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Structure Based Drug Designing and Molecular Docking Studies of Monosubstituted 1-Cyano-2-methyl-3-({[2-(5-methyl-1*H*-imidazol-4-yl)ethyl] sulfanyl}methyl)guanidine (Cimetidine) with Cytochrome P450 (CYP450) Enzyme

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Abstract

1-cyano-2-methyl-3-({[2-(5-methyl-1*H*-imidazol-4-yl)ethyl]sulfanyl}methyl)guanidine (cimetidine) is a drug that helps in the reduction of stomach acidity, pepsin output reduction and promotion of ulcer healing. We carried out molecular docking for six analogous structurally diverse 1-cyano-2-methyl-3-({[2-(5-methyl-1*H*-imidazol-4-yl)ethyl]sulfanyl}methyl)guanidine (cimetidine) with cytochrome P450 1A2 using Patchdock and Firedock softwares. Extensive structure activity relationship studies was carried out with the substituted derivatives and compared with the non-substituted. These molecules were designed by substituting different chemical groups on position 17 of cimetidine. The scoring function (empirical binding free energy) was used to estimate the free binding energy of the protein-ligand complex. The binding energy of cimetidine was -30.96 kcal/mol. The free binding energies of COOH, $COCH_3$, NO_2, CF_3 and $CONH_2$ analogues were -33.12, -31.67, -34.67, -34.73 and -36.58 Kcal/mol respectively. All the monosubstituted analogues showed lower values than the non substituted cimetidine. These lower values indicate that they inhibit CYP450 functional activity. These results suggest that the new inhibitors may cause hepatotoxicity. Synthesis and pre-clinical studies of these monosubstituted derivatives with cytochrome P_{450} 1A2 receptors is recommended in order to confirm their hepatotoxicity.

Keywords: Cimetidine, Docking, Cytochrome P450, Binding affinities, Scoring function.

INTRODUCTION

Cimetidine, a potent CYP450 enzyme inhibitor. It helps in the reduction of stomach acidity, pepsin output reduction and promotion of ulcer healing (Elks, 2014; IDD, 2000, Morton *et al*., 2015; Yusuf, 2007). It is mainly used in the treatment of heartburn, peptic ulcer, abomasal and duodenal ulcers, drug induced erosive gastritis, duodenal gastric reflux and oesophageal reflux healing (Elks, 2014; Morton *et al*., 2015; Yusuf, 2007; Burchum and Rosenthal, 2014*)*. The use of cimetidine has been decreased with the development of other longer acting H_2 receptor antagonists with fewer adverse effects such as famotidine and ranitidine. The monosubstituted derivative of cimetidine could have an improvement with a wider spectrum of activity.

The monosubstituted derivative of cimetidine could also reduce the side effects of cimetidine such as, constipation, rashes, muscle pain at the site of injection, fatigue, dizziness and mental confusion (Ritter *et al*., 2008*).* The changes in the substituent of cimetidine may be very safe in overdose, producing no symptoms even at high overdoses (Dart, 2004*).* Cimetidine is metabolized through *S*-oxygenation by flavin-containing monooxygenases, specifically FMO1 and FMO3 (Cashman, 2000*)*. Cimetidine is a potent but not a universal inhibitor of CYP450 enzyme (Dart, 2004; Lemke and Williams, 2008*)* but it inhibits a broad array of CYP450 isoforms, including CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (Dart, 2004; Lemke and Williams, 2008; Karalliedde *et al*., 2010*)* and it is said to be most potent in inhibiting CYP1A2, CYP2D6, and CYP3A4 (Priskorn *et al*., 1997*)* of which it is described as a moderate inhibitor (Elks, 2014*)* , and this is notable as these three isoenzymes are involved in the majority of CYP450-mediated drug biotransformations (Martínez *et al.,* 1999*)*. As such, cimetidine has the potential for a large number of drug interactions (Dart, 2004; Lemke and Williams, 2008; Karalliedde *et al.,* 2010*).* CYP450 enzymes are involved in the elimination of xenobiotic detoxification of harmful carcinogenic substances. Cytochrome P450 1A2 receptors are mainly expressed in the human liver and participate in the oxidation of drugs.

