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MOLECULAR DOCKING STUDIES OF L-NAME WITH THE NEURONAL NITRIC OXIDE SYNTHASE

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Abstract: Nitric oxide synthase inhibitors have been regarded as beneficial for psychiatric disorders like depression. Many studies indicated that nitric oxide synthase inhibitors have antidepressant-like activity in various animal models. With the objective to design new chemical entities with enhanced inhibitory potencies against nitric oxide synthase enzyme. This study was designed to explore binding affinity and antidepressant-like activity of nitric oxide synthase inhibitor L-NAME with the nitric oxide synthase using Glide docking program and hydrogen-bonding interactions were observed between the inhibitors and the target. L-NAME docked with other nitric oxide synthase inhibitors like L-Nitro Arginine, 7-Nitro Indazole and Methylene blue on the target molecule. The energy minimized final average complex structure suggests that the Glide Extra Precision (XP) derived docked complexes are in a state of near equilibrium. The structure-based drug design strategy described in this study will be highly useful for the development of new inhibitors with high potency and selectivity. From the docking result we can conclude that L-NAME may have good binding affinity toward the nitric oxide synthase enzyme. Also L-NAME having nearly similar results with the L-Nitro Arginine which shows antidepressant like activity so from this result we can conclude that L-NAME may have antidepressant activity like L-Nitro Arginine.

***Keywords*:** L-NAME, Molecular docking, Nitric oxide synthase, L-NA.

Abbreviation: N (G)-nitro-L-arginine methyl ester (L-NAME), L-Nitro arginine (L-NA), 7-Nitroindazole (7-NI), Neuronal nitric oxide synthase (nNOS).

1. Introduction

In conjunction with our first efforts to find potent and selective nitric oxide synthase inhibitors, we first carried out a structure-based molecular modeling study on the recently deposited X-ray structure of nitric oxide synthase in complex with NOS inhibitors. To the best of our knowledge, this is the first report on the prediction of binding modes of recently published nitric oxide synthase inhibitors.

In the second efforts, identification of novel receptor nitric oxide synthase inhibitors continues to be an intense area of investigation in antidepressant research

due the fact that over expression of the nitric oxide which involved in depression. Nitric oxide, a messenger molecule in the brain, synthesized from L-arginine by nitric oxide synthase (NOS), and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidences have shown that the reduction of nitric oxide levels within the hippocampus can induce antidepressant like effects, thus implicating endogenous hippocampal nitric oxide in the neurobiology of stress and depression (Joca and Guimaraes, 2006). Recently, we disclosed the identification of a novel class of nitric oxide inhibitors, a structure-guided us to the identification of this series of molecules. While the interaction of these inhibitors with the nitric oxide synthase protein at the molecular level is not fully understood. The nitric oxide synthase inhibitors have been reported to possess antidepressant-like behavioural properties at doses that are without any effect on locomotor activity (Wegener et al., 2003).

The neuronal isoform of nitric oxide synthase (nNOS), the enzyme responsible for the production of nitric oxide in the central nervous system, represents an attractive target for the treatment of various neurodegenerative disorders (Tylor B.S et al., 1997). X-ray crystal structures of nNOS, led to the discovery of a conserved structural water molecule that was hydrogen bonded between the two heme propionates. Rationalizations for the small increase in potency are consistent with other changes in the crystal structures (Jiwon Seo et al.,2007).

N^G-nitro-L-arginine methyl ester (L-NAME) is a non –specific nitric oxide synthase inhibitor (Cindy M. Pudiak., 1997). Previous studies with L-NAME show that it has antidepressant like action. But very little information is available on it.

2. Materials and methods

2.1. Biological data

The Nitric oxide synthase inhibitory activity of L-NAME which is methyl ester derivative of l-NMMA, l-NNA (Cindy M. Pudiak., 1997).The structures of these inhibitors and enzyme are shown in figure 1 and 2 respectively.

