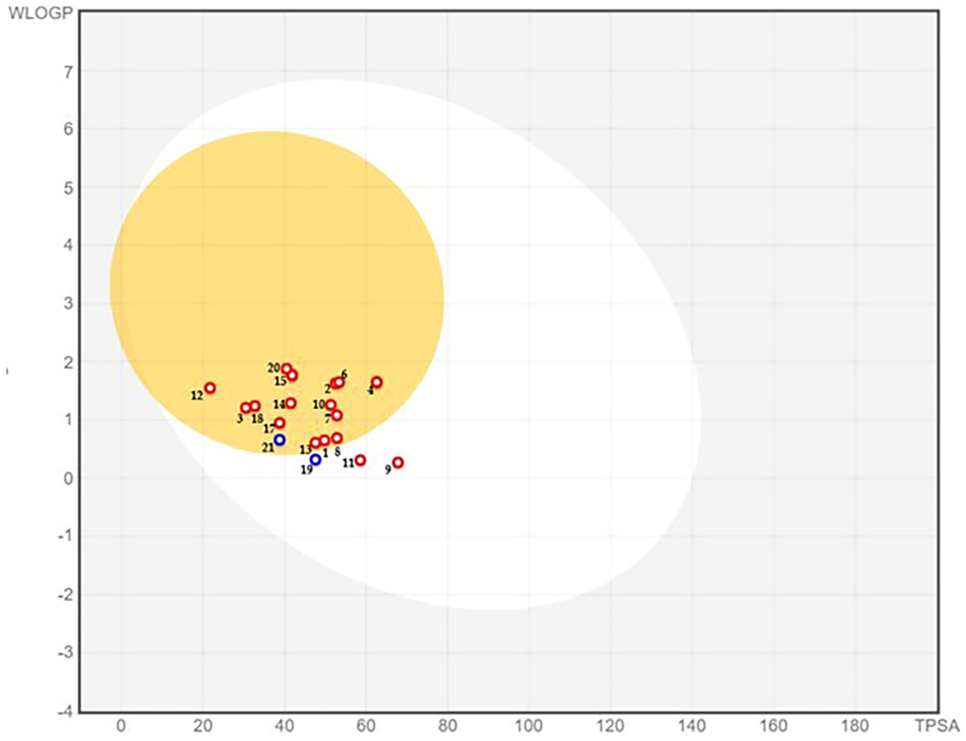

Fig. 5. The boiled egg plot displaying the comparative prediction of BBB permeation ability and absorption of compounds 1–21. Points within the BOILED-Egg's yolk are for the molecules predicted to passively diffuse through BBB, Points within the BOILED-Egg's white are for the molecules predicted to passively absorbed by GIT, Blue/Red-colored dots are for the molecules predicted to/not to be effluated from the CNS through P-glycoprotein.

Table 3. Evaluation of oral toxicity, toxicity class and toxicity status of studied isoquinoline alkaloids.

Toxicity parameters	Ligands									
	1	2	3	4	5	6	7	8	9	10
LD_{50} (mg/kg)	500	3350	1000	400	1000	280	900	900	1123	1000
Prediction accuracy (%)	69.26	70.97	72.9	68.07	68.07	68.07	68.07	68.07	69.26	67.38
Hepatotoxicity	- [a]	-	–	–	–	–	–	–	–	–
Carcinogenicity	–	–	–	–	–	–	–	–	–	+ [b]
Immunotoxicity	–	–	+	–	–	–	–	–	–	–
Mutagenicity	–	–	–	+	+	+	–	–	–	+
Cytotoxicity	–	–	–	–	–	–	–	–	–	–
Toxicity class	IV	V	IV	IV	IV	III	IV	IV	IV	IV

[a] non-toxic.
[b] toxic.

Table 3. (continued) Evaluation of oral toxicity, toxicity class and toxicity status of studied isoquinoline alkaloids.

