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In silico inhibition of GABARAP activity using antiepileptic medicinal derived compounds

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Abstract:
Epilepsy is a neurological disorder affecting more than 50 million people worldwide. It can be controlled by antiepileptic drugs (AEDs) but more than 30% patients are still resistant to AEDs. To overcome this problem, researchers are trying to develop novel approaches to treat epilepsy including the use of herbal medicines. The γ-amino butyric acid type-A receptor associated protein (GABARAP) is ubiquitin-like modifier implicated in the intracellular trafficking of $GABA_AR$. An *in silico* mutation was created at 116 amino acid position G116A, and an *in silico* study was carried out to identify the potential binding inhibitors (with antiepileptic properties) against the active sites of GABARAP. Five different plant derived compounds namely (a) Aconitine (b) Berberine (c) Montanine (d) Raubasine (e) Safranal were selected, and their quantitative structure-activity relationships (QSAR) have been conducted to search the inhibitory activity of the selected compounds. The results have shown maximum number of hydrogen bond (H-bond) interactions of Raubasine with highest interaction energy among all of the five compounds. So, Raubasine could be the best fit ligand of GABARAP but *in vitro*, and *in vivo* studies are necessary for further confirmation.

Key words: Epilepsy, antiepileptic drugs, γ-amino butyric acid type-A receptor associated protein, inhibitors, ligand.

Background:
Epilepsy is a complex neurological disorder characterized by spontaneously occurring seizures; affecting 50 million people around the world; more than 85% people suffering with this disease belongs to developing countries **[1, 2].** Epileptic state represents a dramatic imbalance between excitatory and inhibitory activity; a seizure activity due to altered γ-amino butyric acid type-A receptor ($GABA_AR$) trafficking and/or subunit expression in animal models of temporal lobe epilepsy (TLE), Status epilepticus (SE) and in patients **[3, 4].** The $GABA_AR$ is a ligand gated ion channel receptor which mediates quick inhibitory synaptic transmission into the central nervous system (CNS) and is a potential target of numerous essential neuroactive drugs **[5, 6].** But, different molecular mechanisms regulating the trafficking and function of $GABA_AR$ are yet not clear. The $GABA_AR$ associated protein (GABARAP) is mainly localized in the Golgi apparatus, indicating its central role in the intracellular trafficking of $GABA_AR$ **[7, 8].** The GABARAP binds with the intracellular domain of γ-2 subunit of $GABA_AR$ i*n vitro* and *in vivo* **[9, 10].**

Due to a mutation in the C-terminal (G116A), the cleavage of C-terminal of GABARAP could be blocked, that could distort the phospholipids addition to GABARAP which is quite essential in controlling the trafficking of $GABA_AR$ **[11]**. In comparison to the wild type GABARAP, its co localization and binding with $GABA_AR$ was significantly reduced that caused a decreased expression of $GABA_AR$ in the plasma membrane **[11]**. Studies have elucidated that $GABA_AR$ expression at cell the surface was prohibited due to G116A mutation when checked in oocytes.

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These findings have revealed that glycine 116 is vital for GABARAP C-terminal processing, necessary for GABARAP localization and its trafficking ability **[11].** Few drugs such as vigabatrin can enhance the level of inhibitory neurotransmitter particularly gamma-amino butyric acid (GABA) or can reduce the level of excitatory neurotransmitter such as glutamate **[12]**. Although seizures are controlled with currently available AEDs but more than 30% patients still have medically refractory epilepsy **[13].** Moreover, about 30-40% epileptic patients are still affected by many side effects **[14]**. These conditions have motivated the researchers to develop novel approaches to treat epilepsy like antiepileptic constituents from herbal medicines **[15].** Five medicinal compounds with antiepileptic/ anticonvulsant properties including Aconitine extracted from *Aconitum* species, Berberine from *Berberis* vulgaris, Montanine from *Hippeastrum* vittatum, Raubasine from *Rauwolfia* serpentine and Safranal from *Crocus* sativus L were selected to check their binding ability with different residues of GABARAP. The docking study was carried out by selecting the GABARAP as a drug target because it acts as a receptor by regulating cell surface expression of $GABA_AR$.