2.2. Ligand structure preparation (Glide, 2007)

All the compounds were constructed using the fragment dictionary of Schrödingers graphical user interface Maestro 8.5 provided LigPrep process to perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures. Many of the steps are optional, and are controlled by selecting options in the LigPrep panel or specifying command-line options (Schrodinger, LLC).

2.3. Protein structure preparation (Glide, 2007)

A typical PDB protein complex structure, as downloaded from the Research Collaborator for Structural Bioinformatics (RCSB) web site (http://www.rcsb.org). The PDB complex structure was corrected by the prime software. Schrödinger offers a Protein Preparation facility for use with Glide that is designed to ensure chemical correctness and to optimize protein structures (Richard et al., 2004). This facility is described in full in the Protein Preparation Guide. In most cases, the full preparation of a protein can be done with the Protein Preparation Wizard in Glide. (Schrodinger, LLC)

2.4. Receptor Grid Generation (Glide, 2007)

In the Receptor Grid Generation the receptor structure was defined by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids. Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a “prepared” structure: an all atom structure with appropriate bond orders and formal charges (Schrodinger, LLC).

2.5. Molecular docking (Glide, 2007)

In molecular docking, we attempt to predict the structure of the intermolecular complex formed between two or more molecules. Docking is widely used to suggest the binding modes of protein inhibitors (Andrew., 2001). For the docking of ligands to protein active sites and for estimating the binding affinities of docked compounds, an advanced molecular docking program Glide, version 4.5 was used in this study. Although details on the methodology used by Glide are described elsewhere, (I. Bytheway and S. Cochran, 2004., H. Chen et al., 2006., R.A. Friesner, et al., 2004) a short description is provided below. During the docking process, initially Glide performs a complete systematic search of the conformational, orientational, and positional space of the docked ligand and eliminating unwanted conformations using scoring and followed by energy optimization. Predicting the binding affinity and rank-ordering ligands in database screens was implemented by modified and expanded version of the ChemScore18 scoring function, Glide Score, for use in. For our studies, X-ray crystal structure of neuronal nitric oxide synthase was taken from PDB entry 2FLQ, having resolution of 2.0 A°. Solvent molecules were deleted and bond order for crystal ligand and protein were adjusted and minimized up to 0.30 A° RMSD. Using standard precision (SP) mode of Glide software, docking studies was performed on nitric oxide synthase inhibitors. The performance of the docking method on nitric oxide synthase inhibitors was evaluated by: re-

docking crystal ligand, correlating the docking scores with activity of the L-NAME as inhibitors of nitric oxide synthase (Schrodinger, LLC).

3. Result and Discussion

To investigate the detailed intermolecular interactions between the ligand and the target protein, an automated docking program Glide 4.5 was used. Three-dimensional structure information on the target protein was taken from the PDB entry 2FLQ; having resolution of 2.0 A°. Processing of the protein included the deletion of the ligand and the solvent molecules as well as the addition of hydrogen atoms. Standard precision (SP) mode of Glide 4.5 software was used for the docking studies. The nitric oxide synthase inhibitors were docked into the active site of 2FLQ. A correlation was calculated by Glide score.

Validation of the docking protocol

The most straightforward method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation) predicted by the object scoring function (Andreas., 2001). In the present study, Extra Precision Glide docking procedure was validated by removing compound from the binding site and redocking it to the binding site of nitric oxide synthase. We found a very good agreement between the localization of the inhibitor upon docking and from the crystal structure. Glide docking is reproducing the experimentally observed binding mode for Nitric oxide synthase inhibitor and the parameter set for the Glide docking is reasonable to reproduce the X-ray structure.

For the prediction of results mainly four parameters are considered, which G-score, Glide energy, H-bonds and Good van-der-walls interactions. On the basis of these parameters the binding affinity of ligand towards receptor are discussed.

The more negative value of G-score indicates good binding affinity of the ligand with receptor. The minimum energy for the formation of complex between ligand and receptor indicates good binding affinity. More H-bonds in the structure shows ligand having good binding mode to receptor. Good vdw interaction means ligand structure having large numbers of bulky group due which van-der-waals interactions are formed. H-bond interaction also relates to antagonist and agonist action of ligand with receptor.