Modern approaches to finding new drugs for therapeutics in man and animals are increasingly based on 3-dimensional information about receptors. An effective way to predict the binding structure of a substrate in its receptor is docking simulation, which has been successfully used in many applications *(*Lorey *et al.,* 2001; Dixon and Blaney, 1998). Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, it describes a process by which two molecules fit together in a 3-dimensional space (Dipankar *et al*., 2007). Though still in use cimetidine is no longer among the more widely used H_2- receptor antagonist. Therefore the aim of this research is to determine the substituents that will reduce the inhibition of CYP450 enzyme and recommend these monosubstituted derivatives for synthesis and pre-clinical studies. The structural formula of cimetidine is shown in Figure 1.

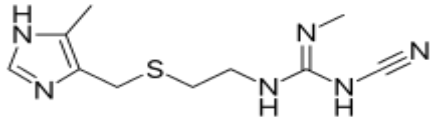


Figure 1: Cimetidine structural formula

MATERIAL AND METHODS

a- Protein preparation

The three dimensional structure of Cytochroome P450 receptor was obtained from the Protein Data Bank, PDB ID – 2HI4 (Figure 2). The protein structure was subjected to a refinement protocol using Molegro Molecular Viewer (Molegro Molecular Viewer, 2012).

b-Designing of structural analogs of Cimetidine

The structure of cimetidine (Figure 1) was drawn with ACD/ChemSketch software (Advanced Chemistry Development, 2008). The structural analogues of cimetidine were developed with structural modifications with different substituents. The methyl group at 17 position of cimetidine was replaced with COOH, $COCH_3$, NO_2, CF_3 and $CONH_2$ analogues. The structures were built with ACD/ChemSketch software and minimized with Arguslab software (Thompson, 2007).

c-Molecular docking

Molecular docking was performed using Patchdock software (Duhovny *et al*., 2002). Patchdock is a molecular docking algorithm based on shape complementarity principles. The docking job was refined in Firedock software (Mashiach *et al.,* 2008; Andrusier *et al*., 2007) and processed with Molegro Molecular Viewer. Lipinski rule of 5 was evaluated using Sanjeevini: a freely accessible web-server for target directed lead molecule discovery (Jayaram *et al*., 2012).

RESULTS AND DISCUSSION

Estimated free energy of binding (FEB) of cimetidine and its analogues is shown in Table 1. Assessment of drug-likeness of the pre-screened ligand is presented in Table 2. Crystal structure of cytochroome P450 receptor is shown in Figure 2, while the docked cimetidine analogues with cytochroome P450 receptor are presented in Figures 3 – 8. The Hydrogen bonding, electrostatic and steric interactions are depicted in Figures 9 -14.

Tabled 1: Estimated free energy of binding (FEB) of 1-cyano-2-methyl-3-({[2-(5-methyl-1*H*- imi dazol-4-yl)ethyl]sulfanyl}methyl)guanidine (cimetidine) and its analogues against cytochrome P450 1A2