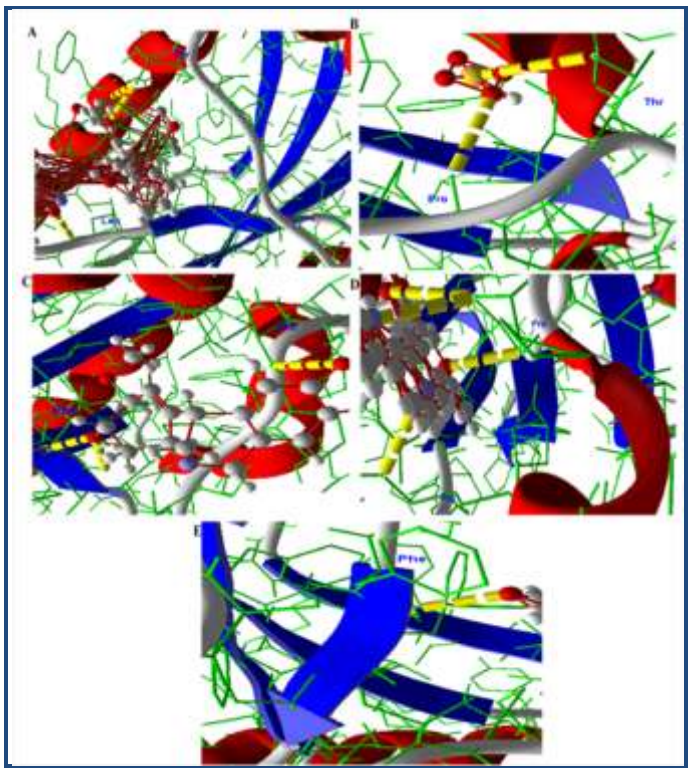

Figure 1: Interaction of GABARAP residues with various ligands. **(A)** Aconitine have shown three interactions; Thr87-O, Thr87-O and Leu76-N; **(B)** Berberine have shown two interactions; Thr87-O and Pro72-O; **(C)** Montanine showed three interactions; Phe78-O, Phe78-N and Thr87-N; **(D)** Raubasine showed four interactions; Arg28-O, Arg22-N, Pro26-O and Pro26-N; **(E)** Safranal showed one interaction with Phe77-N.

Methodology:

Template Search

Template search with Blast and HHBlits has been performed against the SWISS-MODEL template library (SMTL, last update: 2014-11-12, last included PDB release: 2014-11-07). The BLAST was used in search of target sequence **[16]** against primary amino acid sequence contained in the SMTL. Total thirteen templates were observed. An initial profile of HHblits has been built using the outlined procedure **[17],** followed by an iteration of HHblits against NR20. Afterwards, attained profile has been searched against all the SMTL profiles. Total, forty templates were observed.

Template Selection

Quality of each of the identified template has been predicted from the features of target-template alignment. Highest quality templates have then been selected for building the models.

Model Building

Based on the alignment of target-template, the models have been built using Promod-II. The coordinates that are conserved between the target and template have been copied from the template to the model. The insertions as well as deletions have remodeled through fragment library, and the side chains were also rebuilted. Geometry of the final model was regularized using a force field. If the satisfactory results were not achieved through loop modelling with ProMod-II **[18]**; then, an alternate model is needed to build with the MODELLER **[19].**

Model Quality Estimation

Global as well as per-residue model quality was assessed through QMEAN scoring function **[20].** For an improved performance, the weights of individual QMEAN terms have been trained specifically for SWISS-MODEL.

Ligand Modeling

Ligands in the template structure have been transferred to the model on fulfilling the following criteria: **(a)** Ligands are annotated as biologically relevant to the template library, **(b)** ligand-model should be in contact, **(c)** should be no clash between the ligand and protein, **(d)** interacting residues with the ligand are conserved between the template-target. The ligands not satisfying the above mentioned criteria will be excluded from the model. Summary of the model includes information why and which ligand has not been included.

Oligomeric State Conservation

Homo-oligomeric structure of the target protein has been predicted depending upon the analysis of pairwise interfaces of identified template structures. For each relevant interface between polypeptide chains, the QscoreOligomer **[21]** has been predicted from the features like similarity to the target and the observing frequency of this interface in the recognized templates. Moreover, whole complex QscoreOligomer was calculated as the weight-averaged QscoreOligomer of the interfaces. Oligomeric state of the target has predicted to be the same as in the template when QscoreOligomer is predicted to be higher or equal to 0.5.

Protein simulation and validation

The obtained protein structure was refined geometrically to decrease the steric hindrances from side chain using online tool, the Mod Refiner. Mod Refiner is an algorithm for high-resolution protein refinement simulations where the initial starting models closer to their respective native state in terms of backbone topology, hydrogen bonds and side chain positioning. Potential energy of the model was analyzed before

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and after the minimization. The output have further taken for the loop refinement and the stereochemical quality of the structure that was validated by PROCHECK.