Toxicity parameters	Ligands										
	11	12	13	14	15	16	17	18	19	20	21
LD_{50} (mg/kg)	1000	450	1000	928	480	480	625	350	1000	1000	625
Prediction accuracy (%)	69.26	72.9	69.26	70.97	69.26	68.07	69.26	100	69.26	68.07	69.26
Hepatotoxicity	– [a]	–	–	–	–	–	–	–	–	–	–
Carcinogenicity	–	–	–	–	–	–	–	–	–	–	+ [b]
Immunotoxicity	–	–	–	–	–	–	–	+	–	+	–
Mutagenicity	–	–	–	–	–	–	–	–	–	+	–
Cytotoxicity	–	+	–	–	–	–	–	+	–	–	–
Toxicity class	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV

[a] non-toxic.
[b] toxic.

Table 4. Harmine and ligands 1–21 RMSD values, S-score and physical interactions with the active site of MAO-A enzyme.

Compound	RMSD	Binding free energy S	Residues interacting with Ligand through H-Bonding and other interactions
Harmine	0.8004	–5.3670	Gln215, Tyr69, Tyr407,Tyr444, Asn181, Ile207, Ile180, Phe208, Cys323, Thr336, Val210, Ile335, Leu337, Phe352.
1	1.1497	–5.6715	Ile180, Ile335,Ile207, Asn181, Val210, Cys323, Phe208, Leu337, Tyr407, Gln215, Phe352, Tyr69
2	0.7825	–6.2487	Thr336, Phe208, Met350, Val210, Cys323, Ile180, Ile335, Leu337, Gln215, Phe352, Tyr444, Tyr407
3	1.0771	–5.6715	Phe 352,Tyr407, Gln215, Tyr444, Asn181, Ile180, Ile335, Leu337, Ile207, Val210, Thr336, Phe208
4	1.1339	–5.1282	Ile180, Phe208, Phe352, Gln215, Leu337,Ile335, Val210, Thr336, Ile352, Cys323, Ile207, Asn181.
5	1.0929	–4.8637	Ile207, Asn181, Leu337, Ile180, Phe208, Gln215, Tyr69, Phe352, Thr336, Met350, Tyr407, Ile335
6	0.9565	–4.8357	Asn181, Ile207, Tyr407, Val210, Phe208, Thr336, Cys323, Ile335, Leu337, Phe352, Gln215, Ile180
7	0.4454	–4.9835	Val210, Phe352, Cys323, Ile180, Phe208, Asn181, Ile207, Tyr407, Gln215, Ile335, Leu337, Thr336
8	1.0236	–4.5877	Ile335, Tyr 407, Gln215, Tyr444, Ile180, Leu337, Phe352, Thr336
9	1.7489	–6.3694	Leu337, Ile335, Thr336, Cys323, Phe108, Phe352, Ile325, Val210, Leu97, Ala111, Ala110, Phe208, Ile180, Gln215, Tyr407
10	1.7121	–5.2584	Gln215, Val210, Phe208, Ile 207, Tyr407, Ile335, Ile180, Thr336, Phe352, Cys323, Leu337
11	0.9897	–5.4512	Cys323, Thr336, Phe352, Ile335, Leu337, Ile207, Asn181, Ile180, Phe208, Tyr407, Gln 215, Val210
12	1.1609	–6.2807	Asn181, Tyr407, Ile352, Phe208, Val210, Ile207, Leu337, Ile335, Gln215, Thr336, Phe352, Tyr444, Ile180
13	0.9452	–6.0333	Gln215, Tyr69, Phe352, Leu337, Ile335, Met350, Thr336, Cys323, Val210, Ile180, Phe208, Asn181, Tyr407, Tyr444
14	0.8582	–5.2615	Tyr407, Ile335, Thr336, Met350, Cys323, Phe352, Ile207, Val210, Ile 325, Leu337, Phe208, Gln215, Ile180
15	1.2423	–5.4264	Val210, Gln215, Thr336, Tyr407, Phe208, Cys323, Ile207, Asn181, Ile180, Tyr69, Phe352, Leu337, Ile335
16	0.7954	–5.7465	Cys323, Leu337, Ile335, Ile180, Met350, Phe352, Gln215, Asn181, Tyr407, Tyr444, Phe208, Thr336, Val210
17	1.4796	–6.3278	Gln215, Asn181, Leu337, Ile335, Ile335, Ile180, Tyr69, Met350, Cys323, Thr336, Thr336, Leu97, Val210, Phe208, Tyr444, Phe352, Tyr407
18	0.7868	–5.7585	Tyr69, Tyr407, Gln215, Ile180, Cys323, Phe 208, Ile207, Ile325, Thr336, Val210, Ile335, Leu337, Phe352
19	0.7986	–5.1469	Cys323, Thr336, Ile180, Leu337, Ile335, Gln215, Tyr407, Val210, Phe208, Ile207, Ile325
20	2.3	–6.1605	Phe352, Tyr69, Met 350, Leu337, Ile335, Cys323, Thr336, Ile325, Val210, Phe208, Ile180, Gln215, Tyr407
21	1.1940	–5.4917	Phe208, Ile207, Ile180, Gln215, Tyr407, Leu337, Ile335, Cys323, Phe352, Thr336, Val210

