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DOCKING STUDY OF CIPROXIFAN AS A HISTAMINE H_3 RECEPTOR ANTAGONIST BY USING ARGUS LAB SOFTWARE

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ABSTRACT

The Ciproxifan was docked into the active site of histamine N- methyl transferase (HNMT) enzyme and evaluated, based on fitness scoring function A Score available in the Argus lab software. We observed that the A Score correlated well with least binding energy. Such as most active compound Ciproxifan that binds the HNMT enzyme with high of GA Score of –8.04804 K.cal/mole. These results indicate that the GA Score is a better parameter to assess the binding of Ciproxifan analogue as inhibitor of HNMT enzyme.

To optimize and calculate the structure of Histamine H3 receptor antagonist drug , Ciproxifan. Docking of Ciproxifan into the active site of protein PDB ID: 2AOT showed that two hydrogen bonds formed with carbonyl group of ligand with hydroxyl group of Tyrosine (Tyr) 147 and amide group of Glutamine (Gln) 94 . The best scoring function (binding affinity) was found to be -8.04804 K.cal/mol.

Descriptive Statistical Scoring Function. The non-bonded potential energies were computed for Ciproxifan, which is the H_3 receptor antagonist and sedative psychoactive in nature. In the present calculation all the possible pairs of non – bonded interaction have been included for the energy calculation. For the present study, one crystal structure of receptor protein (2AOT) was used to validity A Score scoring function. Docking results were analyzed by Argus Lab software. Docking of Anta 16 into the active site of protein 2AOT showed that two hydrogen bonds formed with carbonyl group of ligand with hydroxyl group of Tyr 147 and amide group of Gln 94. The hydrophobic amino acid residues Gln 94,Gln 143,Glu 89, Gly 60, Gly 61,Gly 62, His 29, Ile 66,Ile 142, Met 144, Pro 90, Ser 91,ser 120,Thr 119, Tyr 147 presents as binding site. The best scoring function (binding affinity) was found to be -8.04804 K.cal/mol.

Key-words: Histamine, H_3 receptor antagonist, Cimetidine derivative, Conformational analysis, Geometry optimization, HNMT, Docking

INTRODUCTION

Cyclopropyl-(4-(3-1H-imidazol-4-yl)propyloxy) phenyl) ketone (Ciproxifan), belongs to a novel chemical series of histamine H3-receptor antagonists. In vitro, it behaved as a competitive antagonist at the H3 autoreceptor controlling H3 histamine release from synaptosomes (Ligneau *et al.,* 1998).

H3 Receptor Antagonist

An H_3-receptor antagonist is a classification of drugs used to block the action of histamine at the H_3 receptor (Nakamura et al., 2000) . Unlike the H_1 and H_2 receptors which have primarily peripheral actions, but cause sedation if they are blocked in the brain, H_3 receptors are primarily found in the brain and are inhibitory autoreceptors located on histaminergic nerve terminals, which modulate the release of histamine (Nguyen et al., 2001). Histamine release in the brain triggers secondary release of excitatory neurotransmitters such as glutamate and acetylcholine via stimulation of H_1 receptors in the cerebral cortex (White and Rumbold, 1999). Consequently unlike the H_1 antagonist antihistamines which are sedating, H_3 antagonists have stimulant and nootropic effects, and are being researched as potential drugs for the treatment of neurodegenerative conditions such as Alzheimer's disease,e,g :A-349, 821, ABT-239, Ciproxifan, Clobenpropit, Thioperamide (Reiner and Kamondi, 1994; Ito, 2004)

Molecular recognition plays a key role in promoting fundamental bimolecular events such as enzyme-substrate, drug-protein and drug-nucleic acid interactions (Yanai, 2001). Detailed understanding of the general principles that govern the nature of the interactions (van der Waals, hydrogen bonding, electrostatic between the ligands and their protein or nucleic acid targets may provide a conceptual framework for designing the desired potency and specificity of potential drug leads for a given therapeutic target. Practical application of this knowledge requires structural data for the target of interest and a procedure for evaluating candidate ligands. To this end, a variety of computational docking methods are available (Ikram *et al*., 2015; Ikram *et al*., 2014; Leurs *et al*., 2002).