Active site prediction

The stabilized macromolecule was validated using tools such as protein quality predictor (ProQ) and Q-site finder to determine the binding site and analyze the protein flexibility, and electrostatic property.

Ligand identification and minimization

Ligands used for this study were selected on the basis of antiepileptic constituents of medicinal plants given in the **Table 1 (see supplementary material)**. Compounds 2D structure and their molecular weights were obtained from PubChem. Using ACD/ChemSketch software, the drugs structures were sketched and MOL file was generated followed by subsequent generation of 3D structures using Web lab viewer program, a molecule converter from MOL file to PDB. By using the Argus Lab 4.0, optimization of the ligand was achieved by applying appropriate force field.

VEGA-QSAR

Virtual models for property evaluation of chemicals within global architecture-quantitative structure-activity relationship (VEGA-QSAR) program analyzed the selected ligands to determine the relationship of physiochemical properties and biological activities of descriptor molecules in various classified QSAR models **Table 2 (see supplementary material).** Toxicity, ecotoxicity and physiochemical predicted properties of ligands such as logP (version 1.1.2), bioconcentration factor (BCF) (CAESAR-version 2.1.13), carcinogenicity model (CAESAR 2.1.8), mutagenicity model (CAESAR version 2.1.12), skin sensitization model (CAESAR-version 2.1.5), developmental toxicity model (CAESAR-version 2.1.6), fathead minnow LC50 96hr (lethal concentration to 50% of the test animals) (Environmental Protection Agency (EPA)-version1.0.6), daphnia magna LC50 48hr (EPA-version 1.0.6), BCF read across (version-1.0.2), ready biodegradability model (version 1.0.8) were determined **[22, 23].** The VEGA-QSAR models were initially derived from CAESAR models, and other models were added to stimulate the already available models, one such model is EPA (US Environmental Protection Agency). The used input formats were SMILES and SDF files.

Molecular Docking

Potential docking between GABARAP and different ligands was carried out by Molegro Virtual Docker (MVD); Software used for drug discovery with graphical user interface. Prior to docking; ligands and target protein was prepared. Best possible interactions were selected; different parameters including MolDock score, docking scores, RMSD values and total number of interactions between ligands and protein residues, and torsions were assigned to get their values **Table 3 (see supplementary material).** The MVD tools were utilized to create grid, calculate the dock score, and evaluate conformers. The non-polar hydrogen atoms have been removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Two types of dock scores such as Mol Dock score and Re-rank score of ligands were calculated in docking **[24].** Docking was performed by following the steps in MVD user manual. Various poses were created for each compound; best pose-wise as well as docking score compound was selected as an effective inhibitor of GABARAP **(Figure 1).**

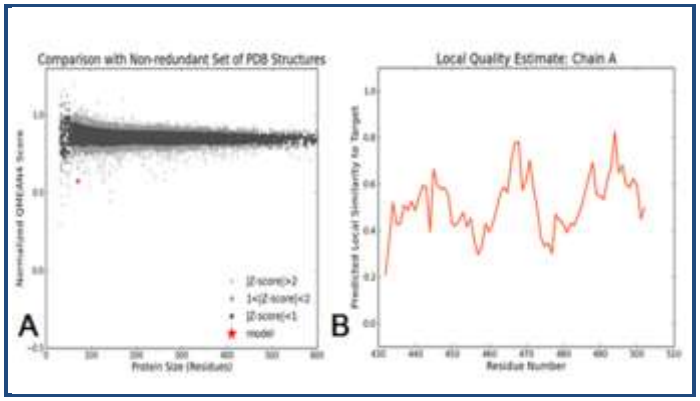

Figure 2: The predicted Z-score and quality of the modeled protein. **(A)** Z-score of the model compared with non-redundant set of PDB structures; **(B)** Predicted residue numbers for similarity and quality of chain A

