Identification of Novel Drug Leads for NMDA Receptor Implicated In Schizophrenia from Indian Traditional Herbs

Preenon Bagchi, and R. Somashekhar

Abstract**—**Schizophrenia is a major debilitating disorder worldwide. Schizophrenia is a result of multi-gene mutation and psycho-social factors. Mutated amino acid sequences of N-Methyl-D-Aspartate (NMDA) have been implicated as factors causing schizophrenia which were retrieved from the National Centre for Biotechnology Information (NCBI). 3D structure of the above receptor was determined by using protein threading technique. Several ayurvedic herbs are implicated as causative factors for schizophrenia. The phyto-compounds information of the above herbs was retrieved from various literature studies. The pharmacophore hypothesis was generated for the reported inhibitors. The phytochemical compounds were screened against the NMDA receptor. Novel ligands were shortlisted based on their fitness & docking score. These shortlisted ligands can be considered for binding assay studies with cell lines with the NMDA receptor in *in-vitro*.

Keywords**—**ADME screening, antagonists, ayurvedic herbs, docking, NMDAR, pharmacophore, schizophrenia, threading.

I. Introduction

SCHIZOPHRENIA is a complex mental disorder. Studies suggest that genetics, prenatal care with environment, neurobiology and psychological and social processes are important causes of schizophrenia. Evidence suggests that genetic vulnerability combined with environmental factors cause schizophrenia. Research suggests that genetic vulnerability to schizophrenia is multifactorial, caused by interactions of several genes [1, 2].

Mutation in genes such as N-Methyl-D-aspartate (NMDA), implicated as factors causing schizophrenia, are taken in this study [3].

The use of phytochemicals as novel, potential lead drug molecules for NMDA receptor is tested *in-silico* in this study.

NMDA receptor: N-Methyl-D-Aspartate (NMDA) receptor is a sub-type of the glutamate receptor, whose function is to mediate fast excitory synaptic transmissions in the central nervous system [4].

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NMDA receptor is one of the main mediators of excitatory neurotransmission, is highly permeable to calcium ions and thus plays a key role in the plasticity of synapses, which is believed to underlie memory and learning, as well as the development of the nervous system [5]. The binding of both glutamate and glycine activates NMDA receptor. The glutamate hypothesis of schizophrenia suggests that NMDA receptor becomes functionless in this disease [4. 5]. NMDA receptors also play an essential role in the development of neural pathways, including cutting of cortical connections during adolescence, making them a critical component of developmental processes whose malfunction may lead to schizophrenia [5].

In this work the 3d structure of NMDA protein is developed using protein threading method and a suitable ligand for NMDA receptor is selected by matching the pharmacophore features of known ligands MPEP (2-Methyl-6-(phenylethynyl)pyridine) [6], MTEP (3-((2-Methyl-4-thiazolyl)ethynyl)pyridine) [7] & aniracetam [8] with the phytocompounds.

Both MPEP and MTEP are NMDA antagonist [6, 7]. Aniractam is a positive allosteric modulators of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) & NMDA receptor [8].

Many neuroleptic drugs are antagonists of the glutamate, serotonin and dopamine receptors. This class of drug is used to treat psychotic disorders, such as schizophrenia.

II. Methodology

A. Predicting the 3D structure of the receptors

The amino acid sequences of the NMDA protein was retrieved from the National Center for Biotechnology Information (NCBI). Using Basic Local Alignment and Search Tool (BLAST) search engine against Protein Data Bank (PDB) the homologous templates for NMDA protein was selected and their crystal structure was downloaded from PDB. Usually using the homologous templates, the 3D structure of the receptor is generated by modeller. But, for NMDA protein, no homologous templates were found using BLAST. I-TASSER server [9] was used to select templates. In I-TASSER server 3D models are built based on multiple-threading alignments.

B. Model Verification

Modeller [10] and I-Tasser generated models are verified using SAVES server's Ramachandran Plot Module to find the

number of residues in core, allowed, generously allowed region and disallowed region [11].