permeation on the other hand has been shown by all the studied compounds, excluding compounds 9, 11, and 19. Compounds 19 and 21 were predicted to be Permeability glycoprotein (P-gp) substrates, while the remaining compounds were not. This efflux pump plays an important role in drug transport in many organs as well as it's overexpressed in many tumor cells [20]. Skin permeability of the studied compounds was also evaluated and expressed as log skin permeability coefficient (log *Kp*); the more negative value of log *Kp*, the less skin permeant is the compound [14]. It is important to point to the interaction of studied compounds with cytochromes P450 (CYP) isoenzymes, especially (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) since many drugs are substrate to these five major isoforms, in addition, these isoenzymes play an important role in drug metabolism and elimination [14]. Results (Table 1) show that compounds 4, 5, 6, 10, 13, 15, 17, 20 and 21 were potential CYP1A2 inhibitors, which means that these might interfere with the metabolism of CYP1A2 drug substrates such as theophylline, clozapine, and tacrine [21], while compounds 2 and 12 were predicted to inhibit CYP2D6 isoenzyme, and hence the enzymatic activation of its drug substrate such as tramadol and Codeine into O-desmethyl tramadol and morphine respectively [22].

4.2. Toxicity analysis

One of the most important medicinal properties of a compound is its non-toxicity, hence, all studied compounds were *in silico* tested for their oral toxicity