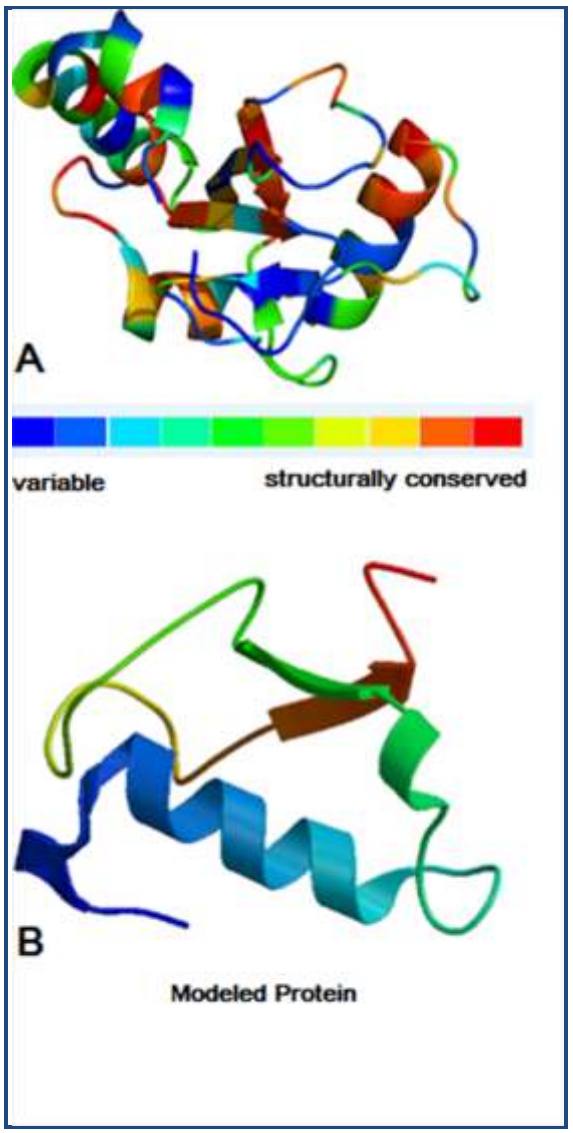

Figure 3: GABARAP protein **(A)** Structure of GABARAP and its conserved regions; **(B)** The three dimensional structure of modeled protein.

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Results:

Modeled Template

The SWISS-MODEL template library (SMTL version 2014-11-12, PDB release 2014-11-07) has been searched with Blast **[16]** and HHBlits **[17]** for evolutionary related structures similar to the target sequence **(Table 1**). The template search details have been explained in materials and methods. Overall 67 templates have been found **(Table 2).** Predicted residue numbers for similarity and quality of chain A and the Z-score of the modeled protein is shown in **Figure 2.**

ProQ and Q site finder results

The LG score **[25]** and MaxSub scores for the mutated GABARAP were obtained from the ProQ server [26] indicating a very good (3.785) and fairly good (0.3790) quality, respectively. The conserved region in GABARAP protein and its three dimensional modeled protein is shown in **Figure 3.**

Ramachandran plot

Ramachandran plot of mutated GABARAP showed 95.2% residues in most favored regions; 4.8% residues were observed in additional allowed region, 0.0% residues were present in generously allowed region and 0.0% residues were seen in disallowed region as shown in **Figure 4.**

Ligand structure

Five antiepileptic constituents from medicinal plants were selected as targeted ligands. Structure of all compounds, their molecular weight and potential functions are given in **Table 1 (see supplementary material)**.

QSAR study

VEGA-QSAR study was carried out for the prediction of different biochemical properties of ligands. Results attained through QSAR models could be effective to evaluate the chemical properties of chosen compounds by decreasing animal tests. Different models were tested against antiepileptic compounds **Table 2 (see supplementary material).** The selected compounds have shown positive predictions being non-mutagenic and non-carcinogenic. Fathead minnow LC50 was predicted less than 6.5 [−log (mol/L)] for all the compounds. All the compounds have been found toxicants except Raubasine **(Figure 4).** Three ligands are non-ready bio-degradable whereas Berberine and Safranal are ready bio-degradable. Skin sensation model predicted sensitizes for Berberine**,** Montanine and Safranal. The log *P* value is a valuable parameter to understand the behavior of drug molecules; log *P* value is higher in Safranal (3.22 log units), Raubasine (2.95 log units), Montanine (1.57 log units), Berberine (-0.97 log units), and Aconitine (-1.62 log units) **Table 2 (see supplementary material).**

Molecular docking

Five antiepileptic constituents from medicinal plants were selected. The results of interaction between GABARAP and compounds **(a)** Aconitine **(b)** Berberine **(c)** Montanine **(d)** Raubasine **(e)** Safranal have shown in **Table 3 (see supplementary material)** and **Figure 4**. Raubasine have shown best interaction carrying 4 H-bonds with GABRAP residues such as: Arg28-O, Arg22-N, Pro26-O and Pro26-N; followed by Aconitine with H-bond interactions: Leu76-N, Thr87-O and Leu76-N. Montanine have shown 3 interactions at Phe78-O, Phe78-N and Thr87-N. Berberine have shown 2 interactions at Thr87-O and Pro72-O. Docking energy of Raubasine was much less compared to other compounds due to heavy ring like structure. The aconitine has maximum number of torsions compared to all other compounds. It can be concluded that Raubasine could be the best fit ligand in the binding pockets of modeled GABARAP protein exhibiting four H-bond interactions within the active sites of GABARAP.