C. Ligand preparation

3-O-Acetyl-a-boswellic acid	Phyllanthin	Rubiadin	Corosolic acid
Demethoxycurcumin	Eupalitin	18a-Glycyrrhetinic acid	3,3',4,4'-Tetrahydroxy-2-methoxychalcone
Lutein	Asiaticoside	Berberine chloride dihydrate	
14-deoxy-11,12-didehydroandrographolide	Picroside I	Rutin	Hypophyllanthin
	Eupalitin-3-O-galactoside	18a-Glycyrrhetinic acid	Crocetin dialdehyde
Mangiferin	Agnuside	Bisdemethoxycurcumin	Ursolic acid
3-O-Acetyl-11-keto-β-boswellic acid	Picroside II	Serratol	4-Hydroxyisoleucine
	Eclalbasaponin I	Glycyrrhizin ammonical hydrate	Curcumin
1,9-Dideoxyforskolin	Alizarin		Vasicinone
Marmelosin	D-Pinitol	Boeravinone B	3'-Hydroxy,4'-methoxygalbretin
3-O-Acetyl-β-boswellic acid	Eclalbasaponin II	Sesamin	
Diosgenin	Allylpyrocatechol 3,4 diacetate	Guggulsterone E	Caffeine
		a-Boswellic acid	Vicine
Methylgallate	Piperine	6-Shogaol	Isoforskolin
Apocynin	Eicosyl caffeate	Guggulsterone Z	Campesterol
Docosyl caffeate	trans-Anethole	β-Boswellic acid	Vasicine
Methyl eugenol	Protocatechuic acid (3,4 dihydroxybenzoic acid)	Shatavarin IV	Isoeugenol
1'-Acetoxychavicol acetate		Gymnemagenin	Capsaicin
2',3'-Dehydrosalannol	Epicatechin	Betaine	Vanillylacetone
3-O-Methylellagic acid	Aristolochic acid I	β-Sitosterol	Isoformonometin
4-Allylpyrocatechol	Estragole	β-Glucogallin	Caryophyllene
7-O-Methylwogonin	Artemisinin	Betulinic acid	Wedelolactone
3,3'-Di-o-methyl ellagic acid-4'-O-β-D-Andrographolide	Psoralen	β-Sitosterol-D-glucoside	Jujubogenin isomer of

Maslinic acid	Ferulic acid	Galangin	bacopasaponin C
xylopyranoside	Atlantone	Caffeic acid	Cedrol
Andrographanin	Punicalagin (a+β)	Stigmasterol	Withanolide A
Menthol	Forskolin	Geraniol	Kaempferol
1-Deoxynojirimycin	Bacopasaponin C	Catechin	Chrysophanol
Apigenin	Pterostilbene	Stevioside	Withaferin A
Maslinyl acetate	Furanoeudesma-1,3-diene	Geranyl acetate	11-Keto-β-boswellic acid
Elemolic acid (α+β)	Bacopaside II	Catechin-5-O-gallate	1,8-Cineole
Arjunetin	Pyrogallol	Tetrahydrocurcumin	Withanolide B
Neoandrographolide	Formononetin	Harmaline	L-Dopa
Elemonic acid (β)	Bacopaside I	Chebulagic acid	Cirsilineol
Arjunic acid	Piperyline	Trigonelline HCl	Withanone
Negundoside	Gallic acid	Harmalol	Lupeol
Arjungenin	Baicoside A3	Chebulinic acid	Cinnamic acid
Ellagic acid	Quercetin dihydrate	1,3,6,-Trigalloyl-β-D-	Withanoside IV
Oleanolic acid	6-Gingerol	Glucose	Luteolin
ar-Turmerone	Bacoside A	Harmine	m-Coumaric acid
Embelin	Rebaudioside	Chlorogenic acid	Withanoside V
1-Octacosanol	A	Tribuloside	Licochalcone A
Eugenol	8-Gingerol	Hexahydrocurcumin	Deacylgymnemic acid
α-Asarone	Bacosine	Colchicine	12-Deoxywithastramonolide
Panduratin-A	Reserpine	Trigoneoside IV a	Lycopene

Epicatechin 3-gallate	10-Gingerol	Hydroxycitric acid Calcium salt	
α-Asarone	Bakuchicin		
Para methoxyethylcinnamate	Rosmarinic acid	Corilagin	
Epigallocatechin 3-gallate	Glabridin	3β-Taraxerol	
Asiatic acid	Bakuchiol	Hydroxycitric acid lactone	

Source: natural remedies, Bangalore.

The 3d structures of the above phyto-compounds were retrieved from various chemical databases.

D. Generating phase database

Now using Application→Phase→Generate Phase Database module of Maestro software phase database of the phyto-compounds was done [12].

