

In Silico Study of N^1 -Alkyltheobromine as Histamine-H1 Receptor Antagonist

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Abstract—Presently, we elucidate the mechanism of N^1 -alkyltheobromine derivatives as histamine-H1 antagonist at a molecular level using computational method (*in silico*). The experiment was set up by docking those N^1 -alkyltheobromines to the crystal structure of histamine-H1 receptor and the results showed that the ligands occupied the active pocket of histamine-H1 receptor by interacting with various amino acid residues such as Thr112, Tyr431, Ser111, Asp107 and Lys191 via hydrogen bonds as well as electrostatic interaction.

Index Terms— N^1 -alkylxanthine, histamine, docking

I. INTRODUCTION

For several decades, theophylline (1, 3-dimethylxanthine) and other xanthine derivatives have been recognized as effective agents for the treatment of reversible and chronic obstructive airways diseases [1]. Theophylline is a weak and nonselective inhibitor of cAMP specific phosphodiesterase (PDE). The inhibition can lead to an increase of intracellular cAMP, with a consequent bronchial relaxation or antiinflammatory effect [1]-[3]. Besides, theophylline has significant side effects that may be related to its A_1 receptor antagonism. It is therefore believed that more potent and selective A_{2B} receptor antagonists will provide enhancing the asthma treatment [4], [5].

Theobromine (3,7-dimethylxanthine) is other xanthine derivatives which was known having diuretic effect had a mild cardiac stimulation rather than theophylline [6]. Based on the structure-activity relationships studies, substitution with a long alkyl chain at the N^1 -position of xanthine nuclei increased the tracheal relaxant activity without leading to positive chronotropic action [7]. Extrinsic bronchial asthma is characterized by an increased airway responsiveness to non-specific and specific stimuli, such as histamine, leukotriene and allergen [8].

The first study of theophylline and theobromine derivatives as antihistamine was done by Pascal *et al.* showed that substitution with piperazine moiety at C4 of xanthine ring gave a bronchorelaxant effect of tracheal bronchospasm induced by histamine in guinea pig [9]. Later on, some N^1 -alkyltheobromine derivatives which showed tracheospasmodic activities against histamine as the spasmogen had been synthesized [10]-[13] (Fig. 1).

The structure-activity relationships study showed that the elongation of alkyl group in the N^1 of theobromine ring increased the tracheospasmodic activity which agreed with the previous study. This result opened the opportunity for theobromine to be developed as antihistamine.

One key computational methodology –docking of small flexible molecules (ligands) to protein binding sites (receptors) - remains a highly active area of research [14]. This method takes advantage of the X-ray structural information of the targeted enzyme to characterize its small molecule inhibitors. Docking study on histamine-H1 receptor had been carried out using homology model H1 receptor on pyrazinopyridoindols [15]. Other docking study was also conducted using histamine-H1 receptor crystal structure (PDB ID 3RZE) on some active compounds from *Aegle marmelos* CORREA [16] and traditional chinese medicine [17]. In this present study, we elucidate the mechanism of N^1 -alkylxanthine derivatives as histamine-H1 antagonists at a molecular level using molecular docking method.

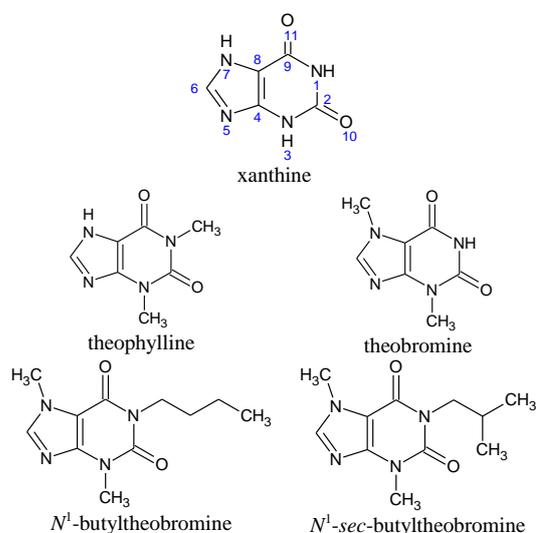


Figure 1. The structure of xanthine and its derivatives [10]-[13].

II. METHODOLOGY

A. Data set Collection and Generation

The 2D structure was sketched using ACD/Chemschetch (www.acdlabs.com) with the basic nitrogen being protonated and in contrast, the acidic oxygen is

deprotonated. The 3D conversion as well as energy minimization was computed using Hyperchem Professional version 8.0 (www.hyper.com). MM+ and Polak-Ribiere (Conjugate Gradient) were used as the force field and algorithm, respectively. The energy cut off for minimization was 0.1 kcal/mol.