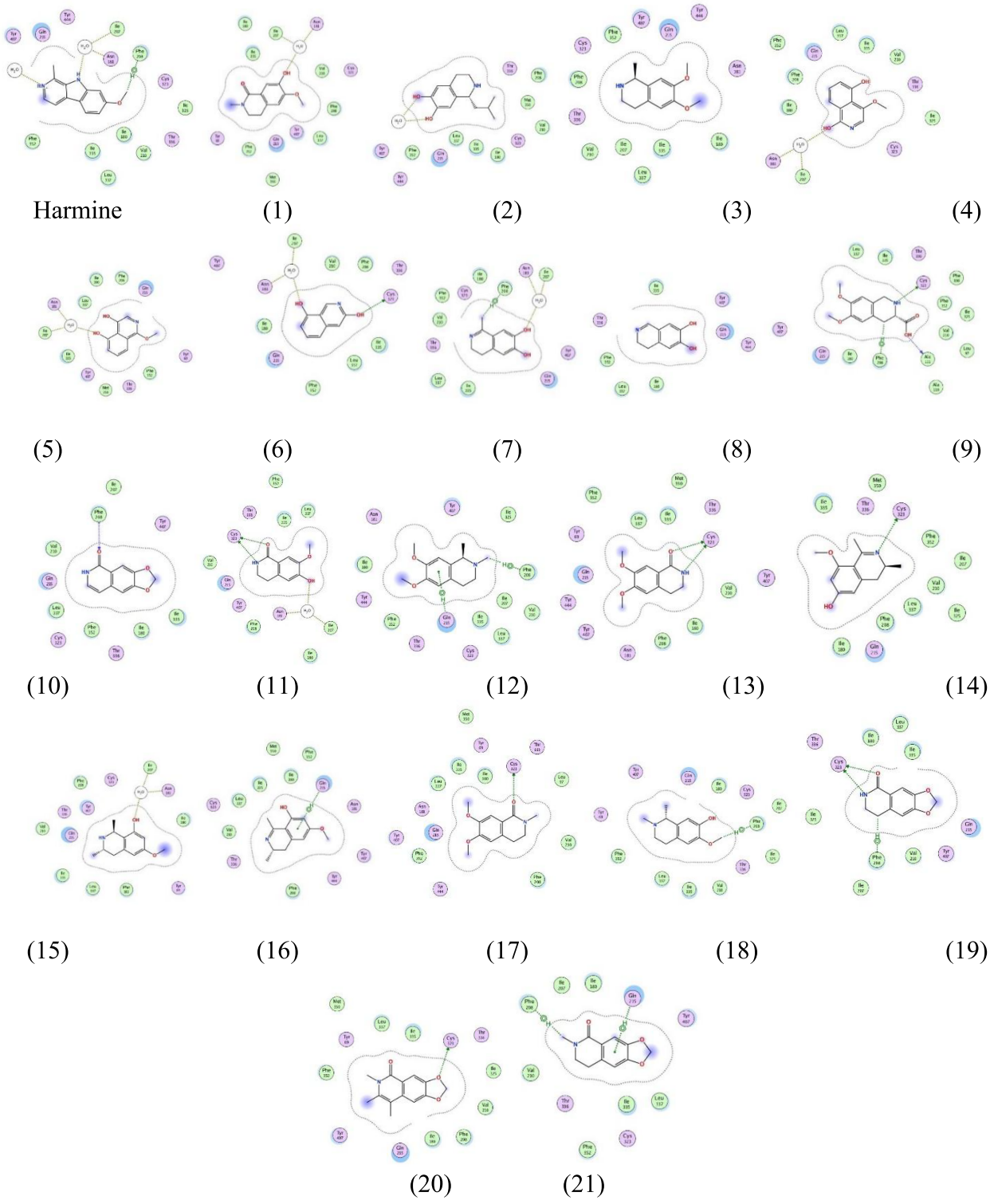

Fig. 6. 2D interactions of Harmine and compounds 1–21 with MAO-A receptor binding sites.

using ProTox II, a web server for the prediction of median oral lethal doses (LD_{50} values) and toxicity classes in rodents. ProTox II prediction is established on the analysis of the two-dimensional (2D) similarity to other compounds with known LD_{50} values and the determination of fragments over-represented in toxic compounds [15, 17]. The toxicity of the compounds is divided into six different classes based on the threshold values LD_{50} of 5, 50, 300, 2000, and 5000 mg/kg body weight [18]. Class I is highly

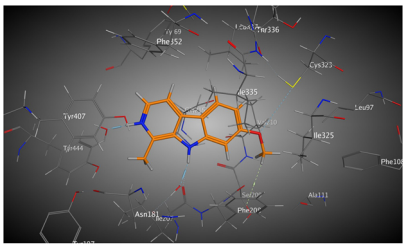

Fig. 7. 3D interactions of Harmine with MAO-A receptor binding sites.

toxic, classes II and III are toxic, class IV is low toxic while classes V and VI were considered safe [19]. According to LD_{50} estimation results, all studied compounds have shown to be within category IV and thus might be considered low-toxic agents, except compound 2, which is safe (class V) and compound 6, which is toxic (category III). No hepatotoxicity was found in any compound; both compounds 10 and 21 have shown carcinogenicity, compounds 3, 18, and 20 have shown immunotoxicity, compounds 4, 5, 6, 10, and 20 have shown mutagenicity, and cytotoxicity was detected for compounds 12 and 18. Toxicity analysis results are listed in Table 3.