E. Selection of ligands for NMDA receptor

Ligand-based pharmacophore model was selected by extracting the common features of the three-dimensional structures of compounds which are known to interact with the target protein (known ligand) [13]. Known ligands were loaded in the Maestro workspace and by using Applications→Phase→Create Hypothesis module pharmacophore features of the known ligands were noted [12].

F. Docking

Protein Preparation

The I-Tasser generated protein is not suitable for immediate use in docking or other molecular modeling calculations. By using Protein Preparation Wizard of Maestro9.1 the I-Tasser generated protein was uploaded for optimization & energy minimization [12].

Active Site Generation

The active site position of the protein was determined by SiteMap module of Maestro [12].

Ligand Preparation

The ligands were selected in Maestro workspace. Using ligprep, the ligands were minimised prepared for docking studies. LigPrep is tool to prepare high quality 3D structure for large number of molecules taking input as 2D or 3D structures and giving output as a single, low energy 3D structure [12].

Receptor Grid Generation

The receptor was loaded in workspace. Using Glide→ Receptor Grid Generation the active or binding site region of the receptor was specified and the receptor was prepared for docking.

Glide Docking

Using module Glide→Ligand Docking module of Maestro the receptor was docked with the selected ligands [12].

G. ADME screening

ADME is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism, and excretion. Using QikProp module the ADME properties of the above ligands was determined [12].

III. RESULTS & DISCUSSION

The receptor protein was selected from the NCBI database (Table I). The 3D structure of NMDA protein was modelled by I-Tasser server [9].

TABLE I
PROTEINS WITH THE NCBI ACCESSION NUMBER

Protein	NCBI Accession Number
NMDA	(NP_001009184)

A. Predicting the 3D structure of NMDA receptor

The 3d structure of NMDA protein was modeled (Fig. 1). Since BLAST search generated no templates, the NMDA amino acid sequence was submitted to I-TASSER server.

I-TASSER server output gives 3d structure of proteins based on protein threading method. Protein threading is a procedure for identifying template proteins from prepared structure databases that have a similar structure or similar structural motif as that of the query protein sequence [14]. The top template (best result) in each threading program is selected for further consideration. The quality of the query protein-template alignments and the difficulty of modeling the query protein are judged based on the statistical significance of the best threading alignment [14]. Ramachandran plot of I-TASSER server output gave 82.0% residues in the core region, 14.5% in the allowed region, 2.4% residues in the generously allowed region and 1.0% disallowed region (Table II).

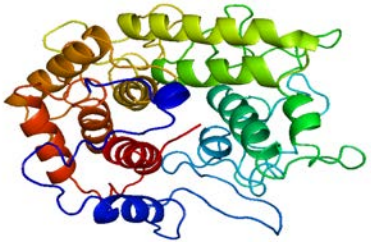


Fig. 1 3d Structure of the selected best NMDA model.

B. Pharmacophore studies

Ligand-based pharmacophore models are selected by extracting the common features of the three-dimensional structures of the known ligands. To do this, possible conformers of compounds should be previously enumerated. Then, we superpose our target compounds by overlapping the three-dimensional structures' common substructures as molecular graphs among the other parts of compounds. So, in this method, since we do not have to enumerate all the conformers of a compound, we usually save much computational time by ligand-based pharmacophore modeling [13].

NMDA receptor

2-Methyl-6-(phenylethynyl)pyridine (**MPEP**) known ligand for NMDA receptor was loaded in the Maestro workspace. Phase generated the pharmacophore features of MPEP as A1, H3, R4, R5 [A=Acceptor, H=Hydrophobic, R=Aromatic Rings] (Fig. 2).

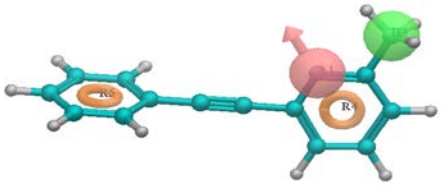


Fig. 2 Pharmacophore features of MPEP.

This pharmacophore features matched with the following compounds in Table II.