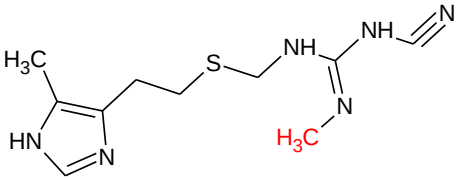
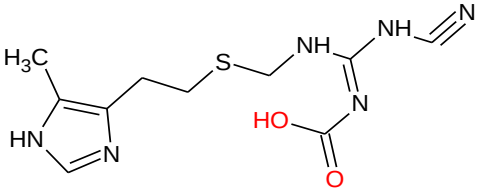
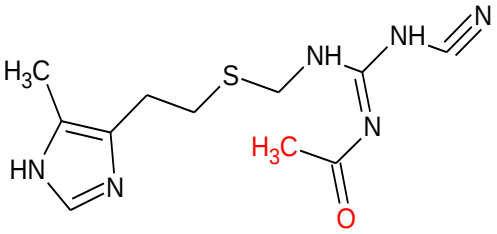
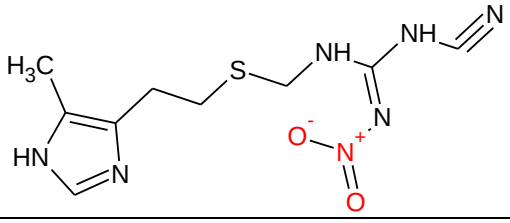
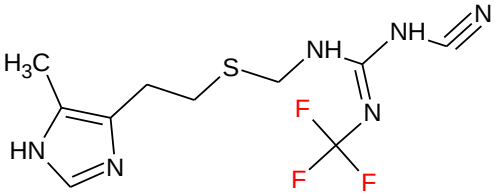
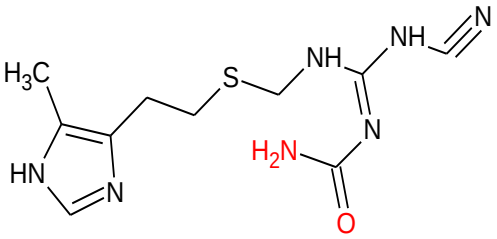
Substituent's	Structure	Docking score (Kcal/mol)
CH_3		-30.96
COOH		-33.12
$COCH_3$		-31.67
NO_2		-34.67
CF_3		-34.73
$CONH_2$		-36.58

Table 2: Assessment of drug-likeness of 1-cyano-2-methyl-3-({[2-(5-methyl-1*H*-imidazol-4-yl) ethyl]sulfanyl}methyl)guanidine (cimetidine) and its analogues

Substituents	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptors:	Lipophilicity LogP	Molar Refractivity
CH_3	250.000	3.000	5.000	1.632	73.720
COOH	278.000	4.000	7.000	0.715	67.960
$COCH_3$	277.000	3.000	6.000	0.827	76.093
NO_2	282.000	4.000	7.000	0.715	67.960
CF_3	305.000	3.000	5.000	2.047	73.581
$CONH_2$	277.000	4.000	7.000	0.844	76.030

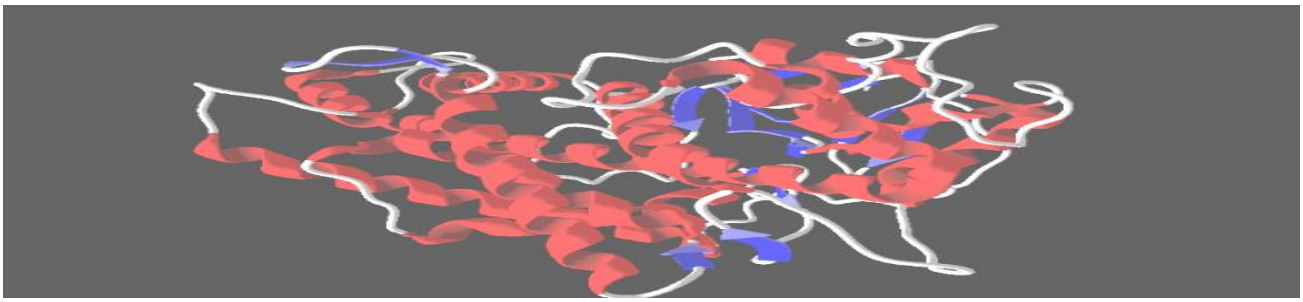


Fig 2: Crystal structure of cytochrome P450 1A2(PDB 2HIA)