Molecular Docking Studies:

For the present study, one crystal structure of Bovine Rhodopsin (2AOT) was used to validity A Score scoring function. Docking results were analyzed by Argus Lab software

Energy minimization of protein:

The crystal structure of protein (2AOT) was obtained from the protein data bank and A chain was selected for docking studies. First we removed from the PDB file all water molecules, ligand atoms. All hydrogen atoms in the protein were allowed to optimize. The hydrogen locations are not specified by the X-ray structure but these are necessary to improve the hydrogen bond geometries. Next hydrogen atoms were added and partial charges were assigned. Minimization was performed by Argus lab4.0.1 software using Hartree-Fock method.

The ligand Structures and optimization:

The Acyl piperazine analogue was built using the Marvin Sketch software. The structure was energy minimized using molecular modelling Marvin Sketch software. Partial atomic charges were calculated by the Mulikan method in Argus lab software.

Docking protocol:

Argus Lab 4.0.1:

Argus lab 4.0.1 requires the receptor and ligand coordinates in any of the following formats: PDB, MOL, SDF or MOL2 format. The active site origin was specified by the center of geometry of the reference ligand. ArgusDock the new drug docking code with both the GADock and ArgusDock docking engines and the AScore scoring function with a preliminary set of parameters.

RESULTS

Conformational Analysis

Prospective views of Ciproxifan are shown in Figure 1. The electron density mapped of atoms by ACD LABS 3D Viewer software in Figure 2, and 3 show Electrostatic potential of molecule ground state with the color map. Figure 3 use a clipping plane showing a cutaway of the same surface revealing the underlying molecular structure. The colour map shows Esp. energy (in hartess) for the various colors. The red end of the spectrum show regions of highest stability for a positive test charge, magenta / blue show the regions of least stability for a positive test charges. Figure 4 shows the occupied molecular orbital of molecule calculated with the ZINDO method and rendered a mesh the positive and negative phases of the orbital are represented by the two colours. The blue region represents an increase in a density the red region a represent a decrease in electron density. The minimum potential energy shows for drug receptor interactive via the geometry convergence map in Figure 5.

Fractional coordinates of molecule are given in Table 1 and bond length and bond angles are given in Table 2 and Table 3 respectively which are taken after geometry optimization of molecular from Argus lab by using molecular mechanics calculation . It is possible that drug in this conformation interact with receptor. The result indicates that the best conformation of the molecule is present at minimum potential energy is found to be 222.7204 kcal/mol. At this point molecule will be more active as imidazol free histamine H3 antagonist (Bano *et al*., 2012).

Docking

The molecular docking study of Ciproxifan into the active site of 2AOT was carried out using the Argus Lab 4.0 software and the docking evaluations were made.

The docking conformations have been analyzed with best GA dock by using two criteria.

a) Ligand binding position.

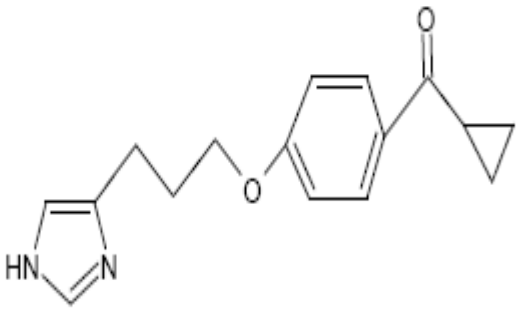
b) AScore comparison.

Ligand binding position:

Docking of Ciproxifan into the active site of 2AOT and showed that one hydrogen bond formed with carbonyl group of imidazole ring of Ciproxifan and N atom of amino acid residue GLN 92 (Figure 5, Table 9). The hydrophobic amino acid residues PHE 131 and HIS 94 near the tetrazine imidazole carboxamide ring of ciproxifan (Table 10). The best scoring function (binding affinity) was found to be -8.04804 K.cal/mole.