4.3. Molecular docking analysis

Molecular docking is considered a powerful computational method with well-known benefits within the field of drug discovery. In this study, MOE 2015 was used to investigate the compound-receptor complexes interactions as well as the prediction of their binding energies (S). Harmine is a reversible inhibitor located in the active center cavity of the MAO-A enzyme. It interacts with Tyr69, Asn181, Phe208, Val210, Gln215, Cys323, Ile325, Ile335, Leu337, Phe352, Tyr407, Tyr444, and FAD [2]. Harmine redocking process revealed that Harmine was bound to its target very similar to the true conformation, in addition, a root mean square deviation (RMSD) value less than 2 Å (RMSD = 0.8 Å) [23] was obtained, which demonstrates the reliability of the docking protocols and parameters. Molecular docking results of the studied alkaloids demonstrated within (Table 4) were listed as the S-scores, and the lower the S value, the stronger the binding [12]. The S-scores values of the selected isoquinolines ranged between –4.5877 and –6.3278 kcal/mol, which is close to that of Harmine (–5.3670 kcal/mol) and considered as an indication of affinity and good fitting of the studied compound to the binding sites of the MAO-A enzyme. Compounds 1, 2, 3, 9–13, 15–18, 20, and 21 have shown even better binding energy than Harmine. Regarding compounds' binding mode with the receptor, compounds 3 and 8 did not show any connection with the receptor binding sites, while compounds 1, 4, 5, 6, 7, 11, and 15 formed solvent contact HOH717. Compound 2, conversely, formed only a solvent connection with HOH882. Compounds 11, 13, and 19 formed two polar hydrogen interactions with Cys323 as a sidechain donor, while compounds 6, 9, 14, 17, and 20 formed single polar hydrogen interactions with Cys323. Compounds 7, 9, 12, 19, and 21 formed an arene-H interaction with Phe208, whereas compound 10 formed one polar hydrogen interaction with Phe-208 acting as a backbone acceptor. In addition, compounds 12, 16, 18, and 21 bound to Gln215 through an arene-H connection, while compound 9 formed a polar hydrogen interaction with Ala111 by acting as a backbone donor. The two-dimensional interactions of Harmine and compounds 1–21 were shown in Fig. 6, while a three-dimensional view of these compounds can be seen within Figs. 7 and 8. These results support the possibility of binding and forming different interactions between the studied isoquinoline alkaloids and the MAO-A receptor and thus, potential MAO-A inhibition-related anticancer activity.

In conclusion, the studied compounds were promising MAO-A inhibitors with potential roles in the treatment of MAO-A-overexpressing tumors. However, further *in vitro* and *in vivo* studies are required to confirm the *in silico* studies' results.

Even though these computer-based simulations indicate that many of these studied isoquinoline alkaloids are promising, with favourable ADME characteristics, generally acceptable safety profile (although compounds 6, 10 and 21 require careful consideration), and strong predicted binding to MAOA enzyme. Despite our models' promising predictions, natural chemicals may have problems in the body; they may not dissolve effectively, absorb poorly, degrade too quickly, or diffuse throughout the body rather than only affecting the afflicted cells. Smart drug delivery technologies are becoming essential to determine these practical challenges and fully realize the therapeutic potential of promising substances like our discovered alkaloids. Small formulations based on nanotechnology, like liposomes, polymeric nanoparticles, or nanomicelles, are particularly helpful. These clever systems can significantly improve how a drug works by helping it dissolve better, stay active longer in the bloodstream, and targeting tumor cells (where MAO-A is often found in higher amounts), and hence, minimize harm to healthy body parts while ensuring the drug gets to its target location [24–26]. This comprehension indicates that the next crucial step for our identified isoquinoline alkaloids is to investigate their efficacy within these advanced delivery methods. Combining our computational findings with these novel drug delivery techniques is crucial for achieving the therapeutic potential of these natural MAO-A.