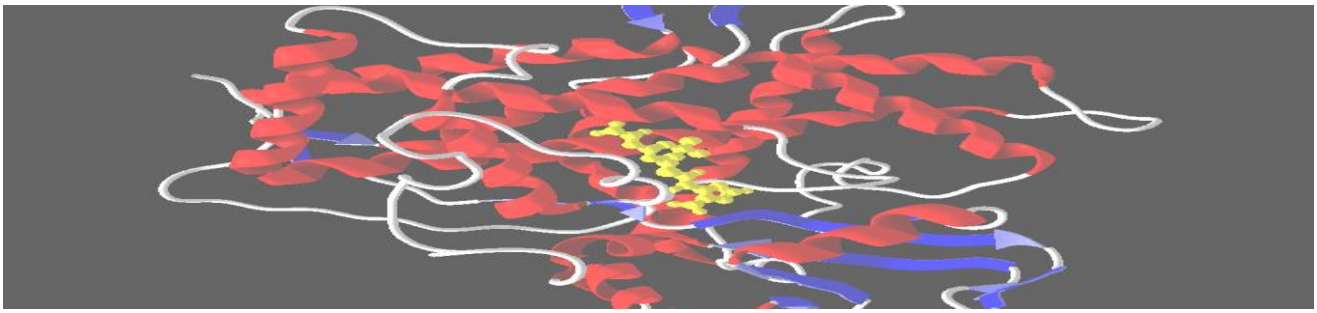


Fig 3: Cimetidine docked with cytochrome P450 1A2

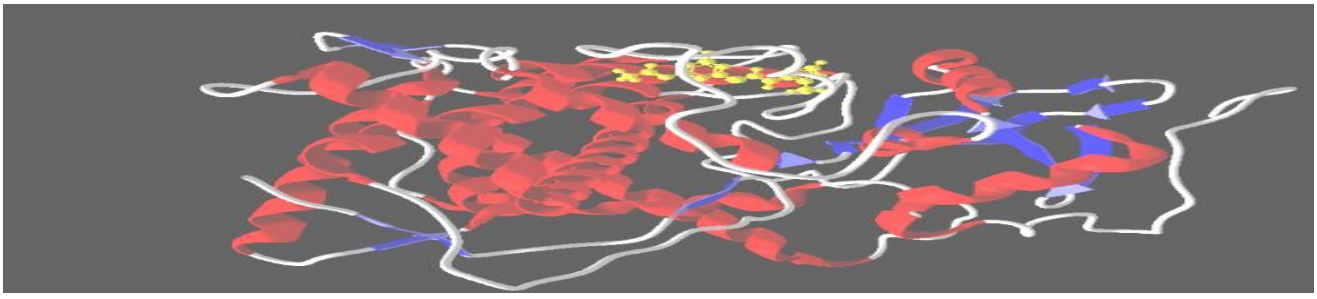


Fig 4: COOH analogue of cimetidine docked with cytochrome P450 1A2

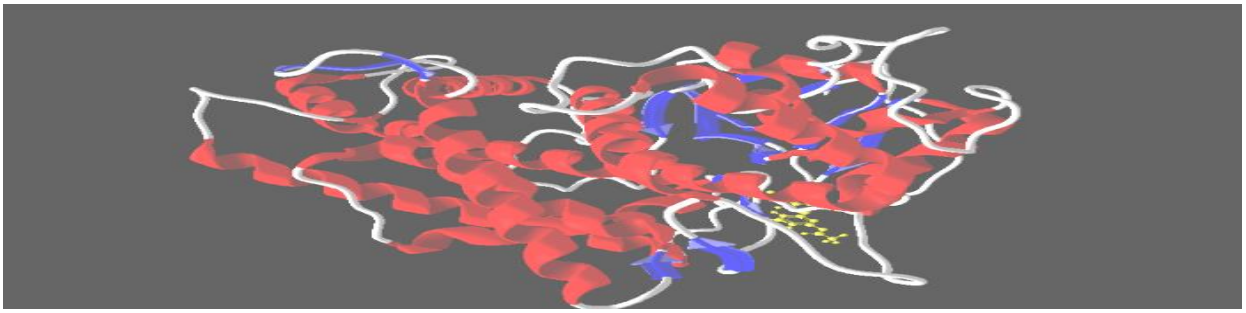


Fig 5: $COCH_3$ analogue of cimetidine docked with cytochrome P450 1A2