We have identified a new class of highly potent and selective antagonists of the human Histamine H_3 receptor by interactive screening of arrays of monoacyldiamines. Because unsophisticated chemistry was chosen at the outset of the project, the initial hits could be optimized quickly, and the preparation of larger amounts of selected

compounds could be accomplished easily. In depth evaluation of the biological properties of these amides is in progress. Finally all geometric variables were completely optimized for compound and the lowest energy conformations were used in molecular modeling studies. In the present potential energy of non-bonded interactive for Ciproxifan is calculated (Table 4, 5 and 6). Total potential energies were calculated by summation of all individual pairs (Table 7). Contours are plotted for visual understanding (Figure 3). The result indicates that the best conformation Ciproxifan is found to be at 222.72 kcal/mol (Table 8) which is minimum potential energy. At this point molecule will be more active as Histamine H3 antagonist.



cyclopropyl{4-[3-(1*H*-imidazol-4-yl)propoxy]phenyl}methanone

Molecular Formula	= $C_{15}H_{18}N_2O_2$
Formula Weight	= 270.32632
Composition	= C(71.09%) H(6.71%) N(10.36%) O(11.84%)
Molar Refractivity	= 76.43 ± 0.3 cm^3
Molar Volume	= 219.5 ± 3.0 cm^3
Parachor	= 601.9 ± 4.0 cm^3
Index of Refraction	= 1.613 ± 0.02
Surface Tension	= 56.5 ± 3.0 dyne/cm
Density	= 1.231 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 30.30 ± 0.5 $10^{-24} cm^3$
Monoisotopic Mass	= 270.136828 Da
Nominal Mass	= 270 Da
Average Mass	= 270.3263 Da
M+	= 270.136279 Da
M-	= 270.137376 Da
[M+H]+	= 271.144104 Da
[M+H]-	= 271.145201 Da
[M-H]+	= 269.128454 Da
[M-H]-	= 269.129551 Da

Fig. 1. Calculated properties of Ciproxifan by ACDC chemsketch.

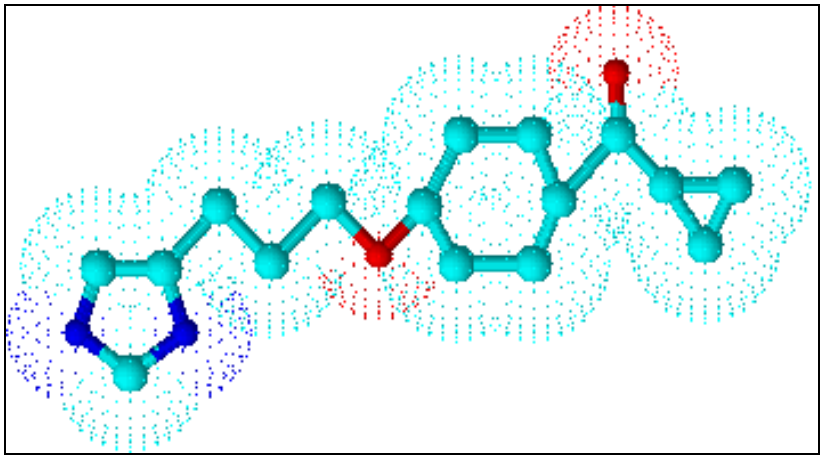


Fig. 2. Ball and stick model of Ciproxifan molecule.

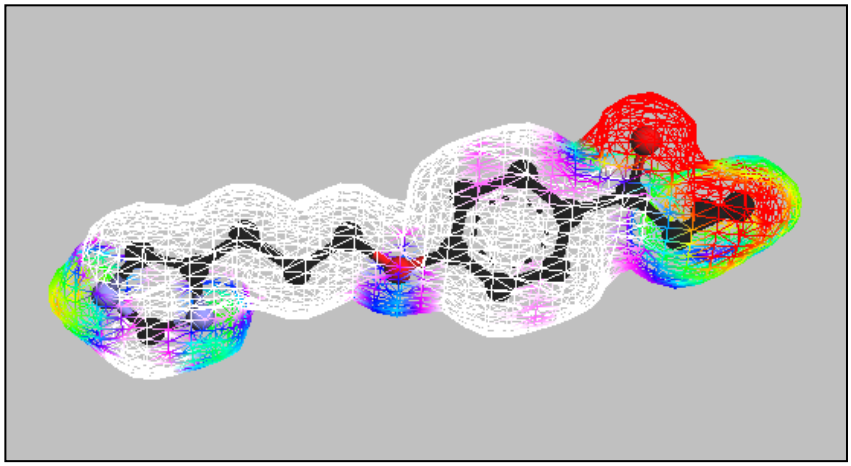


Fig. 3. Electrostatic potential (ESP) mapped electron density surface (mesh).

