Computational Evaluation of 2-Phenyl-4*H*-chromen-4-one Analogues as Antihistamines: Potential Histamine *N*-Methyltransferase (HMT) Inhibitors

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Abstract: Abnormal release of histamine, which is present in relatively high concentration in the lungs, causes serious allergic vasoconstriction and anaphylactic manifestation in human beings. In mammals, a major pathway of histamine metabolism in the lungs is mediated by histamine *N*-methyl transferase (HMT) and diamine oxidase. The need to design a strategy of mechanistic computational evaluation of protein-ligand affinity *i.e.* HMT- 2-phenyl-4*H*-chromen-4-ones, protein complex binding energy has been established. A library of synthesized 2-phenyl-4*H*-chromen-4-ones was docked into the active site cavity of target protein, HMT (Pdb: 2aot). The high-resolution crystal structure of HMT complex with the competitive inhibitor *N* [2 (benzhydryloxy)ethyl] *N,N*-Dimethylamine (Diphenhydramine) revealed a protein with a highly confined binding region that could be targeted in the design of specific anti-histamines. The validation of docking programme by Potential Mean Force was compared with binding energy results of known ligands in the active sites of HMT, diphenhydramine / benadryl, promethazine, cyproheptadine, trimeton / avil *etc.* All the synthesized chromone derivatives showed comparable negative binding energies pointing towards the fact that these molecules could be potent antihistamines.

Keywords: Histamine *N*-methyltransferase (HMT), Docking, 2-Phenyl-4*H*-chromen-4-ones. Computational Evaluation

Introduction

Antihistamines a class of drugs, which blocks the effect of a histamine that is produced by the body, especially the mast cells, in response to an allergic reaction due to antibody-

antigen interaction followed by degranulation of mast cell. The antibody-antigen bonding immunologic mechanism displaces histamine in granules and once formed, it is either stored or rapidly inactivated. Histamine is broken down by histamine *N*-methyl transferase (HMT) and diamine oxidase. Histamine exerts its actions by combining with specific cellular histamine receptors discovered and designated as H1, H2, H3, and H4.

The term antihistamine is traditionally used to refer to drugs that block the H1-receptors. The H1-receptors are found in smooth muscles, endothelium and central nervous system tissue. Abnormal release of histamine binds H1-receptors and its stimulation causes a variety of inflammatory diseases, including asthma, glomerulonephritis, psoriasis, inflammatory bowel diseases, rhinitis symptoms, anaphylactic attack and acute pulmonary injury.

Antihistamines have a much broader use than decongestants. They are used to treat symptoms of allergic reactions such as the sneezing and runny nose of hay fever, itching, swelling and other allergic rashes. HMT and diamine oxidase are two enzymes involved in the metabolism of histamine.

Thus, a new therapeutic approach to treat different inflammatory diseases *viz.* Psoriasis, dermatitis, *etc* may involve the inhibition of various enzymes involved at various stages of allergic pathway. Further many medical reports explained the disappointing results shown by non-steroidal and inflammatory drugs (NSAIDS) against inflammatory dermatitis.

Many types of antihistamines do not end the histamine formation nor do they stop the clash between the IgE and antigen. Thus, antihistamines do not stop the allergic reaction but protect tissues from some of its effects. Many antihistamines often cause mouth dryness and drowsiness hence searches for new potent heterocycles possessing histamine-inhibiting action continues.

Flavanoids represent an awfully important group of organic compounds exhibit significant biological activity, including anti-inflammatory^{1,2} and pharmacological effects. Some flavones have potential as radioligands for imaging the multidrug resistance associated protein (ABCC1/MRP1)³.

In the late 1960, the Fisons Corporation in England introduced a new class of compounds referred to as *bis*-flavones. Later it was found that *bis*-flavones such as cromolyn sodium Figure 1 are effective in preventing but not reversing, antigen-induced bronchospasms^{4,6}.

Figure 1. Cromolyn sodium.

Recent study reveals that numerous types of compounds have been screened for their activity as histamine *N*-methyltransferase⁷ enzyme inhibitors to assess their potential to cure adverse effects produced by histamines and other allergic diseases. Selecting flavones for computational evaluation as anti-histamines focused our investigation for many more reason as their mode of action is not only confined to anti- inflammatory but also act as anti-oxidants, for their affinity towards divalent ions of heavy metals, those catalyze the processes involving free radical generation.

A recent study reveals that histamine *N*-methyltransferase plays a significant role in degrading histamine and in regulating the airway response to histamine. It may be termed as key enzyme involved in allergy and immune responses host defense against infection. It is mainly known for its pathogenic action against allergy related asthma/human allergic bronchospasm.

Recently many molecular modelling techniques are used to predict how any protein interacts with small drug like molecules. The ability of a protein to interact with small molecules governs a significant part of the protein's dynamics which may enhance/ inhibit its biological function. This plays an important role in the rational design of drugs. Present study aims to demonstrate the successful use of synthetic and docking techniques to study the interaction of Histamine *N*-methyltransferase enzyme with previously synthesized⁸ differently substituted 2-phenyl-4*H*-chromen-4-ones in search of new antihistamine pharmacophores.

