# Research Article

# GABAA receptor binding molecules from Traditional Chinese Medicine: An *in silico* approach

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## Article Info

## Abstract

The advents of how anesthesia works have helped in the discovery of anesthetic target protein. One such target protein named Ɣ-aminobutyric acid (GABAA), which is the chief inhibitory neurotransmitter in the mammalian central nervous system. Asparagine at 289 position of GABAA protein within TM2 is important for its anesthetic function. This study explores Traditional Chinese Medicine (TCM) against ASN 289 of GABAA 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 Ɣ- aminobutyric acid (GABAA) is the most studied among them.

Etomidate, propofol, barbiturates, isoflurane, and sevoflurane are the known compounds that significantly increase the activity of GABAA 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 GABAA 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 GABAA , 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<br>(ARG293) NH – O<br>(ASN289) O – OH<br>(ASN289) O – OH<br>(ASP306) O – OH | 1.9<br>3.2<br>2.3<br>2.6<br>2.0 |
| Huperzine | -5.1 | (ASP306) O – NH<br>(ASP306) O – NH | 2.0<br>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.

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.
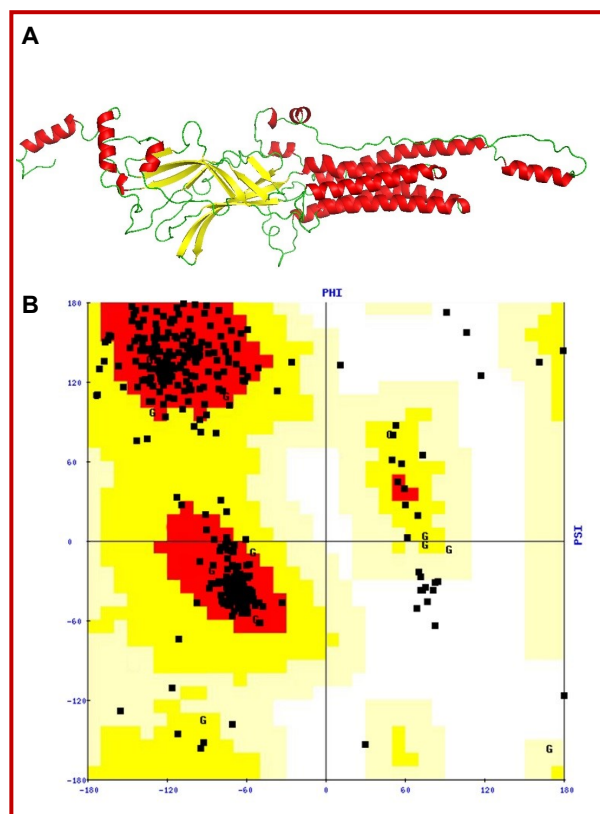


Figure 1: (A) I-TASSER model with the best C-score (B) Ramachandran plot for the modeled protein structure generated by PROCHECK in SAVES

## Conclusion

Out of 1400 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.