B. Protein and Ligand Preparation

The histamine-H1 receptor (PDBID 3RZE) was used as the protein target. This protein was co-crystallized with doxepin in two isomers. The protein was separated from all those ligands using Discovery Studio 2.5 (www.accelrys.com) and then only ((3*E*)-3-(dibenzo oxepin-11(6H)-ylidene)-*N,N*-dimethylpropan-1-amine (Fig. 2) used as the ligand in control docking study. Both protein and ligands were prepared using AutoDockTools 1.5.6. (www.autodock.scrips.edu). The Kollman charge was added to the protein structure while the Gasteiger charge was applied for the ligands. The torsion of ligands was set to four rotatable bonds. The Grid Box was set in number of points $x = 68$, $y = 66$, and $z = 68$ as well as its spacing i.e. 0.375 Å. The center of mass being used in this Grid Box were $x = 16.499$, $y = 34.219$ and $z = 23.803$. The docking parameters were set up as followed: $ga_pop_size = 150$, $ga_num_evals = 2500000$, $ga_num_generations = 27000$, $ga_run = 50$.

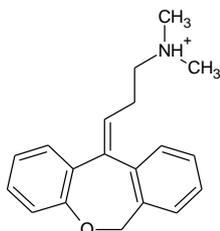


Figure 2. The structure of ((3*E*)-3-(dibenzo oxepin-11(6H)-ylidene)-*N,N*-dimethylpropan-1-amine.

C. Docking

The control docking was carried out by using Lamarckian algorithm in AutoDock4 package. The parameter set-up in control docking was approved if the RMSD of docked pose was less than 2.0 Å [18]. The free energy of binding (FEB) was defined as the sum of final intermolecular energies (van der Waals + hydrogen bond + desolvation energy), final total internal energy, torsional free energy and unbound system's energy.

III. RESULTS AND DISCUSSIONS

Histamine-H1 receptor crystal structure in complex with doxepin, the first antihistaminic generation, was resolved in 3.1Å of resolution [19]. At the initial pose, doxepin sits deeply in the hydrophobic pocket which is surrounded by some well conserved amino acid residues in aminergic receptor such as Asp107, Ile115, Phe424, Trp428 and Phe432 with FEB of -10.45 kcal/mol (Fig. 3). Two other amino acid residues which are not conserved in aminergic group also present, i. e. Trp158 and Asn198.

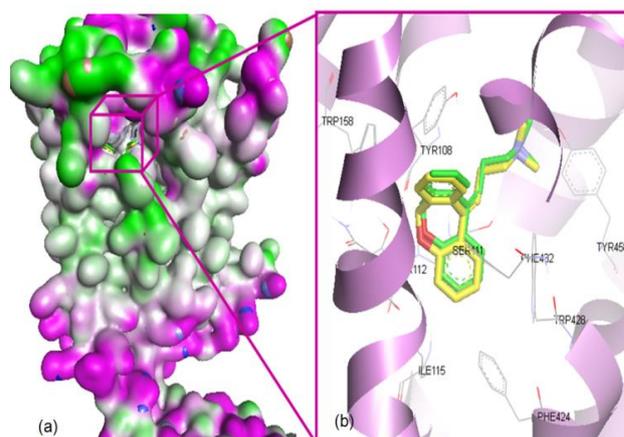


Figure 3. The superimpositions of doxepin at initial and re-docked poses against histamine-H1 presented in (a) surface form and in set (b) magenta ribbon form.

The interaction of doxepin at its amine moiety with histamine-H1 at Asp107 via electrostatic interaction was reported having essential role for this first antihistaminic generation antagonistic mechanism [20]-[22]. On the other hand, the tricyclic dibenzoxepin plays as hydrophobic moieties by interacting with the hydrophobic side chain of Ile115, Phe424, Trp428, Phe432 and Trp158. In addition, the pi-sigma interaction is observed within phenyl ring of doxepin and Ser111 while two pi-cation interactions are observed within protonated amine of doxepin with Tyr108 (data are not shown).

From this control docking, we gain some ideas to develop new models in order to have a comparable insight binding mode. Xanthine ring is obviously capable to be used as the scaffold. The alkylation of N^1 up to five numbers of carbons might gradually decrease the FEB from the unsubstituted theobromine. Furthermore, the amination at the terminal alkyl moiety covers the interaction of ligand with Asp107, one of the key residues in histamine-H1 receptor. The methylation of the amine group showed a lower FEB than the previous free amine (data is not shown), accordingly.