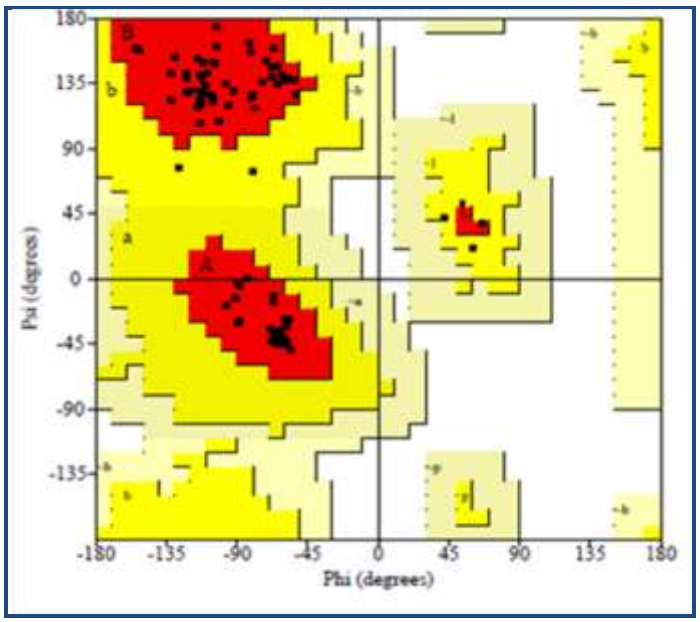

Figure 4: Ramachandran plot of mutated GABARAP. Ramachandran plot of mutated GABARAP have shown 95.2% residues in most favored regions, 4.8% residues in additional allowed region, 0% residues in generously allowed region and 0% residues in disallowed region.

Discussion:

Currently, molecular docking studies have been frequently done in drug designing through an understanding between drug-receptor bindings. Prior studies have revealed that computational analysis could be helpful in making the new possible inhibitors through various mechanisms of interaction between drug and receptor **[27].** Current docking study have been carried out for five herbal antiepileptic compounds toward GABARAP. For the analysis of best interaction between protein and ligands; root mean square distance (RMSD) value was used. Maximum 4-hydrgen bonds were observed between Raubasine and GABARAP whereas Aconitine and Montanine either showed 3-H bonds. Binding of these compounds toward GABARAP was observed to be strong in docking models.

Aconitum alkaloids belong to diterpene alkaloid neurotoxin series which bind with voltage dependent Na^+ channel. These channels have an essential role in the neuronal excitability. Studies have shown antiepileptic activities of aconitum alkaloids on an *in vitro* rat hippocampal slices **[28, 29]**; these studies have depicted that benzoyl ester is an active center of anticonvulsant activities. Aconitine, an important plant alkaloid of *Aconitum* species comprises of benzoyl ester on C-14 position, it could inhibit normal neuronal activity and epileptoform activity **[28, 29]**. The two other compounds; Montanine and Berberine have been investigated against

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seizure inducing chemicals, pentylenetetrazole (PTZ), kainic acid (KA), bicuculline and maximal electroshock (MES). Both of these compounds have shown anticonvulsant activity through neurotransmitter modulation **[30, 31].** Another compound, the Raubasine reduced bicuculline as well as PTZ induced convulsions into the mice; it might be due to its interacting activity with benzodiazepine **[32].** Safranal is another monoterpene aldehyde of Crocus sativus L; it also showed inhibitory effect toward the PTZ induced convulsions in mice through an interaction with $GABA_A$ benzodiazepine receptor complex **[33, 34].**

Based on docking score and H-bond interactions, the Raubasine has strong interaction in comparison to other compounds which reveals its highest interacting ability with GABARAP and it can be considered as a possible ligand of GABARAP.

Conflict of interest:
The authors declare no conflict of interest.

Author contributions:
Performed experiments**: SM, MF, and IQ**; Analyzed data: **SM;** Planned and conceived experiments: **SM, MF, and IQ**; Reviewed the article: **SM, MF, IQ, AA, and TK**; Wrote the article**: SM, MF.** Financial support of this work was provided by the KACST large grant 162-34 to Ishtiaq Qadri.

