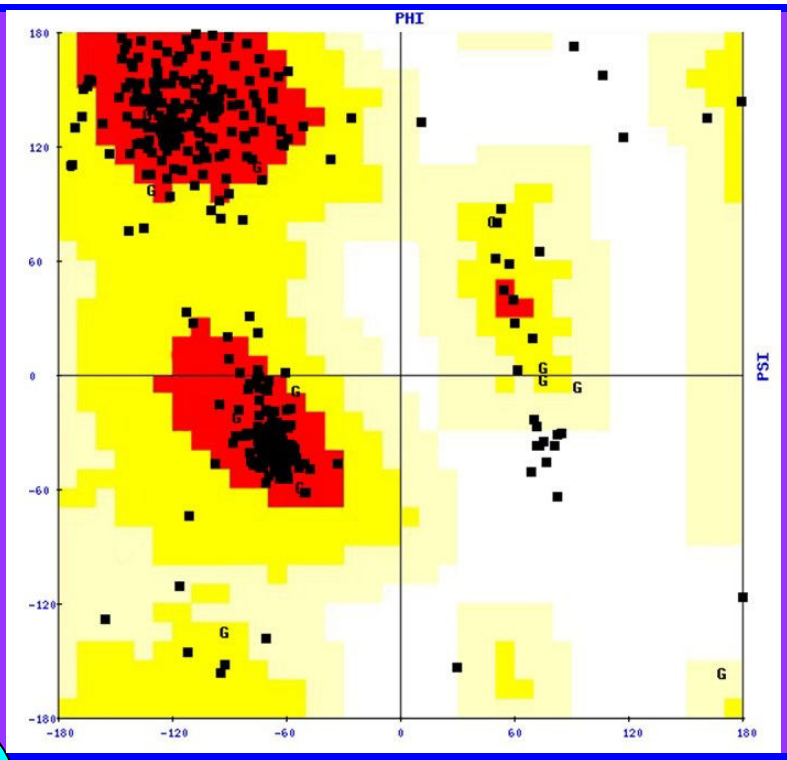

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$GABA_A$ receptor binding molecules from Traditional Chinese Medicine: An *in silico* approach

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Abstract

The advents of how anesthesia works have helped in the discovery of anesthetic target protein. One such target protein named ϒ-aminobutyric acid ($GABA_A$), which is the chief inhibitory neurotransmitter in the mammalian central nervous system. Asparagine at 289 position of $GABA_A$ protein within TM2 is important for its anesthetic function. This study explores Traditional Chinese Medicine (TCM) against ASN 289 of $GABA_A$ for novel anesthetic compounds. The *in silico* approach showed gastrodin out of all compounds to be the best compound to start further analysis. It is a potential anesthetic compound suitable for the development of new drug.

Introduction

The molecular mechanism of general anesthesia is a classical unsolved problem of neuro pharmacology (Miller, 1985), but the advents in the past three decades have shed some insight into how anesthetic drug might work (Urban, 2008). Since general anesthesia discovery, hundreds of compounds have been identified as anesthetic drugs (Urban et al., 2006), out of those only few have been introduced into clinical practice (Hardman et al., 2001). Their mechanism is an arguable aspect and a plethora of work has been done to understand there working (Hopkins et al., 2002; Campagna et al., 2003; Sonner et al., 2003). These works have pointed out ligand-gated ion channels as potential targets for general anesthetics and ϒ- aminobutyric acid ($GABA_A$) is the most studied among them.

Etomidate, propofol, barbiturates, isoflurane, and sevoflurane are the known compounds that significantly increase the activity of $GABA_A$ receptors at clinically relevant concentrations (Krasowski and Harrison, 1999; Yamakura and Harris, 2000). The intravenous anesthetics etomidate and propofol, as well as pentobarbital, have been shown to exert their immobilizing action and in part their hypnotic action through β3-containing $GABA_A$ receptors anesthetic action (Zellar et al., 2008). The action of the etomidate at type A GABA receptors has been shown to be highly dependent upon a single amino acid (ASN-289) residue within TM2 of the β-subunit (Belelli et al., 1997).