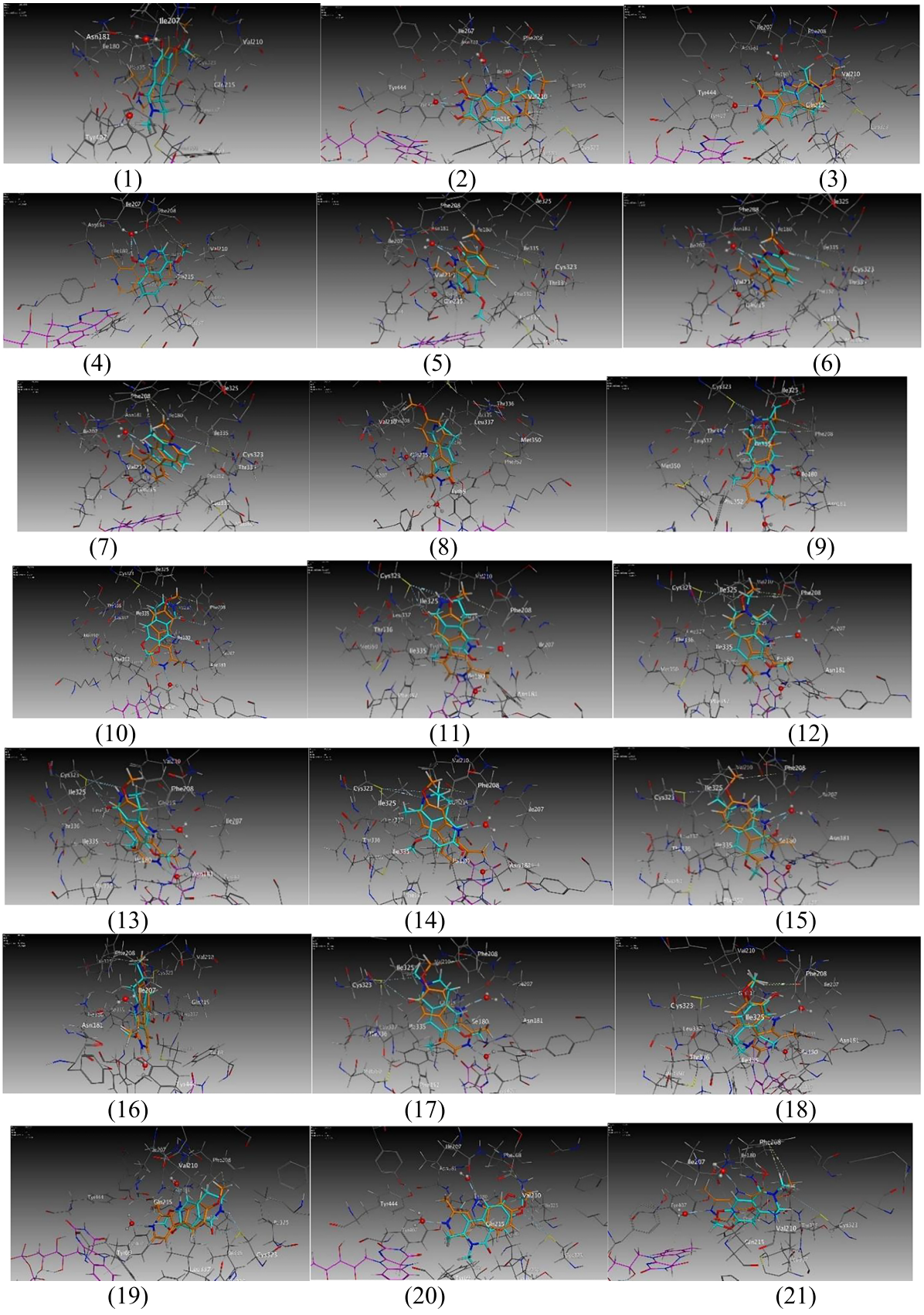

Fig. 8. 3D interactions of Harmine (orange)and compounds 1–21 (Turquoise) with MAO-A receptor binding sites.

5. Conclusion

This study sheds light on the potential of natural isoquinoline alkaloids as promising lead compounds in the developmour computerent of anticancer agents targeting monoamine oxidase A (MAO-A). Computational predictions of drug-likeness and ADME properties suggest that all 21 compounds investigated have favorable pharmacokinetic profiles, indicating their suitability for further drug development. Toxicity assessments showed that most compounds were predicted to have low toxicity overall. Among them, compound 2 showed to be the safest, while compound 6 raised concerns due to its predicted toxicity. None of the compounds were found to be hepatotoxic. However, a few showed specific safety concerns: compounds 10 and 21 exhibited potential carcinogenicity; compounds 3, 18, and 20 showed signs of immunotoxicity; compounds 4, 5, 6, 10, and 20 were flagged for mutagenicity; and compounds 12 and 18 showed possible cytotoxic effects.