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Supplementary material:

Table 1: Ligands, their structure and possible functions

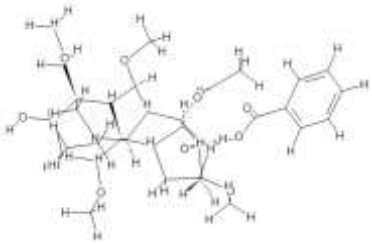
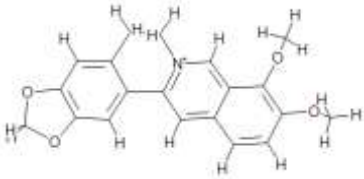
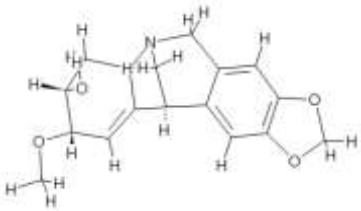
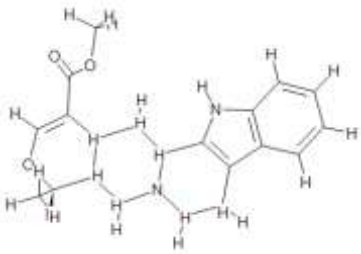
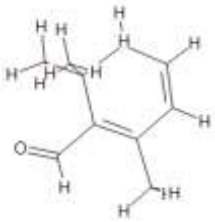
Compound	Molecular weight (g/mol)	Structure	Possible Function	References
Aconitine	645.73708		Inhibits normal neuronal activity and epileptoform activity	[28, 29]
Berberine	433.43176		Anticonvulsant activity through neurotransmitter system modulation	[30, 31]
Montanine	301.33706		Anticonvulsant activity through neurotransmitter system modulation	[30, 31]
Raubasine	352.42686		Inhibits convulsions by interacting with benzodiazepine	[32]
Safranal	150.21756		Binds with $GABA_A$ benzodiazepine receptor complex	[33, 34]

Table 2: Classification of models and predicted values for various structure activity relationships.

QSAR Models	Prediction and applicability domain analysis for models				
	Aconitine	Berberine	Montanine	Raubasine	Safranal
Fathead minnow LC_{50} (96hr) -log (mol/l)	4.76	5.44	4.19	5.57	4.5
Daphnia Magna LC_{50} (48hr) -log (mol/l)	5.71	5.89	5.63	6.62	4.93
Mutagenicity model (CAESAR)	Non-Mutagen	Mutagen	Mutagen	Non-Mutagen	Non-Mutagen
Mutagenicity sarPy model	Non-Mutagen	Non-Mutagen	Non-Mutagen	Non-Mutagen	Non-Mutagen

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Carcinogenicity model	Carcinogen	Carcinogen	Non-Carcinogen	Carcinogen	Non-Carcinogen
Developmental Toxicity model	Toxicant	Toxicant	Toxicant	Non-toxicant	Toxicant
BCF model log(l/kg)	-1.12	1.12	0.45	0.79	0.7
Ready biodegradability model	Non ready biodegradable	Ready biodegradable	Non ready biodegradable	Non ready biodegradable	Ready biodegradable
LogP prediction [log units]	-1.62	-0.97	1.57	2.95	3.22
Skin sensitization model (CAESAR)	Non-sensitizer	Sensitizer	Sensitizer	Non-sensitizer	Sensitizer
BCF read-across log(l/kg)	1.96	2.24	1.3	2.61	1.81

Table 3: Antiepileptic constituents of medicinal plants and their interaction with various GABARAP residues.

Medicinal herbs	Compound	MolDock Score	RMSD	H-bond	Interactions	Torsions	Docking Score
Aconitum species	Aconitine	69.3522	29.7771	03	Thr87-O Thr87-O Leu76-N	86	59.6731
Berberis vulgaris	Berberine	-39.0374	28.4547	02	Thr87-O Pro72-O	00	-43.6295
Hippeastrum vittatum	Montanine	-82.6624	36.1476	03	Phe78-O Phe78-N Thr87-N	01	-81.846
Rauwolfia serpentina	Raubasine	-105.986	22.9307	04	Arg28-O Arg22-N Pro26-O Pro26-N	00	-111.333
Crocus sativus L	Safranal	-90.8032	29.4333	01	Phe77-N	00	-90.9961

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