L-NAME a nitric oxide synthase inhibitor compare with other nitric oxide synthase inhibitors like L-NA, 7-NI, methylene blue for the prediction of binding affinity interactions between L-NAME and possible pharmacological activity by molecular docking study. All nitric oxide synthase inhibitors are docked with Nitric oxide synthase enzyme.

The docking results are shown in the table 1. The G-score, H-Bond, and Glide vdw shows binding affinity of ligand (NOS inhibitor) towards protein (NOS) .The G-score of the L-NAME is -03.02 which is nearly similar to the G-score of L-NA is -03.34. G-score of Methylene blue and 7-NI is -05.93 and -06.12 simultaneously. The minimum energy required for the more binding affinity of ligand and receptor complex. The G-energy for L-NAME is -38.8. L-NAME required minimum energy for the formation of L-NAME – NOS complex which indicates L-NAME have good binding affinity as compare to other NOS inhibitors. If H-bond is more, the binding affinity of the ligand is higher. H-bond in L-NAME and NOS docked molecule is 5 and in L-NA docked molecule is 7. Compare to the other NOS inhibitors, L-NAME and L-NA have more H-bond means L-NAME have more binding affinity towards NOS enzyme. The good vdw interactions indicates more bulky group present in the structure of ligand. L-NAME have more Good vdw interactions 192, as compare with other NOS inhibitors. Result showing L-NAME have bulky group in its structure.

From the H-bonding (Fig 3) and Glidescore energy we can say that L-NAME having good binding affinity with nitric oxide synthase enzyme. Also the docking result of L-NAME are nearly similar with L-NA which have antidepressant-like activity which prove in many literatures, so we can say may L-NAME have antidepressant-like activity.

4. Conclusions

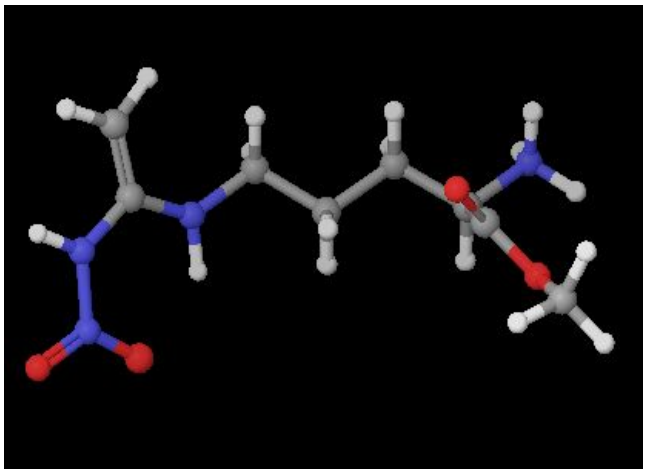
We have developed a docking model for L-NAME a nitric oxide synthase inhibitor. To the best of our knowledge, this is the first report on molecular docking of L-NAME with nitric oxide synthase. Glide 4.5, an automated docking program, successfully reproduced the binding mode of crystal structures of nitric oxide synthase inhibiters. The docking simulations suggested modifications to series of NOS inhibitors that may improve their inhibitory activity and selectivity, thus representing a valuable tool for the structure-based design of future NOS inhibiters. The predicted hydrogen bonds between the NOS and the inhibitors were restored in the energy minimized average structure of the complex.

From the results and discussion we conclude that, L-NAME having Good binding affinity at the binding site of Nitric oxide synthase enzyme by energetically with the minimum energy and Stable complex structure with NOS enzyme structurally with good binding mode.