In the current research area of anti-histamine pharmaceuticals, a huge number of structurally different class of ligands (many of them may not have been synthesized yet) have been screened against various disease strains by means of various techniques of computational chemistry. Seldom synthesis of these target compounds may involve the use of tough and critical reaction conditions, toxic chemicals, sophisticated instrumentation, *etc.* Hence, herein we report an easy, simply synthesized, which may possess structural similarity as cromolyn sodium and ready to dock 2-phenyl-4*H*-chromen-4-one analogues, to inhibit HMT enzyme as the potential antihistamines. The detailed structure is depicted in Table 1& 2.

1(a-j)

Figure 2

Table 1. Detailed structure of 2-phenyl-4*H*-chromen-4-ones.

Ligand	R	R ¹	R ²	R ³	R ⁴	R ⁵
1a	H	H	H	CH ₃	H	H
1b	H	H	H	Cl	H	H
1c	H	Br	H	CH ₃	H	H
1d	H	Br	H	Cl	H	H
1e	Cl	H	H	CH ₃	H	H
1f	Cl	H	H	Cl	H	H
1g	Br	H	H	CH ₃	H	H
1h	Br	H	H	Cl	H	H
1i	H	H	H	CH ₃	H	OCH ₃
1j	H	H	H	Cl	H	OCH ₃

Experimental

In the present work, all the ligands used were made using⁹ Chemdraw 3D Ultra 8.0. Before the docking calculation of the ligands, the structures were fully optimized. As details of the calculations used are available in the literature and therefore, they are not mentioned here. Argus Lab 4.0 was used¹⁰ to perform all the docking techniques. The crystal structure used for the present study was found to be complexed with inhibitor diphenhydramine, was downloaded from Protein Data Bank (<http://www.rscb.org/>) as PDB files.

MT: The file containing the crystal structure of HMT with its selective inhibitor diphenhydramine in the active site (PDB entry 2aot) was downloaded. It is a dimeric structure with chains A and B each consisting of 286 residues. Each chain has one 2PM (*N*-[2(benzhydryloxy)ethyl] *N,N*-Dimethylamine), two molecules of CSO and one molecule of SAH (S Adenosyl Homocysteine).

The chain A with the residues, water and hetero groups within a radius of 3 Å⁰ was refined and further cleaned by ascertaining the hybridization and the valence of each atom of diphenhydramine and introducing *H*-atoms to the protein residues. The cleaned structure of HMT carried no charge and 4831 atoms. The active site residues of HMT are shown in Figure 3.

Figure 3. Active site of HMT with diphenhydramine.
(Hydrogen atoms are suppressed for clarity).

Docking and binding evaluation

In the automated Argus Lab 4.0 system, using a generic algorithm with a fast-simplified Potential of Mean Force (PMF) carried docking of synthesized ligands into active site of HMT. It was assumed that the protein and the ligand docked non-covalently. The standard PMF implementation used UFF potential for this purpose. The docking was carried with flexible ligand into a rigid protein active site. The general procedure for the docking process started with the addition of energy minimized target ligand on the enzyme. The active site and the ligands were specified in the programme. Using 15×15×15 Å⁰ box located at the centre of the target active site optimized the different starting parameters. The whole procedure of docking was repeated until a constant value of docking score was achieved.

Concluding docking results were parameterized in terms of docking score in kcal/mol. The docked flavone ligands **1a-j** complexed with HMT enzyme was interpreted by looking at the H-bonding or hydrophobic interactions of the ligand with the amino acid residues in the active site. The same procedure was adapted for docking different flavone derivatives **1a-j** into the active site of HMT. The results obtained from the docking of these heterocycles into the target active site pocket are summarized in the Table 2.

Table 2. Docking score (kcal/mol) of different flavone ligands **1a-j** with HMT (pdb ID: 2aot)

S.No.	Ligand	Docking score, kcal/mol
1	1a	-12.3516
2	1b	-10.9316
3	1c	-12.9211
4	1d	-11.7521
5	1e	-11.6930
6	1f	-11.5536
7	1g	-11.3279
8	1h	-11.0329
9	1i	-10.7838
10	1j	-10.6195

Compounds **1a-j** has all the carbon atom of the central core (Flavone skeleton) as sp^2 hybridized while the C-2 carry differently substituted aryl group. These synthesized derivatives have some structural similarities with leukotriene biosynthetic inhibitor Cromolyn sodium. Due to decrease in steric repulsion between the C-2 phenyl ring and the flavone core ring provides a degree of freedom to overall structure. As all the ligands showed difference in their binding energies pointing towards the significant role of various substituents in their binding abilities.

Results and Discussion

Validation of PMF method

To validate the programme involved in current study, before docking the test ligands (synthesized flavone derivatives **1a-j**), the docking of diphenhydramine into the active site of HMT was performed. This selective inhibitor binds into the active site cavity with a binding score of -11.0115 kcal/mol and r.m.s deviation of 0.65 was observed. The docked structure of diphenhydramine in the active site of HMT enzyme is shown in Figure 4. The close overlapping of a docked structure with the native ligand (*x*-ray crystal structure) demonstrates the validity of the programme.