TABLE II
LIGANDS WHICH MATCHES WITH THE PHARMACOPHORE OF MPEP AND THEIR FITNESS SCORE

Identified ligands	**Fitness score**	**Plant**
7-o-methylwogonin	1.709	*Andrographis paniculata*
Berberine Chloride dehydrate	1.359	*Berberis aristata*
Cirsilineol	1.698	*Artemisia vestita*
Curcumin	1.316	*Curcuma longa*
Eupalitin	1.218	*Boerhaavia diffusa*
Glabridin	1.768	*Glycyrrhiza glabra*
Hypophyllanthin	1.328	*Phyllanthus amarus*
Licochalcone A	1.593	*Glycyrrhiza uralensis*
Phyllanthin	1.573	*Phyllanthus amarus*
Pterostilbene	1.488	*Pterocarpus marsupium*

Known NMDA ligand **3-((2-Methyl-4-thiazolyl)ethynyl)pyridine** (**MTEP**) was loaded in the Maestro workspace. Phase Create Hypothesis module gave pharmacophore features as A1, A2, H4, R5 and R6 (Fig. 3) which matched with the compounds in Table III.

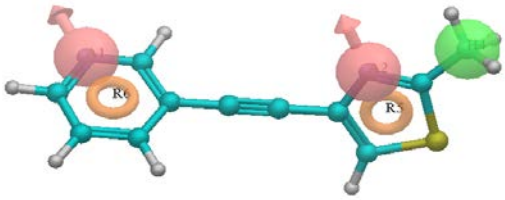


Fig. 3 Pharmacophore features of MTEP.

TABLE III
LIGANDS WHICH MATCHES WITH THE PHARMACOPHORE OF MTEP AND THEIR FITNESS SCORE

Identified ligands	**Fitness score**	**Plant**
Cirsilineol	1.410	*Artemisia vestita*
Curcumin	1.095	*Curcuma longa*
Glabridin	0.829	*Glycyrrhiza glabra*
Hypophyllanthin	1.243	*Phyllanthus amarus*
Licochalcone A	1.010	*Glycyrrhiza uralensis*
Phyllanthin	1.269	*Phyllanthus amarus*

Pharmacophore features of the known ligand NMDA ligand, **Aniractam** A1, A2, A3, H4 and R6 (Fig. 4) matched with the compounds in Table IV.

TABLE IV
LIGANDS WHICH MATCHES WITH THE PHARMACOPHORE OF ANIRACTAM AND THEIR FITNESS SCORE

Identified ligands	**Fitness score**	**Plant**
Isoformononetin	2.055	*Vitis vinifera_*
7-o-methylwogonin	1.972	*Andrographis paniculata*
Wedelolactone	1.962	*Eclipta alba*
Formononetin	1.832	*Trifolium pratense*
Eupalitin	1.802	*Boerhaavia diffusa*
Cirsilineol	1.747	*Artemisia vestita*
8-gingerol	1.747	*Zingiber officinale*
6-gingerol	1.744	*Zingiber officinale*
Hypophyllanthin	1.716	*Phyllanthus amarus*
Picroside –II	1.594	*Picrorhiza kurrooa*
α-Asarone	1.562	*Acorus calamus*
β-Asarone	1.561	*Acorus calamus*
Phyllanthin	1.430	*Phyllanthus amarus*
Colchicine	1.547	*Gloriosa superba*

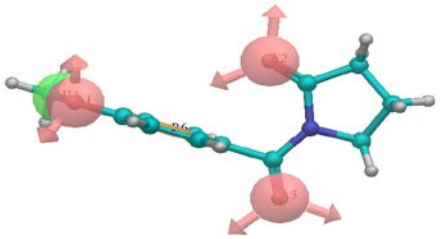


Fig 4 Pharmacophore features of aniractam.

All the above compounds were docked with NMDA receptor.