Next, as studied by Shimamura *et al.* (2011) [19] in molecular docking, the second generation of H1 antihistamine, olopatadine, reduced the CNS depressant effect of the first generation by interacting with Lys191 as well as Lys178. Therefore, the model 1 has attempted to gain this type of binding mode by introducing carboxylate group in the particular xanthine ring. Initially, we still utilize the lead model (N^1 -*n*-butyltheobromine) by extending the butyl chain up to pentyl chain. Afterall, the introduction of the carboxylate group at the N^7 linked by methylene group (-CH₂-) was carried out. The model 2 was designed by shortening the pentyl down to propyl moiety. The last model was designed by extending the methylene linker up to ethylene linker. The structure of Model 1-3 and Olopatadin are presented in Fig. 4. The values of docking results for these three models were presented in Table I.

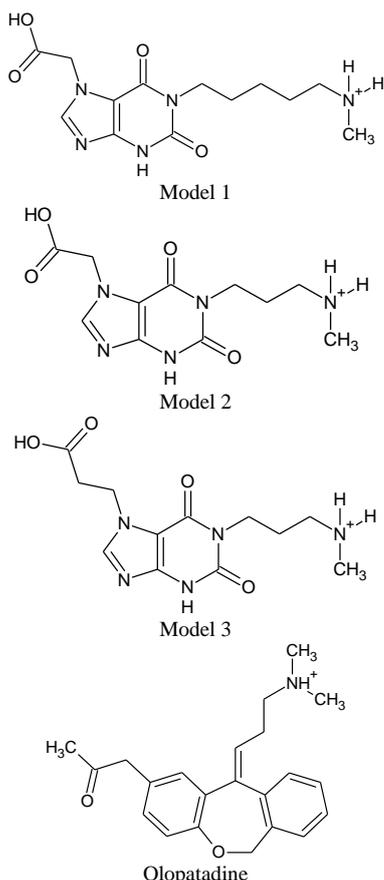


Figure 4. The structure of Model 1-3 and olopatadine.

TABLE I. THE FEB OF ALL MODELS, DOXEPINE AND OLOPATADINE

Models	FEB (kcal/mol)
1	-8.89
2	-9.13
3	-10.33
Doxepin	-10.45
Olopatadine	-12.47

As listed in the Table I, those docked ligands cover from higher to lower free energies of binding (from -8.89 to -10.45 kcal/mol). Some hydrogen bonds as well as electrostatic interactions are observed and predicted having contributions to its *in vitro* activities. The strong hydrogen bond interactions were defined when the distance between hydrogen and its corresponding heteroatom at a proximity distance less than 2.0Å.

In model 1, the 1, 1-methyl-pentylamine group is able to interact with Asp107 but the long pentyl moiety makes it bending down to the pocket defined by the side chains of helices III and then pushes away the carboxylate group from the pocket defined by Lys191 of helices VI (Fig. 5). However, some H-bond interactions such as interactions between carbonyl oxygen at C9 with Tyr431, carboxylate ion with Tyr108 as well as amino with Tyr458 still give contributions to the ligand-receptor affinity.

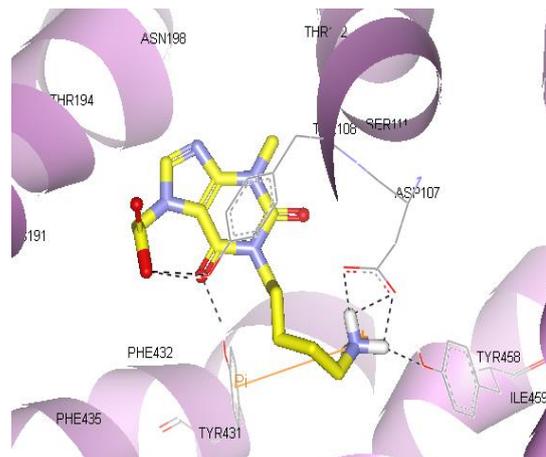


Figure 5. The docking pose of Model 1 against histamine-H1 receptor.

In order to minimize the steric effect, the length of N¹ alkyl chain must be reduced. In the next model (Model 2), the pentyl group was shortened down to propyl group by estimating that if the nitrogen of amine was linked away by eight numbers of atoms (an approximately distance is 8.4Å) to the carboxylate group, it will adapt its conformation and move forward to Lys191. As predicted, the docking pose showed a new H-bond interaction between carbonyl oxygen with Lys191 while maintaining the H-bond interaction between amine and Asp107 (Fig. 6). In line with this, the FEB of Model 2 with its propyl moiety is lower than that of Model 1.

