Available online on 25.12.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

# MOLECULAR MODELING STUDIES OF *N, N* -DISUBSTITUTED DERIVATIVES AS POTENT UREASE INHIBITORS

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## ABSTRACT

The discovery of *Helicobacter pylori* in the human stomach by Professor W. Jaworski has encouraged many scientists to study these bacteria, their characteristics, and their effect on human health. In 1982, Marshall and Warren showed experimentally that *H. pylori* infection is responsible for human gastritis. The pathogenic urease found in *H. pylori* causes infection not only in the stomach, but also in the duodenum, uterus, and urinary tract, among others. To determine the efficacy and potency of newly synthesized compounds in treating *H. pylori* infection, docking studies were performed with the 1E9y protein. During these studies, the Arg338, Ala365, Asn168, and Hie221 amino acids were observed to actively participate in bond formation with the studied compounds. In this series of compounds, only the R2 –substituted group is responsible for the observed biological activity. In this study, the results have been revaluated with MD simulations, in which the potency of the compounds was assessed by the percent contribution of hydrogen bonds at the end of the simulation. To calculate the free energy, the present study employs MMGBSA and finds the deviation of the RMSD value.

**Cite this article as:** Gupta S, Bajaj A, Molecular modeling studies of N, N -disubstituted derivatives as potent urease inhibitors, Journal of Drug Delivery and Therapeutics. 2017; 7(7):158-160

## INTRODUCTION:

*Helicobacter pylori* bacteria are gram-negative, spiral/helix-shaped, and[1] micro-aerophilic [2]. *H. pylori*, both genotypically and phenotypically, are highly heterogeneous bacteria that can survive in highly adverse environments [3]. In 1980, Marshall and Warren[4] discovered that *H. pylori* infection is the primary cause of gastric cancer, a discovery for which both scientists were awarded the Nobel Prize in Physiology or Medicine. The author, motivated by this discovery, has become interested in studying the carcinogenic properties of *H. pylori*. In addition to gastric cancer, *H. pylori* have been found to cause gastritis, peptic ulcers, and duodenal ulcers.

N, N'-disubstituted thiourea, a basic and important derivative of thiourea, can be found in either white crystalline leaflets or grey powder. Due to the remarkable number of its potential derivatives, N, N'-disubstituted thiourea has become pharmacologically and industrially significant and continues to be an essential compound for future drug development.

To determine the pharmacological action of these compounds, each must go through the drug design process, of which molecular modelling is an essential step. Though it is the least time-consuming approach to molecular modelling, docking studies, falling within the scope of computational quantum chemistry, provide sufficient explanatory power for study needs and generate results (molecular visualization and graphics) that are easily interpreted. For the newly synthesized series, docking had to be done initially to determine the protein-ligand binding pose, the orientation of the ligand on the protein binding site, the bonds that form between the protein and the ligand, the contributions to bond formation made by hydrophobic and polar regions of the active site. After for checking the results for accuracy, MD-simulations (advanced docking) were performed to determine the stability and consistency of each bond via its duration as determined from the snapshots. Although MD simulations are time-consuming and costly, their use remains widely accepted and suitable. As long as computer power and advanced quantum algorithms continue to improve, the future of computer-aided drug modelling seems to be very promising, though study should overcome the limitations of the simulations themselves. In particular, simulations are useful for finding the RMSD, RMSF, and Gyration Radii. Following the simulation, MMGBSA, an attractive approach used to revaluate the results obtained by virtual screening or docking, is used to calculate the binding free energy between the protein and the ligand.

## MATERIAL AND METHODS:

Structures of compound of Reported series (Table 1) was draw using ChemSketch software, thereafter all structure were optimized. All proteins were downloaded from PDB database and processed with Schrodinger software. Docking was carried by using Extra Precision Glide module and then MMGBSA studies were performed. MD simulation was performed with Desmond software utility.