## Financial Support

## Conflict of Interest
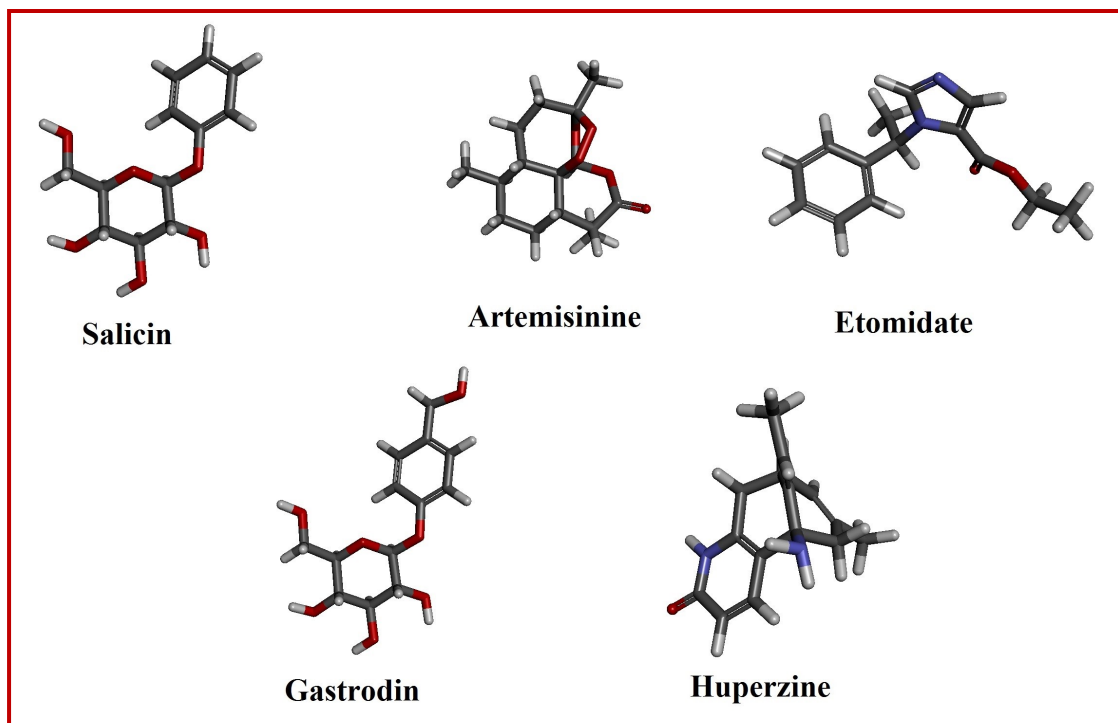
Authors declare no conflict of interest

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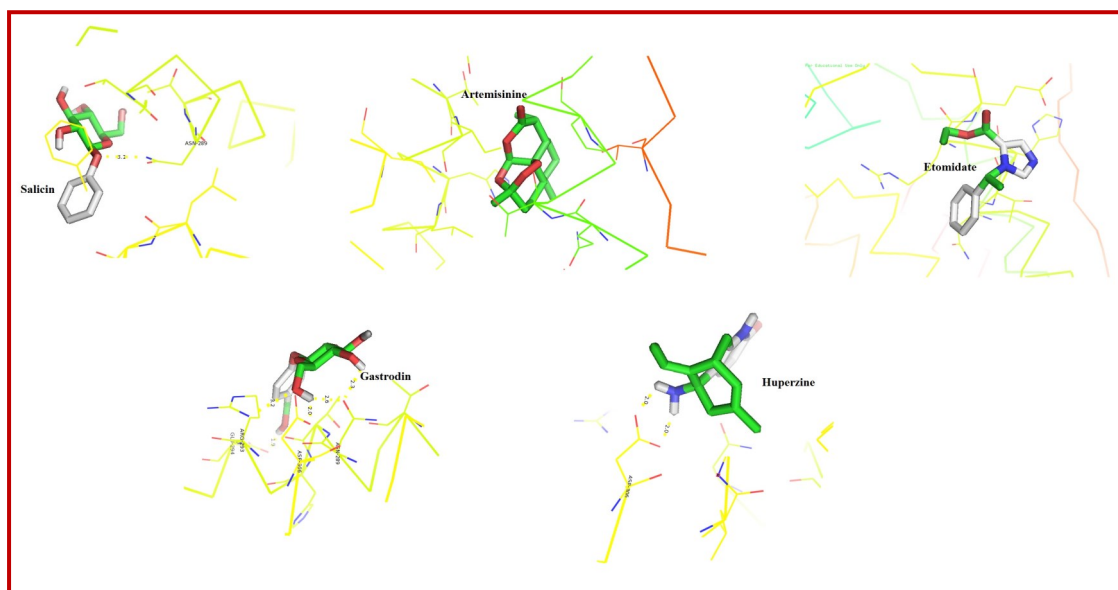


Figure 3: The interactions of Traditional Chinese Medicinal Compounds with GABA$_A$, the hydrogen bond interactions spotted in yellow dotted lines and their bond lengths for each ligand generated in Pymol

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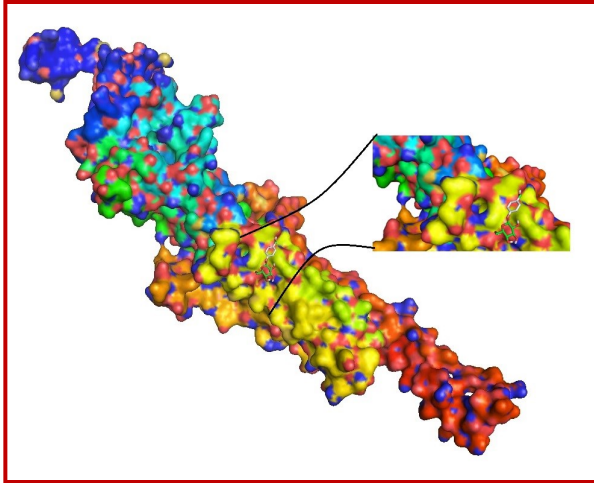
Figure 4: Molecular surface of GABA$_A$ showing the ligand in binding cavity

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