In silico approach can be used to discover new, potent anesthetic drug (Eckenhoff et al., 2008). In this study modern computational tools are used to design and analysis some Traditional Chinese Medicine (TCM) against the proposed anesthetic binding site of $GABA_A$, the results generated are then compared with that of etomidate, to give us an idea about the effectiveness of the proposed drug before going for further *in vitro* and *in vivo* analysis.

Materials and Methods

Protein preparation

Table I

Molecular and drug likeness property of top five traditional Chinese medicine compounds

TCM	Drug likeness model score	MolLogP	MolLogS (mg./L)	TPSA	Number of stereo centre	Rule of five violation
Salicin	-1.4	-1.2	-1.5	80.1	5	0
Artemisinine	-0.6	2.6	-5.1	54.0	6	0
Etomidate	-0.3	2.7	-3.3	35.7	1	0
Gastrodin	-1.2	-1.7	-1.0	97.2	5	0
Huperzine	-0.9	1.5	-3.3	55.1	2	0

Table II

ADMET properties of the Ligand molecules obtained from PreADMET server

TCM	Donor HB	Acceptor HB	Mol. Wt (Da)	Ames Test	Carcinogenicity (Mouse)	Carcinogenicity (Rat)
Salicin	4	6	256.1	Non-mutagenic	Non-carcinogen	Non-carcinogen
Artemisinine	0	5	282.1			
Etomidate	0	3	245.1			
Gastrodin	5	7	286.1			
Huperzine	3	3	242.3			

Permissible ranges are as follows: mol wt.: (>500 Da); donor hb: (0.0–6.0); accept hb: (2.0–20.0)

The $GABA_A$ protein sequence was retrieved from Uniprot (http://www.uniprot.org/). The protein contains 512 amino acids and molecular weight of 59,150 Daltons. The $GABA_A$ 3D structure models were generated by I-TASSER software by Zhang. I-TASSER (Zhang, 2008). The structure validation was done in SAVES server by PROCHECK (Laskowski et al., 1993).

TCM selection

In house TCM database was used for the study, the database constitutes around 1,400 com-pounds of natural origin. The compounds were screened against the active site of $GABA_A$ protein using AutoDock vina (Vina, 2010).

ADME/T and drug likeliness prediction

The top hundred compounds were checked for their pharmacokinetic properties using online server named pre-ADMET. Only compounds strictly following absorption, distribution, metabolism and elimination are selected for further analysis.

Molecular docking analysis

Molecular docking analysis of the $GABA_A$ with the top five TCM compounds was done using AutoDock 4.2 (Morris et al., 2009). The tool uses binding free energy evaluation to find the best binding mode between the compound and the protein, the energy values are calculated by the characterization of intermolecular energy, internal energy of ligand, and torsional free energy. All the visualizations were generated by Pymol (Pymol, 2009).

Result and Discussion

The 3D structure of the protein was modeled using I-TASSER, an online server which uses threading method. The models proposed by the server have good quality and resolution, out of the five generated models, the one with the best C-score shown in Figure 1a was selected for the study. The C-score is a measure to observe the quality of generated models. The best modeled structure of $GABA_A$ was subjected to PROCHECK in SAVES server the result depicting the psi-phi distribution is shown in Figure 1b, the amino acids arranged in most favored regions in plot were 88.8% and some of the amino acids in disallowed regions of 3.2%.

The generated structure was used virtual screening, where the in house database of TCM was screened against ASN289 using AutoDock vina. Top hundred compounds were selected for further drug likeness and ADME/tox analysis. The results generated were used to shorten the number of compounds for further molecular docking study, only five compounds fulfilled the criteria of drug likeness and Lipinski rule of five (Lipinski, 2004). Table I and II shows the results generated for the top five compounds. The five compounds shown in Figure 2 are all Chinese traditional medicine, used for different diseases. Hydrogen bond interaction provide a very important contribution to the binding affinity for ligands. Three of the five selected compounds are showing hydrogen bond interaction with $GABA_A$. Table III shows the results generated by AutoDock 4.2. All the interactions

Table III

Docking Results generated by AutoDock 4.2 and Pymol of top five traditional Chinese medicine compounds

TCM	Binding energy (K.Cal/Mol)	H-Bond interactions	Bond length (A˚)
Salicin	-1.4	(ASN289) HN – O	3.1
Artemisinine	-1.1	NIL	NIL
Etomidate	-2.0	NIL	NIL
Gastrodin	-1.7	(GLU294) O – OH (ARG293) NH – O (ASN289) O – OH (ASN289) O – OH (ASP306) O – OH	1.9 3.2 2.3 2.6 2.0
Huperzine	-5.1	(ASP306) O – NH (ASP306) O – NH	2.0 2.0

are shown in Figure 3, which are generated in Pymol, the distances of the interactions are calculated using Pymol in built measurement tool.