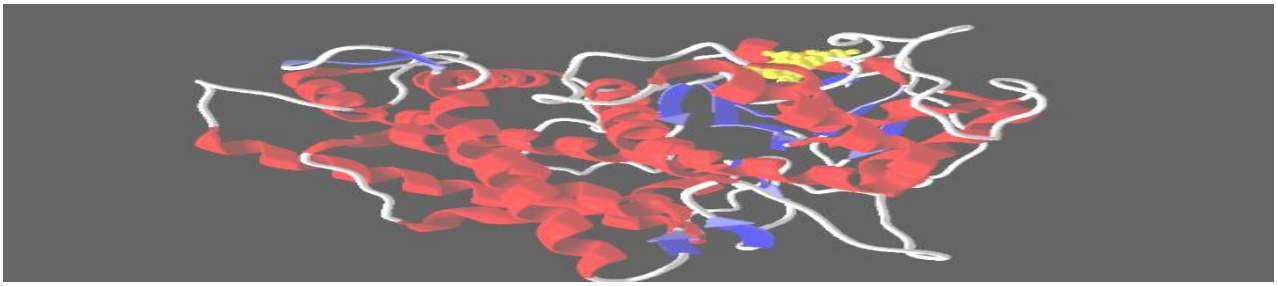


Fig 6: NO_2 analogue of cimetidine docked with cytochrome P450 1A2

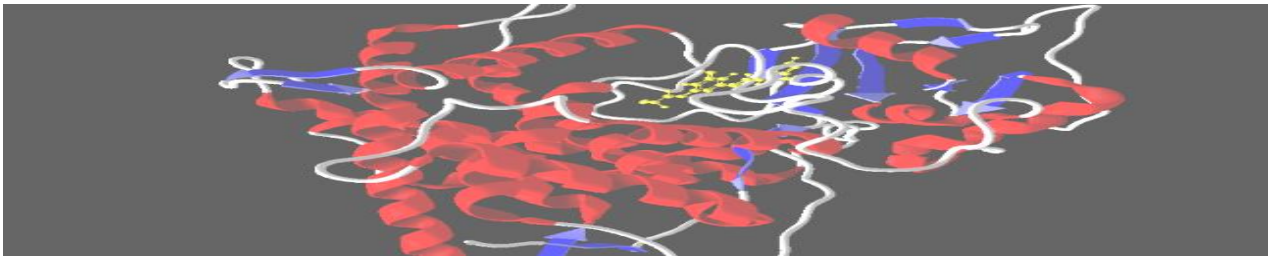


Fig 7: CF_3 analogue of cimetidine docked with cytochrome P450 1A2

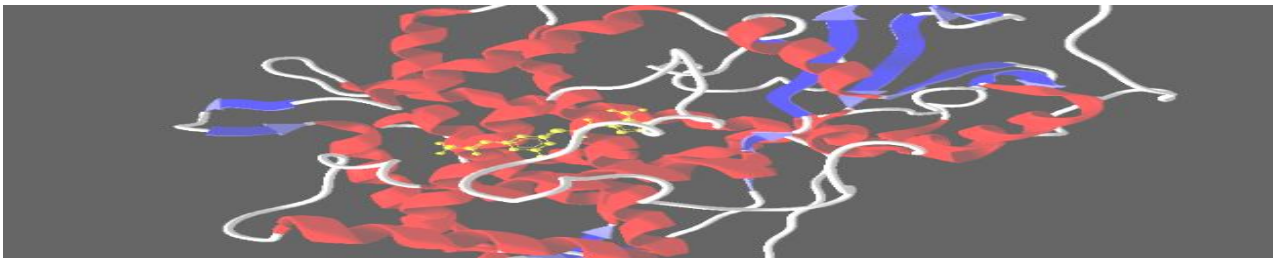


Fig 8: $CONH_2$ analogue of cimetidine docked with cytochrome P450 1A2