## RESULTS AND DISCUSSION:

**Active site:** Within each protein, there is a specific active space, called the binding site, which is responsible for attaching and fitting incoming ligands. Cytosolic urease, a $Ni^{2+}$-containing enzyme, is found in *H. pylori*. Structurally, each protein consists of a regular, precise, and systematic pattern of amino acids, forming by two chains, A and B, in which the α and β sub-units are present. In α sub-unit, there exists a catalytic cavity in which the two ligands Ni and HAE are found attached with a coordinated bond. The structural arrangement of ligands, both their geometry and orientation, makes the site suitable to bind incoming ligands.

**Docking Studies:** To determine the potency of the selected compounds and find the combinations that are most effective as urease inhibitors, three different crystalline proteins, 1E9Y, 4UBP, and 4AC7, were imported from PDB into the Schrödinger glide module. Once the proteins have been imported, the XP glide docking process is applied. Compounds are selected randomly, such that some have an experimental IC50 close to that of standard Thiourea while others have an experimental IC50 that differs significantly from the standard. Compounds 2, 24, 30, and 37 are selected for docking, which was performed with each of the three proteins mentioned above (Table 2). The docking score for each compound-protein pair was recorded and analysed. The first best dock score was determined by docking the compounds with 1E9Y. To ensure the accuracy of the results, the RMSD for each pair of compounds and proteins was calculated by superimposing the initial 3D structure of the ligand and the docked pose. The average RMSD was then calculated, from which the minimum deviation was found to be when compounds were docked with 1E9Y. Thus, the 1E9Y protein was selected for further detailed study, from which the docking results for the reported series were found.

In Table 3, the XP glide score, docking score, interacting amino acids, and hydrogen bonds are shown. The three compounds with the best dock score (when docked with 1E9Y) and the best experimental IC50 have been selected for detailed analysis. Compound 24 presents the best glide score due to its impressive affinity, perfect bonding, and favourable orientation. The pyridine ring in compound 24 enters the active site cavity, in which ring's nitrogen atom forms a bond with the polar portion of the cavity. Nitrogen, [N···$HN^+$], makes a side chain H-bond with the Hie221 amino acid. Similarly, the ring makes [Pi- cation ···$H^+$-$N^-$] and [Pi-Pi stackingC···N-H] bonds with the Arg338 amino acid. Additionally, the NH group of thiourea forms a backbone hydrogen bond [NH···O=$C^-$] with Ala338, keeping the compound inside the cavity. The compound's phenyl ring was found to be oriented towards the mouth of the cavity, but no bond was formed.

In the case of compound 2, which has the second best dock score, the phenyl ring, representing the $R^2$-substituted group, enters the cavity. For this compound, only the phenyl ring participates in the formation of [Pi-cation···$H^+$-$N^-$] and [Pi-Pi stacking···C-N-H] bonds with Arg338. The ring could not fit into the cavity due to the inability of its chlorine group to form a bond. The NH group of thiourea also forms a backbone hydrogen bond [NH···O=C-] with Ala365.

Compound 6, which has the next best dock score, enters the cavity primarily vertically, though tilted slightly towards the inner side. The compound's meth-oxy-phenyl ring reaches deep within the cavity, in which the oxy- group makes a side chain hydrogen bond [O---N=C-] with Arg338 amino acids. The phenyl ring forms a [Pi cation···$H^+$-$N^-$] bond with Hie322, and the NH group of thiourea forms a backbone hydrogen bond [NH···O=$C^-$] with Arg168. The compound's second chloro-phenyl ring is oriented away from the cavity mouth, preventing it from participating in bond formation.

For compound 30 (Figure 1), which was found to have the best IC50, the complete pyridine ring reaches deep within the cavity and forms [Pi cation···$H^+$-$N^-$] and [Pi-Pi stacking···C-N-H] bonds with Arg338. As before, the NH group of thiourea also forms a backbone hydrogen bond [NH···O=C-] with Ala365. All bonds conveniently keep the compound in the cavity.

**Simulation Studies:** Over the past decade, computational methods have proven to be instrumental to the field of biochemical and drug modelling, allowing scientists to investigate protein-ligand companionship at the atomic level. In this sense, computational simulations have acted as a bridge between the microscopic world observed in the laboratory and the microscopic length and time scales provided computationally. Fundamentally, MD simulations are merely statistical mechanics, showings all protein-ligand contacts and their stability through an impressive pattern found on each snapshot.