Gastrodin a TCM originating from *Gastrodia elata* Blume, a well known medicinal plant of china, is showing five hydrogen bond interactions with the $GABA_A$ protein and out of those interactions one is with ASN 289. Huperzine, the other compound, showing two hydrogen bond interactions comes from *Huperzia serrata,* a Chinese medicinal plant. Both the interactions formed by huperzine are with ASP 306. Salicin is another TCM compound which is forming single hydrogen bond interaction with ASN 289 of $GABA_A$ protein. The general binding pose between the compounds and $GABA_A$ is depicted in Figure 4.

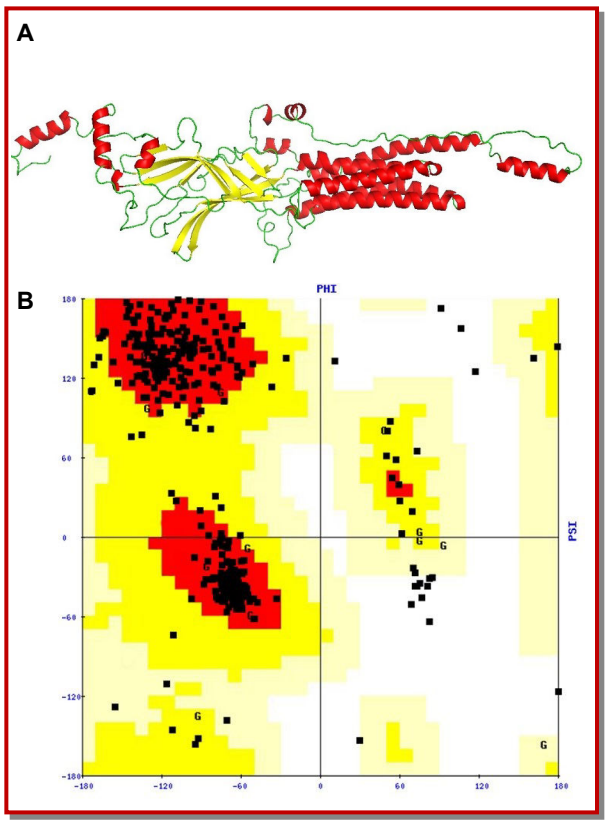

Figure 1: (A) I-TASSER model with the best C-score (B) Ramachandran plot for the modeled protein structure generated by PROCHECK in SAVES

Based on the binding energies, Huperzine is showing the least binding energy of -5.1 kcal/mole. Etomidate is the second best compound based on binding energy of -1.92 Kcal/Mole. The third best TCM based on binding energies is gastrodin, its top interaction with $GABA_A$ is of -1.69 Kcal/mol. The other two compounds salicin and artemisinine are both showing binding energy less than -1.5 Kcal/mol.

Conclusion

Out of fourteen hundred compounds, five were shortlisted based on drug likeness and binding energy. Out of five selected compounds only three compounds showed hydrogen bond interactions with $GABA_A$. Gastrodin out of all compounds is the best compound to start further analysis. Its generated result suggests that it is a potential anesthetic compound suitable for the development of new drug.

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References

Belelli D, Lambert JJ, Peters JA, Wafford K, Whiting PJ. The interaction of the general anesthetic etomidate with the γ-