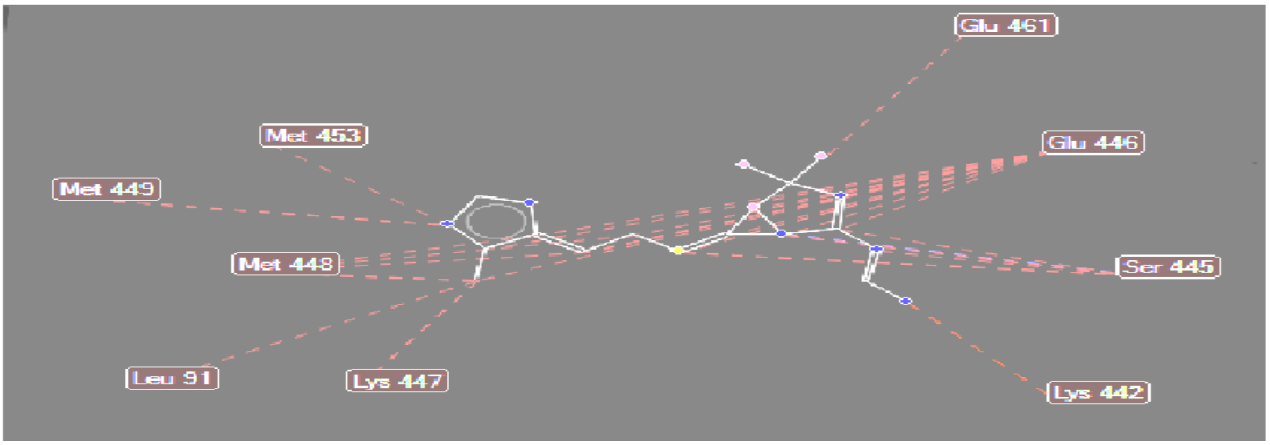


Fig. 9. Hydrogen bonding, electrostatic and steic interactions of CF_3 analogue of cimetidine docked with cytochrome P_{450} 1A2

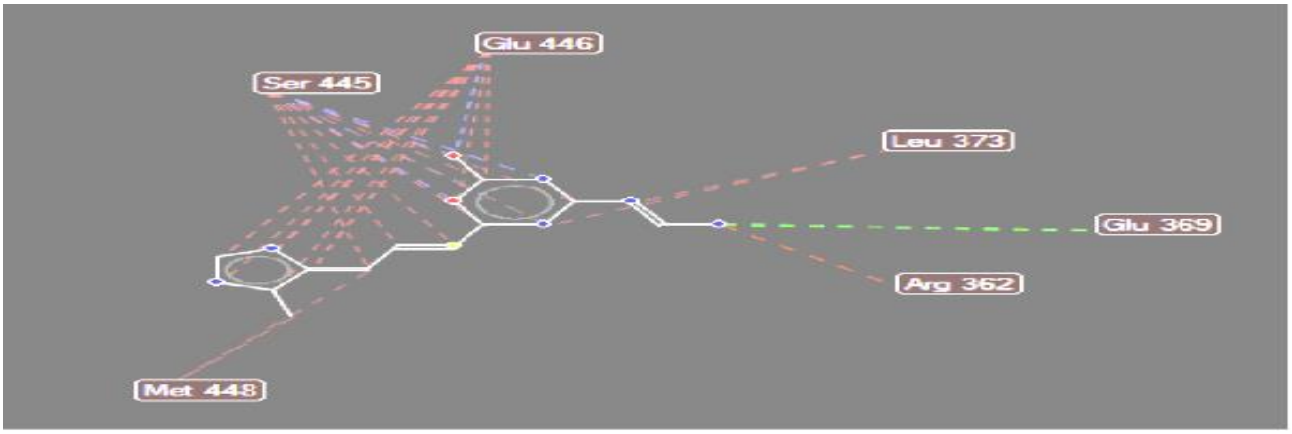


Fig. 10. Hydrogen bonding, electrostatic and steic interactions of COOH analogue of cimetidine docked with cytochrome P_{450} 1A2