Four compounds, 30, 24, 6, and 2, were selected for detailed study according to factors such as biological activity, docking score, and free energy. Figure 3 shows that the ligand-ligand RMSD for each compound is 0.96, 1.61, 0.90 and 0.82, respectively, indicating that no more changes in orientation were observed after binding.

For compound 30 (Figure 2), the three bonds were formed primarily through the water bridge, in which only the nitrogen atom of the pyridine ring was seen to participate in bond formation. 80% of the bonds with His138 are preserved, 32% of the bonds with Asn168 are preserved, and 40% of the bonds with Ser363 are preserved. Although Ala365 and Arg338 are also seen to be participating in bond formation at the beginning of the docking simulation, these bonds had disappeared by the last snapshot.

Similarly, only one water bridge formed between compound 24 and Thr251, of which 35% was preserved.

The nitrogen atom in the pyridine ring participated in bond formation. For compound 6, one hydrogen bond formed with Asn168 in the starting snapshot but disappeared before the final snapshot. In compound 2, at the beginning of the simulation, compound 2 formed two hydrogen bonds with Arg338 and Ala365. Later in the simulation, approximately half (57%) of the bonds with Ala169 were preserved.

In short, compound 30 was found to be the most biologically active and shows stable bond interactions throughout the simulation. To this end, the analysis presented in this study supports the experimental results. Compound 24 did not show biological activity but displayed the best result during docking. Upon re-examination via simulation, bonds were preserved up to 35%. This result shows that the bioactivity of compound 24 depends only on the behavior of the hydrogen bond. If the R group is replaced by an activating or electron-donating group, the potency may be improved. Similarly, compound 6, which displays the minimum free binding energy in MMGBSA, did not preserve its potency for the duration of the simulation. This result is comparable to the manner in which compound 2 displayed the second-best dock score, but did not preserve efficacy through the end of the simulation.

**Free Energy Studies:** MMGBSA was carried out as a post-scoring approach for the reported series. The various energies derived in the course of this study are listed in Table 4 to this end; the present study provides a valuable correlation between the experimentally determined activity of a compound and the activity predicted via docking.

The calculated free binding energies of the N, N'-disubstituted thiourea compounds range from -4.7 to -30.83. According to the energies obtained from this analysis, the primary contributions to bond formation are made by the Van der Waals and non-polar solvation energies ($\Delta G_{solSA}$). Conversely, the polar solvation ($\Delta G_{solGB}$) and Coulomb energies oppose bond formation. The data show that compound 30 presents an MMGBSA term of -30.83k/Cal in addition to its excellent experimentally-obtained activity. Thus, there is a high correlation between the values obtained via different approaches. The polar solvation energy term, which strongly opposes bond formation, should positive per the table. As such, the value obtained for compound 30 was 65.42 k/Cal; the overall range is from 21.63 to 65.42 k/cal.

As mentioned above, the Coulomb energy term also strongly opposes bond-formation. In this study, however, compound 24 was found to have a coulomb energy of -2.54 k/Cal despite its excellent docking scores. Contrarily, the experimental data show that this compound was not biologically active.

The other contributory energies, Van der Waals and bind lipo or $\Delta_{solSA}$, were found to have energies that range from -27.7 k/Cal to -42.23 k/Cal, and -6.73 k/Cal to-14.90 k/Cal respectively.

## CONCLUSION:

In this manuscript, the ligand-protein docking pose, their geometrical orientation, constituent bonding interactions, and binding free energy were discussed. Each method was performed separately, step by step, using different modules of Schrödinger and subsequently tested. MD simulations were used to determine many of the details that were seen in our initial studies, allowing for the examination of processes that experimental methods have been unable to study. In the field of drug modeling, further research regarding the development of advanced forms of quantum-virtual methods may help in the search for and discovery of novel, potent, and efficacious pharmaceutical agents.

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