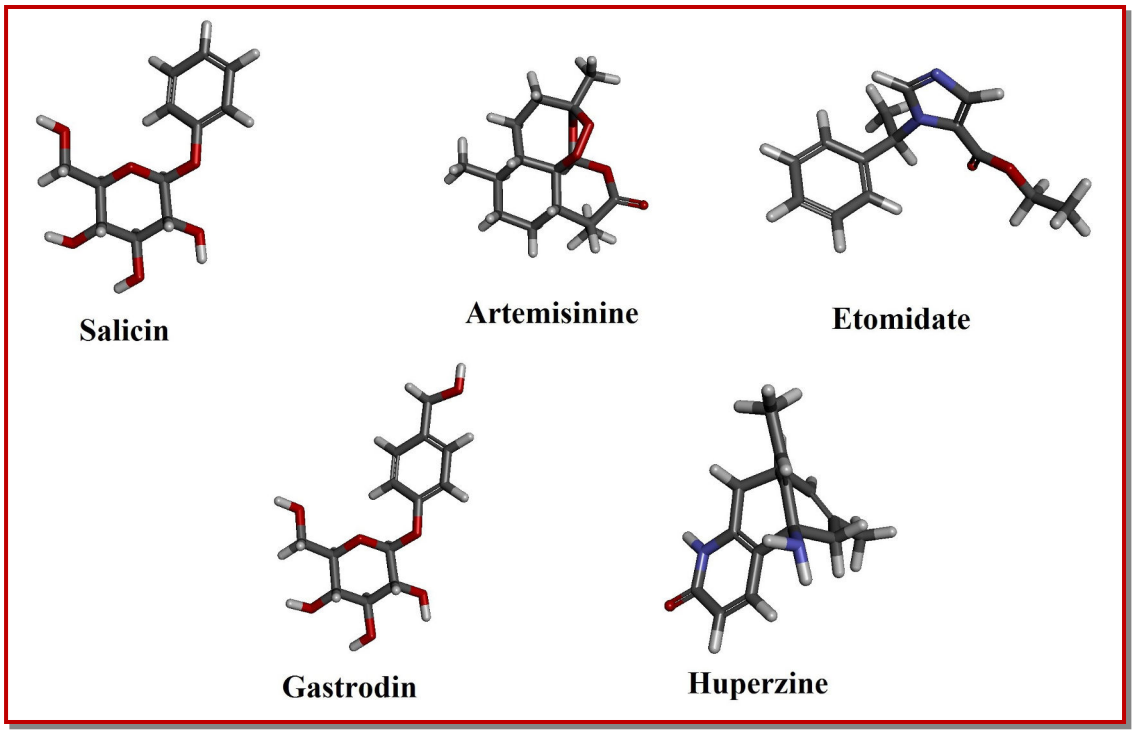

Figure 2: Top five traditional Chinese medicinal compounds selected on their drug likeness

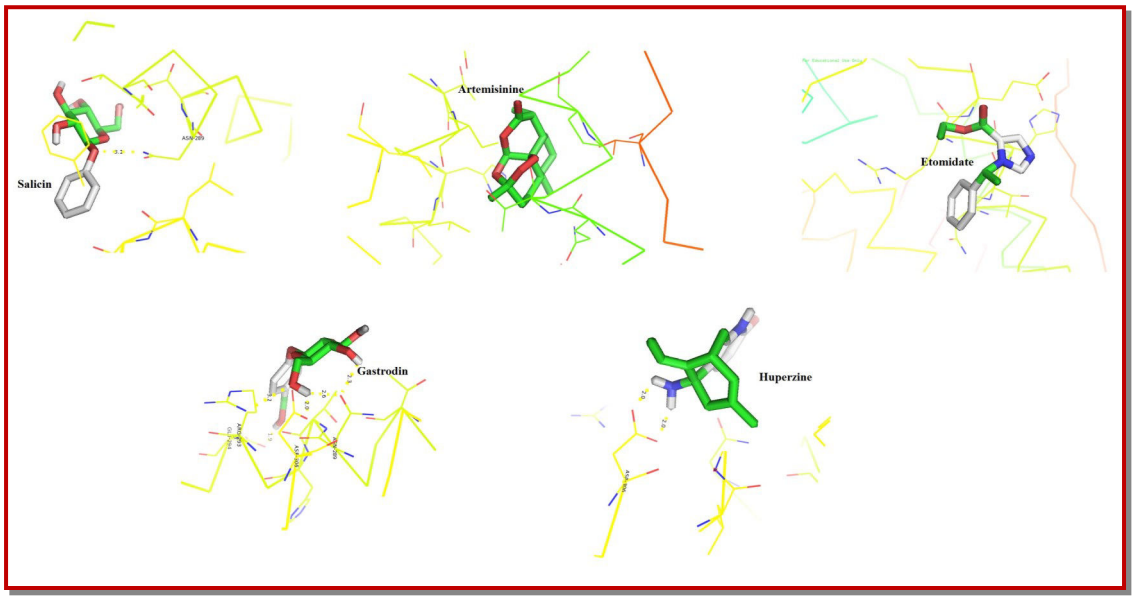

Figure 3: The interactions of Traditional Chinese Medicinal Compounds with $GABA_A$, the hydrogen bond interactions spotted in yellow dotted lines and their bond lengths for each ligand generated in Pymol

aminobutyric acid type A receptor is influenced by a single amino acid. Proc Natl Acad Sci. 1997; 94: 11031-36.