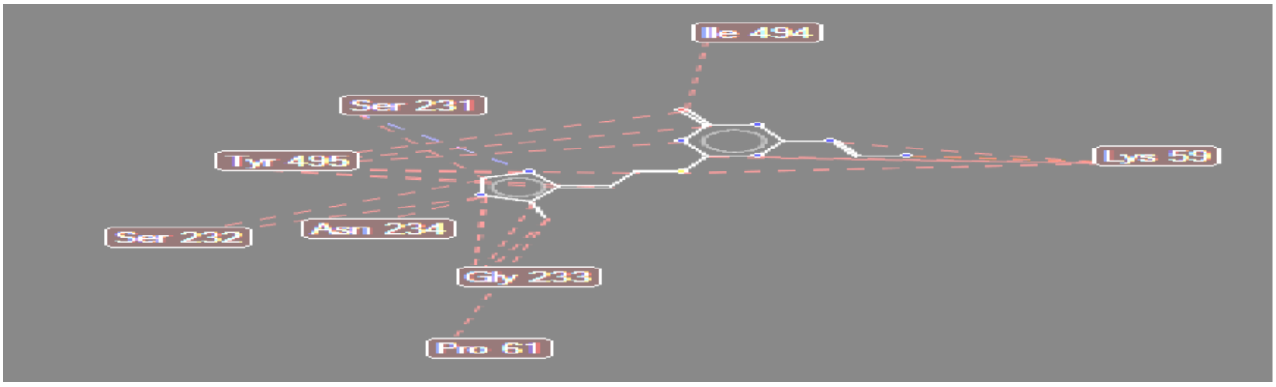


Fig. 11. Hydrogen bonding, electrostatic and steic interactions of $CONH_2$ analogue of cimetidine docked with cytochrome P_{450} 1A2

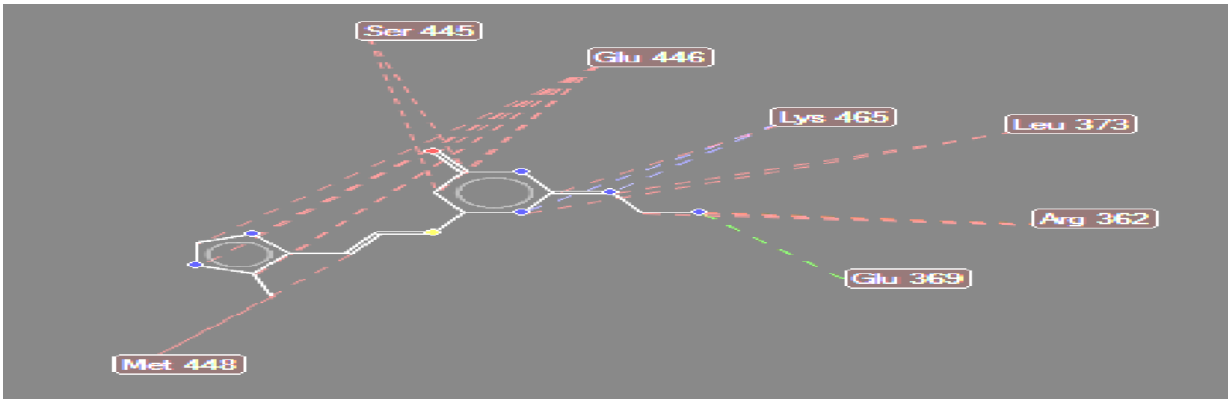


Fig. 12. Hydrogen bonding, electrostatic and steric interactions of $COCH_3$ analogue of cimetidine docked with cytochrome P450 1A2

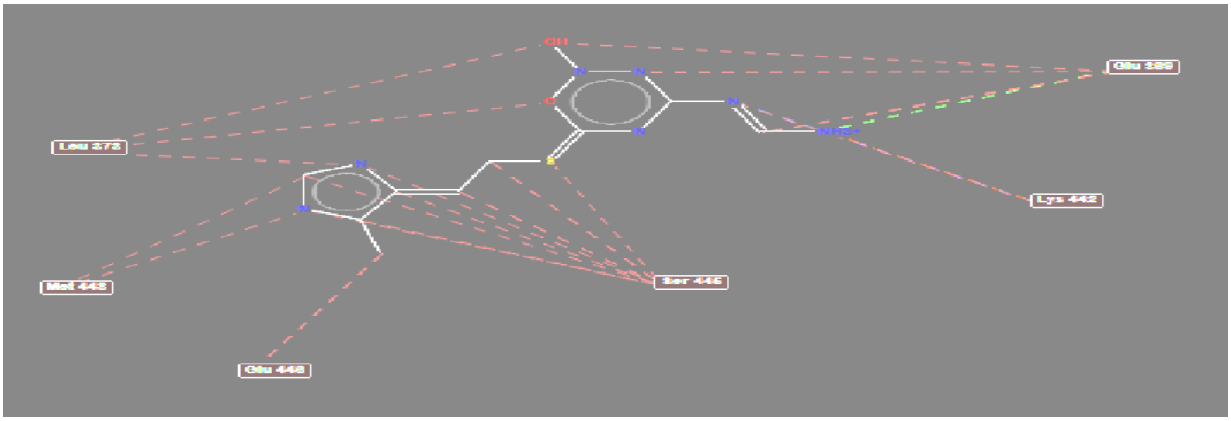


Fig. 13. Hydrogen bonding, electrostatic and steric interactions of NO_2 analogue of cimetidine docked with cytochrome P450 1A2

