Answers to reviewers

Reviewer #1:

Specific Comments to Authors:

This manuscript describes the molecular modeling studies on Amurensinine as NMDA receptor antagonists. A few concerns for the authors.

1. The chemical structure of Amurensinine and Ifenprodil need to be shown.

The structure of Ifenprodil is shown in Figure 1C.

2. The authors showed that Amurensinine had binding affinity to NMDA receptor, but having binding affinity to NMDA receptor does not necessarily mean that it will antagonize the receptor.

The reviewer is right. The fact that Amurensinine binds to the receptor as well as Ifenprodil (antagonist) does not prove that and it acts as an antagonist as well. Therefore, we only suggest this hypothesis and claim that more studies are needed, as we point out on page 10 in CONCLUSION: "The structural and receptor interaction similarity between Amurensinine and Ifenprodil suggest that this isopavine could behave as a receptor inhibitor; therefore, this compound could present potential biological application, which needs to be evaluated by *in vitro* and *in vivo* assays."

3. The authors claimed that Amurensinine will have similar binding affinity to NMDA receptor as Ifeprodil because of the similar calculated affinity energies from molecular modeling studies, which is not very much convincing.

There is a misunderstanding in the reviewer's interpretation. On page 7 we claim the opposite: "Upon coupling of Ifenprodil to ATD, an energy affinity = -8.2 Kcal/mol was obtained, a value very close to that obtained for protonated Amurensinine/GluN1A/GluN2B NMDA receptor complex; even the same number of bonds was observed (six bonds) (2.6 Å - 4.9 Å; average = 3.67) as shown in Table 3; however, the geometry of the bonds is different (Figure 2). This result is consistent, since the structures of Amurensinine and Ifenprodil are similar, but not identical (Figure 3)." The affinity energies obtained to Amuerensinine and Ifenprodil are similar (not identical), one of the likely explanations is because the structures of the two compounds are similar but not identical. We do not claim at any point that the affinity energies are similar because the structures are similar, because this claim is not plausible.

4. Figure 2, the amino acid residues that making interactions with the ligands need to be shown. Otherwise, it is difficult for the readers to see the difference between the binding modes of different ligands.

We tried to indicate the name of the residues in the figure, however the figure became too polluted; the names of the residues overlap and make it difficult to see. The tables 2 and 3 indicates the residue that interacts with each atom of the drug in order to be able to identify it.

Reviewer #2:

Specific Comments to Authors:

1. Are there controversies in this field? What are the most recent and important achievements in the field? In my opinion, answers to these questions should be emphasized. Perhaps, in some cases, novelty of the recent achievements should be highlighted by indicating the year of publication in the text of the manuscript.

The aim of the study was to analyze the potential interaction of an isopavine - Amurensinine, with the NMDA receptor due to its involvement in the onset of neurological diseases. Isopavines are alkaloids with biological activity for the treatment of neurological disorders (DOI 10.1021/ja0651815). In the literature, the number of studies on isopavines is scarce, so our study contributes to increase the source of information on this subject. Regarding the indication of date (year of publication) in the main text, these were not placed in order to respect the rules of the journal.

2. The results and discussion section is very weak and no emphasis is given on the discussion of the results like why certain effects are coming in to existence and what could be the possible reason behind them?

Regarding the question: "why certain effects are coming in to existence and what could be the possible reason behind them", we did not emphasize this issue because it is not the focus of the study and also because bioinformatics data do not assess such elaborate physiological issues. This kind of approach should be done only after in vitro or in vivo studies; the *in silico* study is preliminary. This type of study helps direct *in vitro* and *in vivo* studies to avoid wasted time and financial burden. Despite being important and presenting reliable data, molecular docking studies cannot point to behavior in a biological environment;

molecular dynamics is able to make predictions in this area, but we did not use it because it was not our goal.

3. Conclusion: not properly written.

The authors would like to state or suggest more hypotheses about the results, however, although reliable in the computational field, in the biological field it is not possible to make more conjectures, otherwise we would be being frivolous. The conclusion is drawn only from what can be concluded from bioinformatics data.

4. Results and conclusion: The section devoted to the explanation of the results suffers from the same problems revealed so far. Your storyline in the results section (and conclusion) is hard to follow. Moreover, the conclusions reached are really far from what one can infer from the empirical results.

Answer 4 can be used to clarify the reviewer, since the explanation would be basically the same; and about the storyline, it is indeed a bit difficult to follow the reasoning when the methodologies are not of the reader's expertise. However, we point out that this is the way the data is exposed in this area, so we kept the standard. In fact, we have gone into a little more detail to make it easier to understand.

5. The discussion should be rather organized around arguments avoiding simply describing details without providing much meaning. A real discussion should also link the findings of the study to theory and/or literature.

The reviewer is right: "A real discussion should also link the findings of the study to theory and/or literature." However, the literature on the subject is very scarce, we have no comparative parameters, and we worked with what we had. Unfortunately, we had difficulty discussing the data because there are no recent studies on isopavines in the scope of the paper, and studies on Amurensinine are even scarcer.