C. Glide Ligand Docking

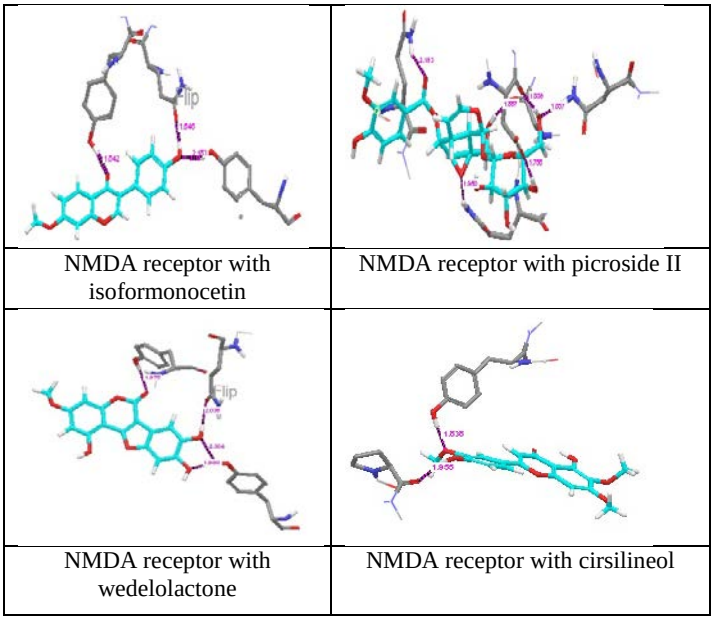
NMDA receptor

Using sitemap the active site residues of the NMDA receptor was determined. As per the output sitemap_site_2 with SiteScore 1.023 (2nd highest score) and size 120 was used to determine the active site of the modelled protein. The sitemap_site_1 was not taken since its size was almost equivalent to the size of the protein (403) and hence contains non-specific active site residues.

LYS155, ASP143, ALA149, ASN151, ILE153, THR150, ASP146, SER140, GLU135, GLU136, GLY137, THR133, ASN144, PRO148, GLN145, PHE147, TYR141, GLY92, GLN91, PRO138, THR133, PRO93, PHE120, GLN123, ASP126, GLN116, VAL119, THR94, TYR84, GLY82, PRO83, TYR79, GLN81, PRO75, GLN78, TYR52, GLN76, GLY77, TYR74, PRO73, PRO70, TYR69.

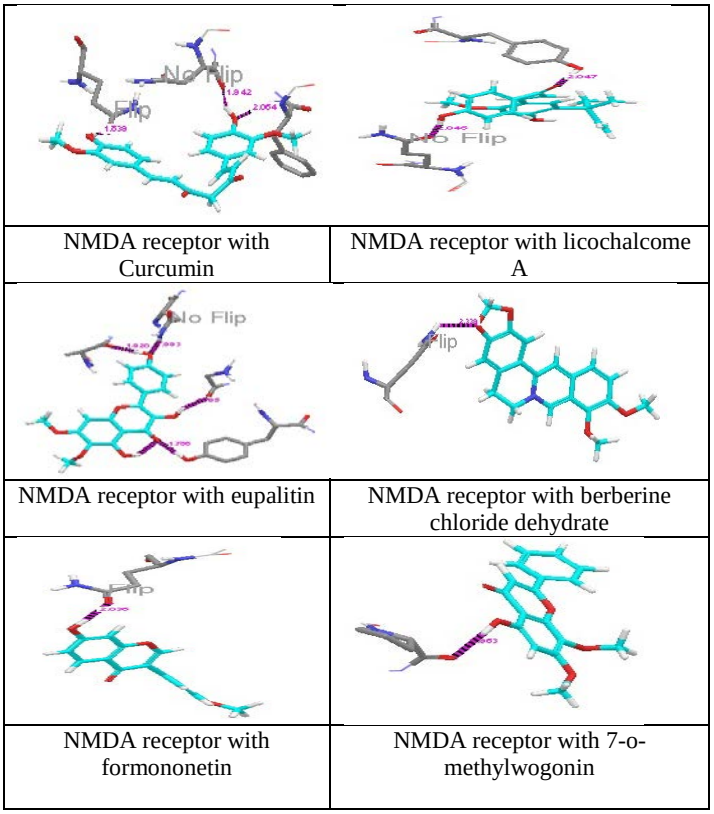
Using Receptor Grid Generation the active site or binding site region of the receptor was assigned and grid for the receptor was generated.

Using Glide Ligand docking the NMDA receptor was docked with the compounds in Table II, III and IV (Fig. 5, Table V).