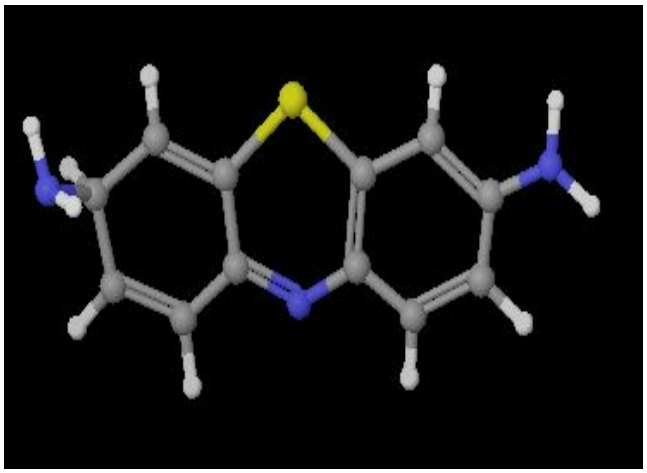
The antidepressant-like effect of L-NAME has positive correlation with docking result of 7-NA. This is open possibility to use and develop L-NAME as antidepressant.

Table 1 Docking results of Nitric oxide synthase inhibitors with Nitric oxide synthase

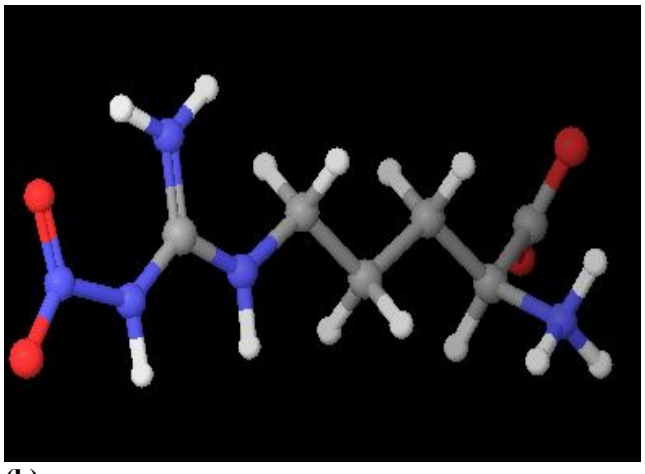
Sr. no	Drug	G-Score	Energy	Good vdw	H-bond
1	L-NAME	-03.02	-38.8	192	5
2	7-NA	-03.34	-34.1	133	7
3	Methylene Blue	-05.93	-34.4	138	2
4	L-NI	-6.12	-27.0	171	1



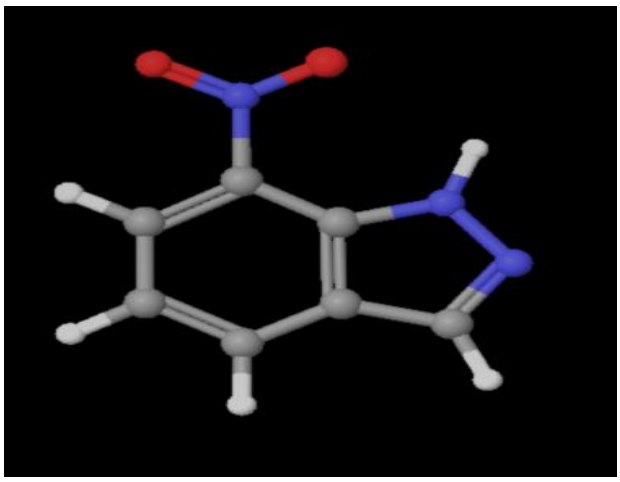
(a)



(c)



(b)



(d)

Figure 1 3D structure of Nitric oxide synthase inhibiters (a) L-NAME (b) L-NA (c) Methylene blue (d) 7-NI