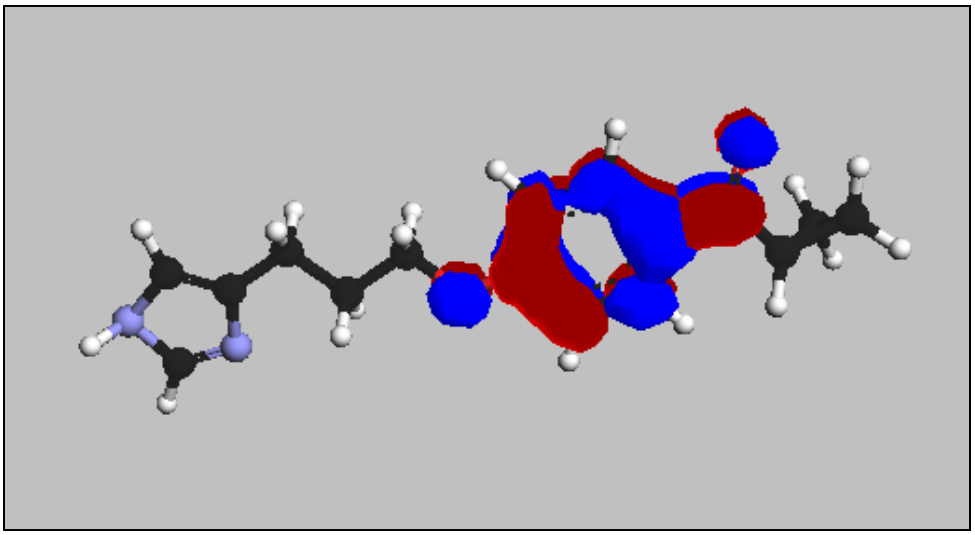


Fig. 4. Visualize molecular orbital of molecule, blue shows positive and red shows negative.

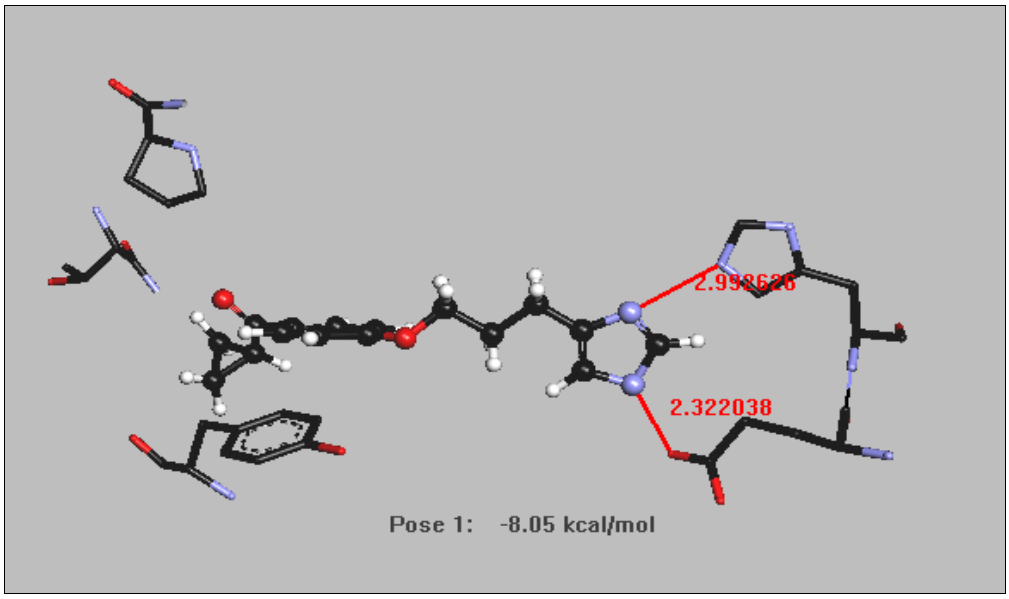


Fig. 5. Ciproxifan docked in 2AOT protein.