Molecular docking results revealed that all compounds had reasonable binding affinity to MAO-A, with compounds 1, 2, 3, 9–13, 15–18, 20, and 21 showing strong and stable interactions, exceeding the reference inhibitor, Harmine, in several key aspects. These findings suggest that selected isoquinoline alkaloids could serve as effective MAO-A inhibitors with potential therapeutic value.

While these *in silico* results are promising, further *in vitro* and *in vivo* experiments are essential to confirm biological activity, evaluate safety profiles, and explore therapeutic potential. Ultimately, this study provides a valuable foundation for the experimental development of MAO-A-targeted therapies, especially in the context of cancer and neurological disorders.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

This study needed no ethical approval.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Financial support and sponsorship

This research received no external funding.

Author contributions

The research concept and literature review were developed by Shaker A. N. Al-Jadaan, Reham A. Al-Anssari, Anwar H. Ali, and Ola Adel Al-Saad. The theoretical studies and the original draft of the manuscript were prepared by Shaker A. N. Al-Jadaan and Reham A. Al-Anssari, while all authors contributed to the critical review and editing of the final version.

Acknowledgment

The author would like to express thanks of gratitude to Dr. Rita S Elias in the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah for her continued help, advice and support in performing this research.

References

1. Mirzaei M, Nazemi H. *In silico* interactions between curcumin derivatives and monoamine oxidase-a enzyme. *Biointerface Res. Appl. Chem*. 2022;12(3):3752–3761. https://doi.org/10.33263/BRIAC123.37523761.
2. Son SY, Ma J, Kondou Y, Yoshimura M, Yamashita E, Tsukihara T. Structure of human monoamine oxidase A at 2.2-Å resolution: the control of opening the entry for substrates/inhibitors. *Proc. Natl. Acad. Sci. U. S. A*. 2008;105(15):5739–5744. https://doi.org/10.1073/pnas.0710626105.
3. Aljanabi R, Alsous L, Sabbah DA, Gul HI, Gul M, Bardaweel SK. Monoamine oxidase (MAO) as a potential target for anticancer drug design and development. *Mol*. 2021;26(19): https://doi.org/6019.10.3390/molecules26196019.
4. Saddique FA, Zaib S, Jalil S, Aslam S, Ahmad M, Sultan S, Naz H, Iqbal M, Iqbal J. Synthesis, monoamine oxidase inhibition activity and molecular docking studies of novel 4-hydroxy-N′-[benzylidene or 1-phenylethylidene] -2-H/methyl/benzyl-1,2-benzothiazine-3-carbohydrazide 1, 1-dioxides. *Eur. J. Med. Chem*. 2018;143:1373–1386. https://doi.org/10.1016/j.ejmech.2017.10.036.
5. Sblano S *et al.* The potential of MAO inhibitors as chemotherapeutics in cancer: A literature survey. *European Journal of Medicinal Chemistry*. 2024:117159. https://doi.org/10.1016/j.ejmech.2024.117159.
6. Mahdianipur S, Maralani M, and Davoodi H. Monoamine neurotransmitters in breast cancer: Progression, immunomodulation, and therapeutic strategies. *Jorjani Biomedicine Journal*. 2024;12(2):23–28.
7. Michaela RJ, Jennifer EO, Hyun OC, Chun-Peng L, Boris AK, Jonathan EK, Mitchell EG, Charles EM. Synthesis and anti-cancer potential of potent peripheral MAO-A inhibitors designed to limit blood:brain penetration. *Bioorg. Med. Chem*. 2023;92:117425. https://doi.org/10.1016/j.bmc.2023.117425.
8. Chaurasiya ND, Leon F, Muhammad I, Tekwani BL. Natural products inhibitors of monoamine oxidases—Potential new drug leads for neuroprotection, neurological disorders, and neuroblastoma. *Mol*. 2022;7(13):4297. https://doi.org/10.3390/molecules27134297.