Campagna JA, Keith WM, Stuart A. Mechanisms of actions of inhaled anesthetics. New Engl J Med. 2003; 348: 2110-24.

DeLano WL. Pymol. Schrodinger LLC, Portland, OR, 2009.

Eckenhoff RG, Zheng W, Kelz MB. From anesthetic mechanisms research to drug discovery. Clin Pharmacol Ther. 2008; 84: 144-48.

Hardman JG, Limbird LE, Gilman AG. The pharmacological basis of therapeutics. New York, McGraw-Hill, 2001.

Hopkins PM, Lambert DG, Urban BW. Postgraduate issue: Molecular and basic mechanisms of anaesthesia. Br J Anaesth. 2002; 89: b1-183

Krasowski MD, Koltchine VV, Rick CE, Ye Q, Finn SE, Harrison NL. Propofol and other intravenous anesthetics have sites of action on the gamma-aminobutyric acid type A

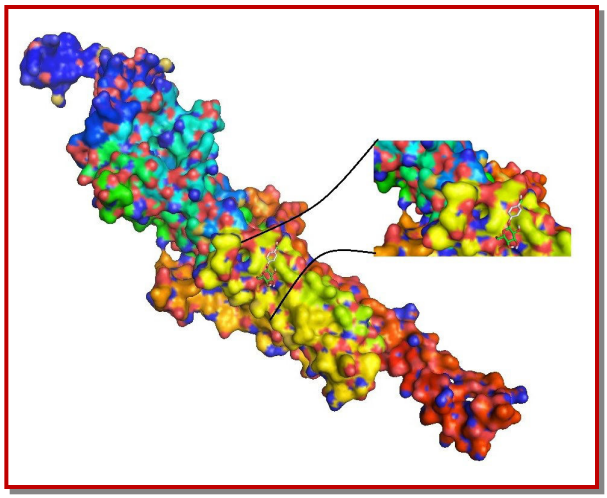

Figure 4: Molecular surface of $GABA_A$ showing the ligand in binding cavity

receptor distinct from that for isoflurane. Mol Pharmacol. 1998; 53: 530–38.

Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK-A program to check the stereochemical quality of protein structures. J App Cryst. 1993; 26: 283-29.

Lipinski CA. Lead-and drug-like compounds: The rule-of-five revolution. Drug Discov Today Technol. 2004; 1: 337-41.

Miller KW. The nature of the site of general anesthesia. Int Rev Neurobiol. 1985; 27: 1-61.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30: 2785-91.

Sonner JM, Antognini JF, Dutton RC, Flood P, Gray AT, Harris RA, Homanics GE, Kendig J, Orser B, Raines DE, Rampil IJ, Trudell J, Vissel B, Eger EI 2nd. Inhaled anesthetics and immobility: Mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth Analg. 2003; 97: 718-40.

Urban BW. The site of anesthetic action. In: Modern anesthetics. Berlin, Heidelberg, Springer, 2008, pp 3-29.

Urban BW, Markus B, Martin B. Interactions of anesthetics with their targets: Non-specific, specific or both? Pharmacol Ther. 2006; 111: 729-70.

Trott O, Olson AJ. Vina A: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31: 455-61.

Yamakura T, Harris RA. Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: Comparison with isoflurane and ethanol. Anesthesiology 2000; 93: 1095-101.

Zeller A, Jurd R, Lambert S, Arras M, Drexler B, Grashoff C, Antkowiak B, Rudolph U. Inhibitory ligand-gated ion channels as substrates for general anesthetic actions. In: Modern anesthetics. Berlin Heidelberg, Springer, 2008, pp 31-51.

Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 9: 40.

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