Table 1. Fractional Coordinates of Ciproxifan.

S. No.	Z	Y	X	ATOMS
1	0.317437	2.197309	-1.286726	C
2	0.367087	1.389611	-0.142724	C
3	0.433345	-0.000299	-0.279488	C
4	0.333167	1.615556	-2.557312	C
5	0.399133	0.219657	-2.695485	C
6	0.449323	-0.582415	-1.550585	C
7	0.348714	0.335491	-5.163756	C
8	0.401730	-0.629012	-6.329043	C
9	0.298158	0.144016	-7.626636	C
10	0.416421	-0.388901	-3.947702	O
11	0.352775	1.977768	1.202416	C
12	0.287704	3.227874	1.363451	O
13	0.574969	-0.462458	-10.034943	C
14	0.365119	-0.801388	-8.775100	C
15	0.127678	-2.186158	-8.680985	N
16	0.217945	-2.651719	-9.881080	C
17	0.516875	-1.639439	-10.876345	N
18	0.414875	1.084273	2.391850	C
19	-0.835326	1.273457	3.224917	C
20	0.462078	1.927089	3.649124	C

Table 2. Bond length of Ciproxifan.

S. No.	BOND LENGTH	ATOMS
1	1.301961	N1-C2
2	1.433804	N1-C3
3	1.433804	C2-N4
4	1.323387	C3-C5
5	1.486000	C3-C6
6	1.433804	N4-C5
7	1.514000	C6-C7
8	1.514000	C7-C8
9	1.436155	C8-O9
10	1.407689	O9-C10
11	1.458000	C10-C11
12	1.323387	C10-C15
13	1.323387	C11-C12
14	1.458000	C12-C13
15	1.323387	C13-C14
16	1.461000	C13-C16
17	1.458000	C14-C15
18	1.461000	C16-C17
19	1.260307	C16-O20
20	1.458000	C17-C18
21	1.458000	C17-C19
22	1.458000	C18-C19

Table 3. Bond angles of Ciproxifan.

S. No.	BOND ANGLES	ATOMS
1	120.000000	C2-N1-C3
2	120.000000	N1-C2-N4
3	120.000000	N1-C3-C5
4	120.000000	N1-C3-C6
5	120.000000	C2-N4-C5
6	120.000000	C5-C3-C6
7	120.000000	C3-C5-N4
8	109.470000	C3-C6-C7
9	109.470000	C6-C7-C8
10	109.470000	C7-C8-09
11	104.510000	C8-O9-C10
12	120.000000	O9-C10-C11
13	120.000000	O9-C10-C15
14	120.000000	C11-C10-C15
15	120.000000	C10-C1-C12
16	120.000000	C10-C15-C14
17	120.000000	C11-C12-C13
18	120.000000	C12-C13-C14
19	120.000000	C12-C13-C16
20	120.000000	C14-C13-C16
21	120.000000	C13-C14-C15
22	120.000000	C13-C16-C17
23	120.000000	C13-C16-O20
24	120.000000	C17-C16-O20
25	120.000000	C16-C17-C18
26	120.000000	C16-C17-C19
27	120.000000	C18-C17-C19
28	120.000000	C17-C18-C19
29	120.000000	C17-C19-C18

Table 4. Torsional angle of Ciproxifan.

S. No.	TORSION ANGLES	ATOMS
1	2.000000	C5-C6-C3-N1
2	2.000000	C11-C15-C10-O9
3	2.000000	C14-C16-C13-C12
4	16.666667	C17-O20-C16-C13
5	2.000000	C18-C19-C17-C16

Table 5. Dihedral angle of Ciproxifan.