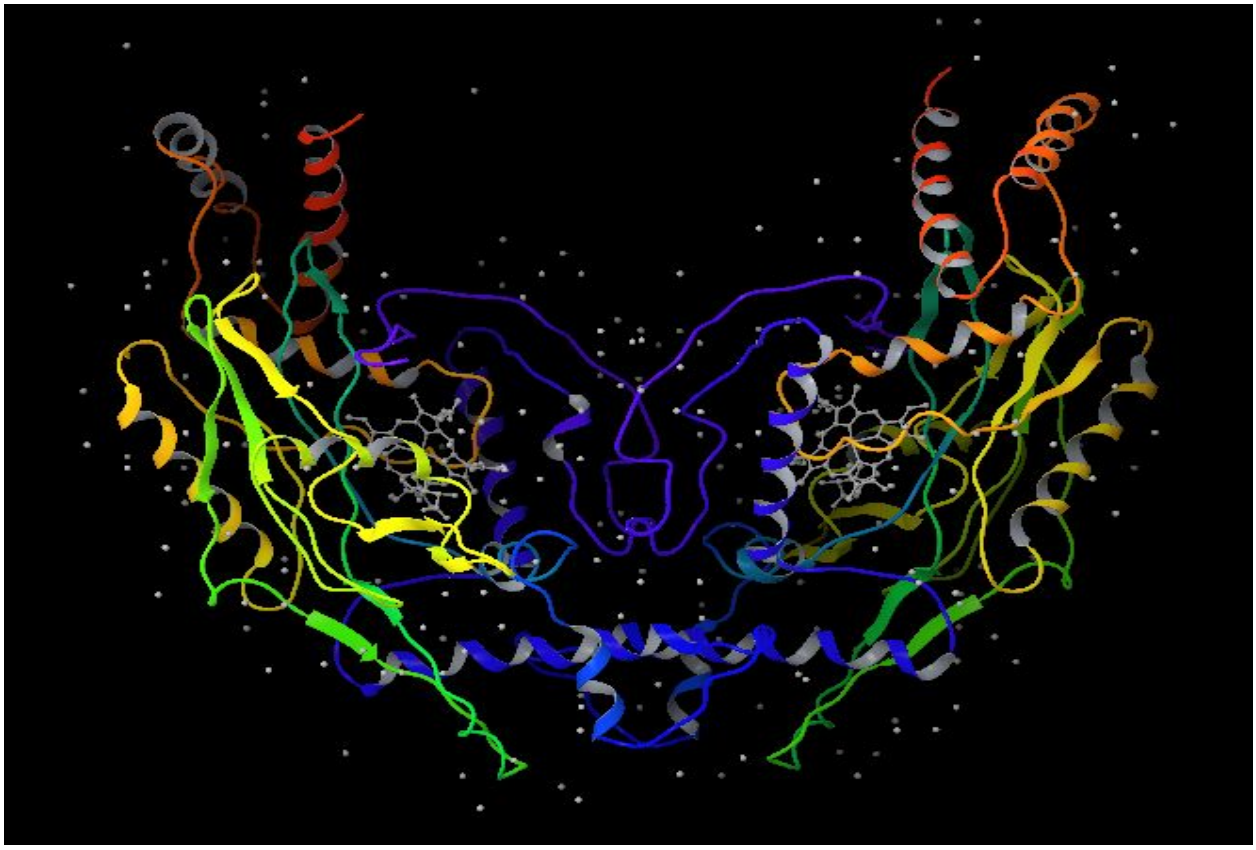
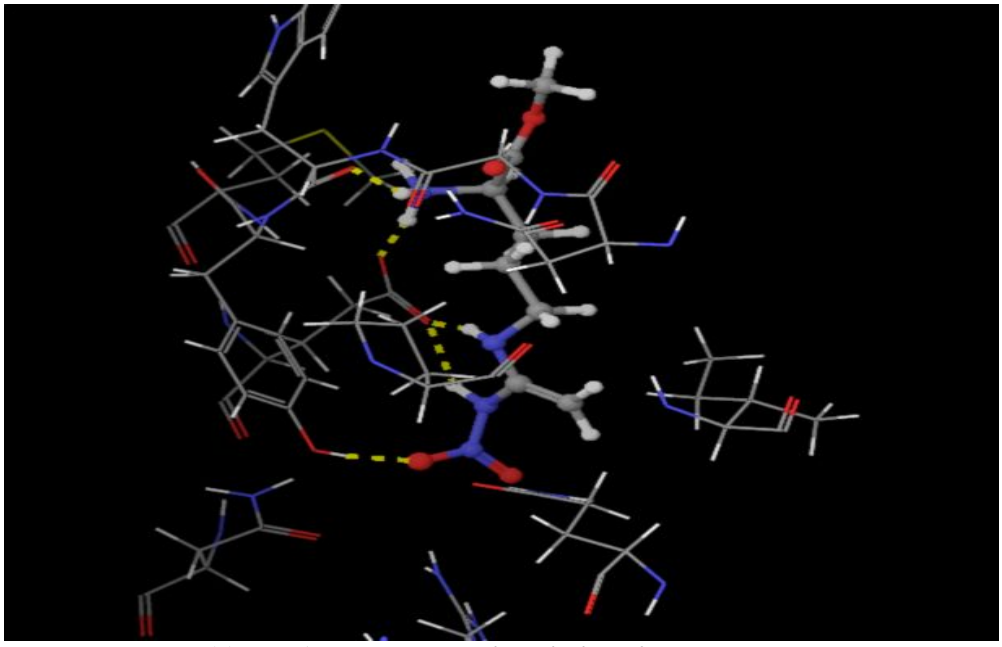
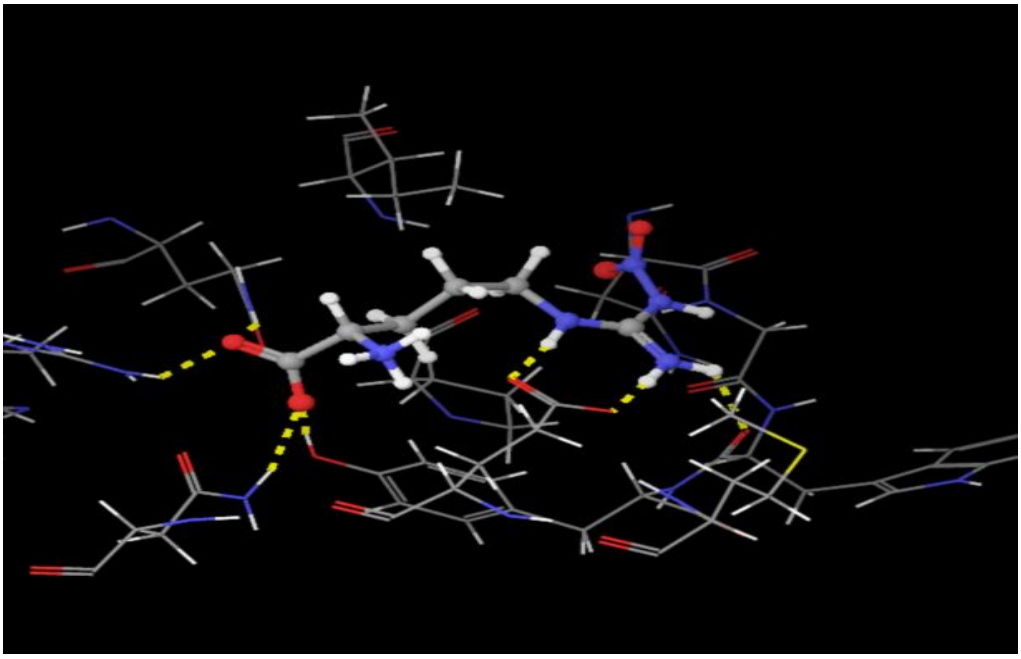


Figure 2 Crystal structure of nitric oxide synthase enzyme



(a) L-NAME docked with nitric oxide synthase.



(b) L-NA docked with Nitric oxide synthase

Figure 3 XPGlide-predicted pose for representative NOS inhibiters with the active site of nitric oxide synthase. For clarity, only the polar hydrogens are shown. Hydrogen bonds are shown as dotted yellow lines.

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