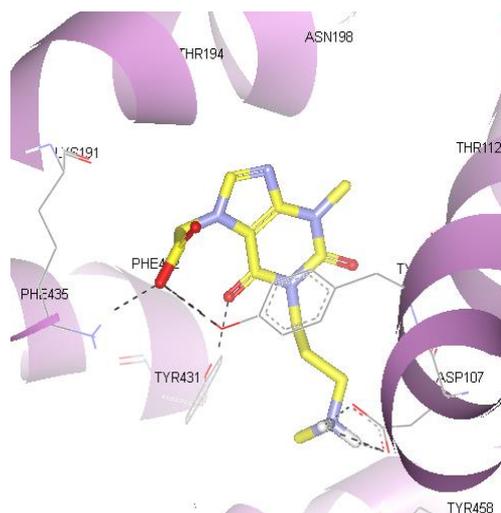


Figure 6. The docking pose of Model 2 against histamine-H1 receptor.

The effort to produce the docking conformation with FEB being more comparable with doxepin was attempted by extending the alkylene link in carboxylate moiety to N⁷. Subsequently, ethylene link was utilized in Model 3 to substitute the previous methylene in Model 2. As observed, the FEB is dramatically decreased down to -10.33 kcal/mol. This energy is mainly contributed by a 0.2220Å and 0.4881Å shorter proximity distance of H-bond interaction between amine and Asp107 as well as carboxylate with Lys191, respectively, than Model 2 (Fig. 7).

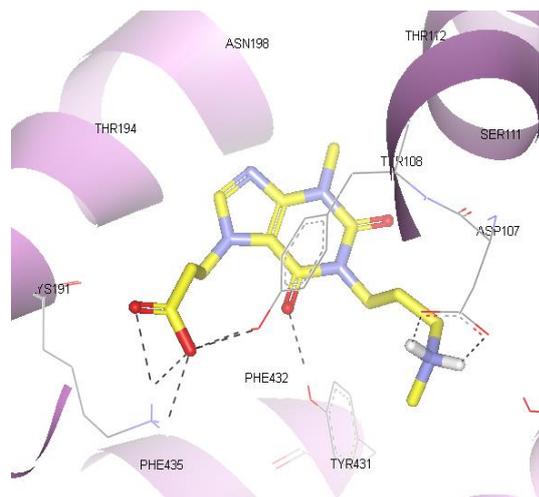


Figure 7. The docking pose of Model 3 against histamine-H1 receptor.

In addition, in this conformation, the ligand is able to form three H-bond interactions with Lys191 might be due to the reason why the affinity of Model 3 to histamine-H1 is higher than that of Model 2 and 1.

As visualized in Fig. 8, the docking pose of olopatadine as the reference for the second antihistamine-H1 generation, is relatively similar to the docking pose of Model 2 as well as Model 3 even though the FEB of those two models are still higher than that of olopatadine. Nevertheless, Model 3 can be used as the new designed molecule having a predicted activity comparable with doxepin in term of antihistaminic activity as well as olopatadine in term of the less CNS depressant side effect. Hence, the synthesis of this new model is highly recommended to prove the concept of this *in silico* study.

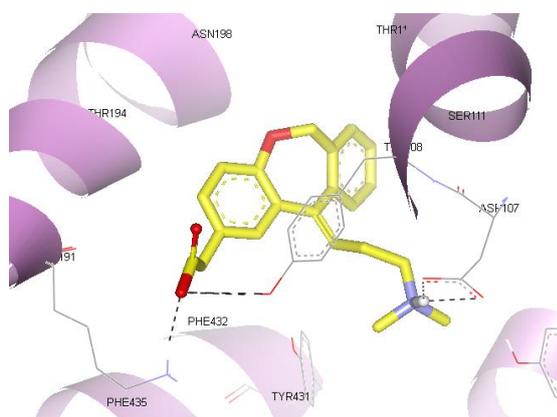


Figure 8. The docking pose of olopatadine against histamine-H1 receptor.

IV. CONCLUSION

Model 3 can be used as the new designed molecule having a predicted activity comparable with doxepin in term of antihistaminic activity and olopatadine, in term of the less CNS depressant side effect. Hence, the synthesis of this new model is highly recommended to prove the concept of this *in silico* study.

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