Figure 4. The close overlapping shown by the docked structure of diphenhydramine with its crystal structure with the r.m.s value 0.65.

Docking of well known anti-histamines in the active site of HMT enzyme

A comparative study involving the interaction of well-known antihistamines *viz.* diphenhydramine, promethazine, cyproheptadine and trimeton in the active site pocket of HMT was made for better understanding of their antihistamine action. Docking score presented in Table 3.

Table 3. Docking scores (kcal/mol) of various antihistamines with HMT (pdb ID: 2aot).

S.No.	Ligand	Docking score, kcal/mol
1.	Diphenhydramine / Benadryl	-11.1393
2.	Promethazine	-10.9324
3.	Cyproheptadine	-12.3789
4.	Trimeton / Avil	-10.3993

Figure 5. Docked structure of cyproheptadine into the active site of HMT enzyme.

In case of HMT selective cyproheptadine Figure 5, the negative binding energies are in agreement with its HMT selectivity as reported in several literatures. During binding of this cyproheptadine in the binding pocket of HMT the conformational placement of amino acid residues in the active site was observed.

Cyproheptadine was found to bind in the active site of HMT through hydrophobic interactions. One of the six membered rings of cyproheptadine is enveloped by Q143, F22 while seven membered ring is surrounded by V173, Y146. Lower nitrogen containing six membered ring is found to be bordered by Y147, Q197; while nitrogen atom is surrounded by F9, F243, and F22. Rest portion of drug is encircled with other amino acid residues like Q197, W183 and W179.

Docking of synthesized flavone ligands 1a-j into HMT active site

All the 10 synthesized flavone ligands **1a-j** showed binding in the HMT active site with the binding scores between -10.6195 and -12.9211 kcal/mol, Table 4. These data clearly indicated their potency as anti-histamine heterocycles.

Compound **1c** and **1a** showed the highest binding score with HMT enzyme active site cavity with comparison to other ligands including well known anti histamines. Further rationalization of mode of binding of these flavone molecules in active site of HMT has been

based upon the amino acid residues present around the ligand. Depending upon the structural features essential for binding in the cavity, flavone molecules could be divided into two segments *viz.* C-2 phenyl and differently substituted main flavone central core structure. For ligand **1c**, (Figure 6) the C-2 phenyl ring is surrounded by the amino acid residues like Q143, F243, F22, Y173 and W179 while its central skeleton is being enveloped by amino acid residues like W183, Y198, Q197, Y146, Y147, C196, Y198 and F9. The oxygen attached to the C-4 of the central structure was found to have characteristic orientation with the active site. The binding score of **1c** indicated as potential histamine inhibitor.

Table 4. Amino acid residue around both segment of flavone derivative when docked into the HMT (pdb ID: 2aot) active site.

S.No.	Ligand	2-Phenyl	Central flavone core
1	1a	Y147, C196, Y146, Q197, F9, W183, Y198	W179, Q143, F22, V173, F243, F9
2	1b	Q143, W179, F243, F22, W173	W179, W183, C196, F9, Y198, Q197,
3	1c	Q143, F243, F22, Y173, W179	W183, Y198, Q197, Y146, Y147, C196, Y198, F9
4	1d	Q197, Y198, C196, Y146, W183, Y147, F9	F22, Q143, V173, W179, F243
5	1e	Q197, Y198, W183, C196, Y147, Y146, F9	W179, Q143, F22, V173, F243
6	1f	Y146, Y198, F9, Y147, Q197, C196	Q143, V173, W183, W179, F243, F22
7	1g	Q143, V173, F22, Y146, W179, W183	Q197, Y147, C196, F243, F9, V198
8	1h	Y146, Y147, Q197, C196, Y198, F9, W183	W179, V173, F243, F22, Q143, W179
9	1i	Q197, C196, Y198, Y147, Y146, W183, F9	Q143, V173, W179, F22, F243
10	1j	Q143, F22, F243, W179, V173	Y146, Y147, Q197, C196, F9, W183, Y198

Figure 6. Docked structure of **1c** into the active site of HMT.

Figure 7. **1a** docked in the active site of HMT.

In case of **1a**, the C-2 aryl ring is encircled by Y147, C196, Y146, Q197, F9, W183 and Y198 while its central core is enveloped by amino acid residues like W179, Q143, F22, V173, F243, and F9 (Figure 7).

Conclusion

From the inferred results the following conclusions can be drawn.

- Flavone derivatives **1a-j** could be a unique class of histamine inhibitors. As many flavanoids are naturally occurring and possess anti-inflammatory and anti-oxidant activity thus may show synergistic effect along with H₁-histamine inhibition.
- The effect of substituents affects the negative binding energy and selectivity in the HMT active site cavity as well.
- The close overlapping shown in figure 4 defines the best choice of docking package; according to results obtained in this work.
- The data may open the new way of investigation of some novel potential anti-histamines.
- The negative binding scores, green procedure of synthesis, easy work up and isolation led chromone analogues as potential candidates for further research.

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