S. No.	DIHEDRAL AGLES	ATOMS	S. No.	DIHEDRAL AGLES	ATOMS
1	38.973552	N4-C2-N1-C3	9	1.000000	C5-C3-C6-C7
2	5.000000	C2-N1-C3-C5	10	2.119000	C3-C6-C7-C8
3	5.000000	C2-N1-C3-C6	11	2.119000	C6-C7-C8-O9
4	10.000000	N1-C2-N4-C5	12	0.195300	C7-C8-O9-C10
5	19.486776	N1-C3-C5-N4	13	5.000000	C8-O9-C10-C11
6	1.000000	N1-C3-C6-C7	14	5.000000	C8-O9-C10-C15
7	10.000000	C2-N4-C5-C3	15	5.000000	O9-C10-C11-C12
8	19.486776	N4-C5-C3-C6	16	19.486776	O9-C10-C15-C14

Table 5 Cont'd…….

S. No.	DIHEDRAL AGLES	ATOMS	S. No.	DIHEDRAL AGLES	ATOMS
17	5.000000	C12-C11-C10-C15	28	2.500000	C14-C13-C16-O20
18	19.486776	C11-C10-C15-C14	29	2.500000	C13-C16-C17-C18
19	38.973552	C10-C11-C12-C13	30	2.500000	C13-C16-C17-C19
20	10.000000	C10-C15-C14-C13	31	2.500000	C18-C17-C16-O20
21	5.000000	C11-C12-C13-C14	32	2.500000	C19-C17-C16-O20
22	5.000000	C11-C12-C13-C16	33	5.000000	C16-C17-C18-C19
23	19.486776	C12-C13-C14-C15	34	5.000000	C16-C17-C19-C18
24	2.500000	C12-C13-C16-C17	35	5.000000	C19-C18-C17-C19
25	2.500000	C12-C13-C16-O20	36	5.000000	C18-C17-C19-C18
26	19.486776	C15-C14-C13-C16	37	10.000000	C17-C18-C19-C17
27	2.500000	C14-C13-C16-C17			

Table 6. Bonded Topology.

1	22	Bonds
2	29	Bond Angles
3	37	Dihedral Angles
4	5	Improper Torsions
5	51	NB Exclusion List
6	70	Initial NB List

Table 7. Initial Energy Components (au).

1	0.18571662	MM Bond
2	0.76191435	MM Angle
3	0.01752960	MM Dihedral
4	0.00000000	MM ImpTor
5	0.22534595	MM vdw
6	0.00000000	MM Coulomb
	1.19050651 au	Total
	747.05478986 kcal/moL	Total

Table 8. Final Energy Components (au).

1	0.18571662	MM Bond
2	0.76191435	MM Angle
3	0.01752960	MM Dihedral
4	0.00000000	MM ImpTor
5	0.22534595	MM vdw
6	0.00000000	MM Coulomb
	1.19050651 au	Total
	747.05478986 kcal/moL	Total

Table 9. List of Hydrogen Bonding Interactions between the Ciproxifan analogue and HNMT Enzyme by AScore.

S.NO	**Molecule Name**	**No. Of H bonds**	**Protein Residue Atom**	**Ligand Atom**	**H bond Distance Å**
1	Ciproxifan	2		C=O	2.945 2.0876

Table 10. List of amino acids residues contributing to the hydrophobic pocket in docking of ciproxifan analogue into 2AOT protein by AScore.

S.NO	**Molecule Name**	**Hydrophobic amino acids residues**	**Binding energy K.cal/mole**
1	Ciproxifan	PHE 131 , HIS 94 , THR 199	-8.04804

Conclusion

In the present potential energy of non-bonded interactive for Ciproxifan is calculated. Total potential energies were calculated by Arguslab software at PM3. Contours are plotted for visual understanding. The result indicates

that the best conformation Ciproxifan is found to beat 222.7204 kcal/moL., which is minimum potential energy at this point molecule will be more active as Histamine H3 antagonist.

We have identified a new class of highly potent and selective antagonists of the human Histamine H_3 receptor by interactive screening of arrays of monoacyldiamines. Because unsophisticated chemistry was chosen at the outset of the project, the initial hits could be optimized quickly, and the preparation of larger amounts of selected compounds could be accomplished easily. In depth evaluation of the biological properties of these amides is in progress.

Finally all geometric variables were completely optimized for each compound and the lowest energy conformations were used in molecular modeling studies.

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