9. Talreja S, Tiwari S. Current trends in alkaloid research: From natural sources to synthetic pathways. *Asian Journal of Pharmaceutical Research and Development*. 2024;12(5):11–20. https://doi.org/10.22270/ajprd.v12i5.1469.
10. Gutiérrez-Grijalva EP, López-Martínez LX, Contreras-Angulo LA, Elizalde-Romero CA, Heredia JB. Plant alkaloids: Structures and bioactive properties. in Swamy M. (Eds). Plant-Derived Bioactives: Chemistry and Mode of Action. *Springer, Singapore*. 2020:85–117.
11. Shang XF, Yang CJ, Morris-Natschke SL, Li JC, Yin XD, Liu YQ, Guo X, Peng JW, Goto M, Zhang JY. Biologically active isoquinoline alkaloids covering 2014–2018. *Med. Res. Rev*. 2020;40(6):2212–2289. https://doi.org/10.1002/med.21703.
12. Sadeghi M, Miroliaei M. Inhibitory effects of selected isoquinoline alkaloids against main protease (Mpro) of SARS-CoV-2, *in silico* study. *In Silico Pharmacol*. 2022;10:5. https://doi.org/10.1007/s40203-022-00122-4.
13. Wang D. *et al.* Biologically active isoquinoline alkaloids covering 2019–2022. *Bioorganic Chemistry*. 2024;145:107252. https://doi.org/10.1016/j.bioorg.2024.107252.
14. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep*. 2017;7:42717. https://doi.org/10.1038/srep42717.
15. Banerjee P, Eckert A O, Schrey A K, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2018;46(W1):W257–W263. https://doi.org/10.1093/nar/gky318.
16. Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *Chem.Med. Chem*. 2016;11(11):1117–1121. https://doi.org/10.1002/cmdc.201600182.
17. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: A web server for the *in silico* prediction of rodent oral toxicity. *Nucleic Acids Res*. 2014;42(W1):W53–W58. https://doi.org/10.1093/nar/gku401.
18. Mutiah R, Dewi TGD, Suryadinata A, Qonita K. Inhibition of human epidermal growth factor receptor-2 (HER-2) from Pomelo (Citrus maxima) flavonoid compounds: An *In Silico* approach. *ISCC*. 2021;12(3):148–160.
19. Zhu YC, Yao J, Adamczyk J, Luttrell R. Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (Apis mellifera). *PLOS*. 2017;12(6):e0178421. https://doi.org/10.1371/journal.pone.0178421.
20. Finch A, Pillans P. P-glycoprotein and its role in drug-drug interactions. *Aust Prescr*. 2014;37:137–139. https://doi.org/10.18773/austprescr.2014.050.
21. Zhu Y *et al.* The alteration of drug metabolism enzymes and pharmacokinetic parameters in nonalcoholic fatty liver disease: current animal models and clinical practice. *Drug Metabolism Reviews*. 2023;55(3):163–180. https://doi.org/10.1080/03602532.2023.2202359.
22. Nahid NA *et al.* Use of CYP2D6 inhibitors with CYP2D6 opioids: Association with emergency department visits for pain. *Clinical Pharmacology & Therapeutics*. 2024;116(4):1005–1012. https://doi.org/10.1002/cpt.3314.
23. Marahatha R, Shrestha A, Sharma K, Regmi BP, Sharma KR, Poudel P, Basnyat RC, Parajuli N. *In silico* study of alkaloids: Neferine and berbamine potentially inhibit the SARS-CoV-2 RNA-dependent RNA polymerase. *J. Chem*. 2022; 2022:7548802. https://doi.org/10.1155/2022/7548802.
24. Xu M, Li, S. Nano-drug delivery system targeting tumor microenvironment: A prospective strategy for melanoma treatment. *Cancer Letters*. 2023;574:216397. https://doi.org/10.1016/j.canlet.2023.216397.
25. Dutt Y *et al.* Liposomes and phytosomes: Nanocarrier systems and their applications for the delivery of phytoconstituents. *Coordination Chemistry Reviews*. 2023;491:215251. https://doi.org/10.1016/j.ccr.2023.215251.
26. Kumar PV, Narayanaswamy H. Nano-Phytoconstituents and its recent advancement in Anticancer efficacy. *Research Journal of Pharmacy and Technology*. 2023;16(1):447–452. https://doi.org/10.52711/0974-360x.2023.00076.