NMDA receptor with isoformonocetin | NMDA receptor with picroside II

NMDA receptor with wedelolactone | NMDA receptor with cirsilineol



NMDA receptor with Curcumin | NMDA receptor with licochalcome A

NMDA receptor with eupalitin | NMDA receptor with berberine chloride dehydrate

NMDA receptor with formononetin | NMDA receptor with 7-o-methylwogonin

Fig. 5 Docking results of NMDA receptor with the selected ligands

TABLE V
DOCKING RESULTS OF NMDA RECEPTOR WITH THE SELECTED LIGANDS

Ligand	Docking score/ glide g score	Doner	Distance in Å	Interaction
Picroside II	-14.595	GLN81 GLY92 GLN86 ASN151 ASP146	2.193 1.887 1.960 1.857 1.859 1.756	GLN81(OH)...O(UNK) GLY92(OH)...O(UNK) GLN86(OH)...O(UNK) ASN151(OH)...O(UNK) ASP146(OH)...O(UNK) ASP146(OH)...O(UNK)
Wedelolactone	-12.046	TYR141 TYR94 GLN123	1.978 2.304 1.990 2.036	TYR141(OH)...O(UNK) TYR94(OH)...O(UNK) TYR94(OH)...O(UNK) GLN123(OH)...O(UNK)
Cirsilineol	-11.187	TYR141 PRO75	1.838 1.955	TYR141(OH)...O(UNK) PRO75(OH)...O(UNK)
Curcumin	-11.126	ASN144 PHE147 GLN123	1.942 2.064 1.839	ASN144(OH)...O(UNK) PHE147(OH)...O(UNK) GLN123(OH)...O(UNK)

Licochalcome A	-10.955	TYR141 GLN81	2.047 2.045	TYR141(OH)...O(UNK) GLN81(OH)...O(UNK)
Eupalitin	-10.537	GLY92 ALA149 ASN151 TYR94	1.795 1.920 1.993 1.786	GLY92(OH)...O(UNK) ALA149(OH)...O(UNK) ASN151(OH)...O(UNK) TYR94(OH)...O(UNK)
Berberin Chloride Dihydrate	-9.309	GLN123	2.339	GLN123(OH)...O(UNK)
Formononetin	-9.168	GLN123	2.036	GLN123(OH)...O(UNK)
7-o-methylwogonin	-8.756	PRO75	1.963	PRO75(OH)...O(UNK)
Isoformononetin	-8.554	TYR141 GLN123 TYR94	1.842 1.646 2.183	TYR141(OH)...O(UNK) GLN123(OH)...O(UNK) TYR94(OH)...O(UNK)

D. ADME screening

ADME is an acronym for **a**bsorption, **d**istribution, **m**etabolism, and **e**xcretion. QikProp is a quick, accurate, easy-to-use ADME prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of molecules [12, 15].

QikProp generated the following output (Table VI, VII):

TABLE VI
PRINCIPAL DESCRIPTORS CALCULATED BY QIKPROP SIMULATION [15] (RANGE 95% OF DRUGS)

Lead molecules	Molecular weight [a] (g/mol)	Molecular volume [b] (Å)	PSA [c]	HB [d] donors	HB [e] acceptors	Rotatable bonds [f]
Picroside II	512.466	1381.689	205.533*	5.000	18.100	13.000
Wedelolactone	314.251	873.124	120.671	3.000	6.000	4.000
7-o-methylwogonin	298.295	912.044	68.460	0.000	3.750	3.000
isoformononetin	268.268	840.385	64.747	1.000	4.000	3.000

A * indicates a violation of the 95% range.

[a] Molecular weight of the molecule
[b] Total solvent-accessible volume in cubic angstroms using a probe with a radius of 1.4 Å
[c] Van der Waals surface areas of polar nitrogen and oxygen atoms
[d] Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer
[e] Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer
[f] Number of rotatable bonds

TABLE VII
PHYSIOCHEMICAL DESCRIPTORS CALCULATED BY QIKPROP SIMULATION [15] (RANGE 95% OF DRUGS)

Lead molecule	QP log P(o/w) [a]	QP log S [b]	QP PCaco [c]	QP log HERG [d]	QP PMDCK [e]	% Human oral absorption [f]
Picroside II	-1.357	-2.051	18	-5.285	6M	3
Wedelolactone	0.883	-2.877	103	-4.724	42	68
7-o-methylwogonin	3.091	-3.652	1268	-4.925	639	100
isoformononetin	2.604	-3.438	1289	-5.141	651	100

A * indicates a violation of the 95% range.

An M indicates MW is outside training range.
[a] QP log P for octanol/water (−2.0, – 6.5)
[b] Predicted aqueous solubility, log S. S in mol dm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (−6.5, – 0.5)
[c] Apparent Caco-2 permeability (nm/s) (<25 poor, >500 great)
[d] log HERG, HERG K+channel blockage (concern below −5)
[e] Apparent MDCK permeability (nm/s) (<25 poor, >500 great)
[f] % Human oral absorption in GI (±20%) (<25% is poor)

IV. CONCLUSION

NMDA: The phytocompounds picroside II, wedelolactone, 7-o-methylwogonin and isoformononetin having the best fitness score, docking score and most interactions with the NMDA receptor are considered for binding assay studies with NMDA receptor *in-vitro*.

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