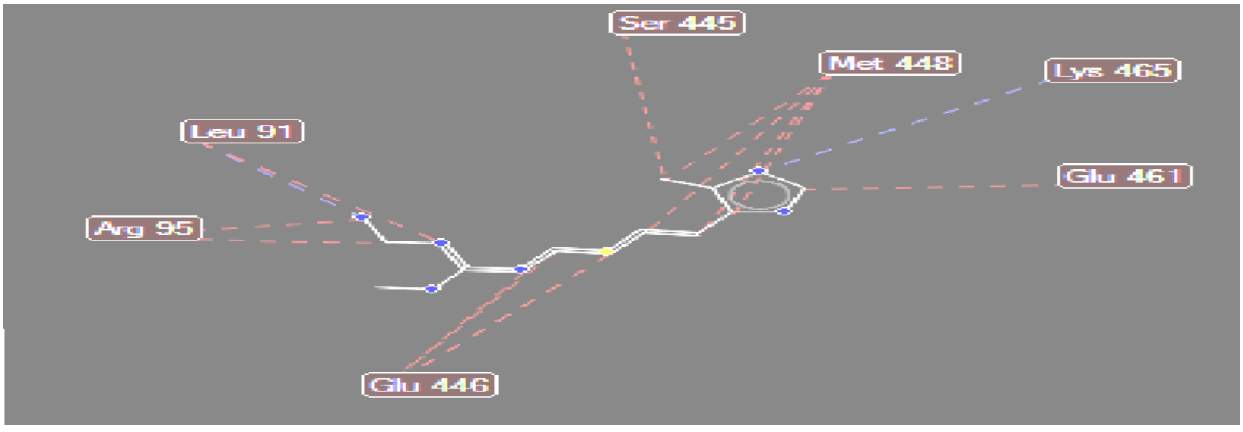


Fig. 14. Hydrogen bonding, electrostatic and steric interactions of cimetidine docked with cytochrome P450 1A2

The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of cytochrome P450 receptor, as is evident from the superposition of all the six analogues in Figures 3 - 8. The hydrogen bonding, electrostatic and steric interactions of

cimetidine and its analogues with cytochrome P450 1A2 occurred at Met 453, Met 449, Met 448, Leu 91, Lys 447, Glu 461, Glu 446, Ser 445, Lys 442, Leu 373, Glu 369, Arg 362, Tyr 495, Ser 231, Lys 59, Glu 233, Pro 61, Ser 232, Gly 232, Asn 234, Lys 465 and Arg 95.
The calculated free energy of binding of the six cimetidine analogues was -30.96, -33.12, -31.67, -34.67, -34.73 and -36.58 Kcal/mol (Table 1). This confirms that the structural modification implemented in this study is significantly related to their activity. Also, this proved the reasonability and reliability of the docking results. It can be seen that substitution of NH_2 functional group of cimetidine with CH_3, COOH, $COCH_3$, NO_2 ,CF_3 and $CONH_2$ analogues at positions 17 lead to an increase in the binding affinity of modified analogues which is even more intense than that of cimetidine. The binding energy of cimetidine was -30.96 kcal/mol. These results clearly indicated that before synthesis and biochemical testing of new analogues one can use molecular docking based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis.

Lipinski rule of 5 (Lipinski, 2004) helps in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500 Dalton; High lipophilicity (expressed as LogP less than 5);Less than 5 hydrogen bond donors ;Less than 10 hydrogen bond acceptors ;Molar refractivity should be between 40-130. Cimetidine and its modified analogues obeyed the Lipinski rule of 5 (Table 2). These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures.

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