

Full Length Research Paper

Generating a 3D structure model of Histamine-4 receptor for Antiinflammatory Drug Design

Jutti Levita*, Nyi Mekar Saptarini and Ardi Ardiansyah

Pharmacochemistry Division, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km.21 Jatinangor, West Java, Indonesia 45363.

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Histamine-4 receptor (H4R) belongs to the GPCRs (G protein-coupled receptors) family. The H4R involved in allergic pathogenesis, autoimmune diseases such as arthritis, type-I allergic diseases such as asthma, conjunctivitis, and rhinitis. This macromolecule has never been crystallized yet. In drug design, the need to obtain 3D structure of macromolecules is to bridge the gap of ligand- and receptor-based methods. Three-dimensional structure of the target is essential for defining the active site and also for designing, improving, and docking of small ligands to the complex target protein. This study was aimed to generate H4R 3D structure that fulfilled the quality parameters of DOPE and QMEAN by using homology modeling principles. The method used in this work was performed in several steps as follows: (1) searching for the H4R sequence data and identifying protein structures similar to the data to be used as templates for H4R; (2) automatic sequence alignment to calculate the similarity between the templates and H4R sequence; (3) building the H4R 3D structure models using SWISS-MODEL and MODELLER 9v7; and (4) validating the 3D structure models by using DOPE, QMEAN, and Ramachandran plot. This study produced four H4R models that were validated by Ramachandran plot. The best model was applied to dock histamine by using AutoDock 4.0. It could be concluded from this work that 3D structure of H4R was able to be generated and applied to dock histamine in its predicted active site. The active site consists of six amino acid residues which are Asp94, Tyr95, Glu182, Trp316, Tyr319, and Phe344. Its agonist, histamine, is bound in the binding site of this protein via the formation of two hydrogen bonds with Asp94 and Tyr319.

Key words: H4R 3D structure, homology modeling, histamine.

INTRODUCTION

Histamine is neurotransmitter and local mediator that regulates some cell functions. The diverse biological effects of histamine are mediated through different histamine receptors, named G-protein-coupled receptors (GPCRs). Four different histamine receptors, namely, the H1, H2, H3, and H4 receptors, have been identified. The H1 receptor mediates symptoms of allergic reaction, including smooth muscle contraction, vasodilatation, and sensory nerve activation. The H2 receptor enhances gastric acid secretion in the stomach. The H3 receptor regulates the release of histamine and neurotransmitters by neurons (Hill *et al.*, 1997). The H4 receptor restricted to cells of haematopoietic lineage, in particular, mast

cells, basophils, and eosinophils (Oda *et al.*, 2000; Morse *et al.*, 2001). The hH4R gene has been cloned on the basis of its relatively high homology with the H3R (Liu *et al.*, 2001). Gantner and colleagues showed that H4R and H2R controlled the induction of interleukin-16 which was released by CD8 T-cells (Gantner *et al.*, 2002). Antagonists specific for H4R have been generated and they are valuable tools for dissecting the biological roles of H4R (Jablonowski *et al.*, 2003). The H4R antagonists showed *in vivo* anti-inflammatory activities (Robin *et al.*, 2004). H4R is also involved in itch pathogenesis, autoimmune diseases such as arthritis rheumatoid, type-I allergic diseases such as asthma, conjunctivitis and rhinitis (Thurmond *et al.*, 2008). The physiological role of H4R is still unclear.

In drug design, the need to obtain 3D structure of macromolecules is to bridge the gap of ligand- and

*Corresponding author. Email: jutti.levita@unpad.ac.id

Table 1. The best template candidates for H4R modeling

No	Hit	Name	Probability	Score	SS	E-value	P-value	Cols	Query HMM	Template HMM
1	2rh1 A	Beta-2-adrenergic receptor	100.0	410.8	23.6	0	0	380	3-386	28-488 (500)
2	3em1 A	Human adenosine A2A receptor	100.0	398.8	24.1	0	0	365	16-382	22-474 (488)

receptor-based methods. Three-dimensional structure of the target is essential for defining the active site and also for designing, improving, and docking of small ligands to the complex target protein. Experimental determination of protein structure through X-ray crystallography or nuclear magnetic resonance spectroscopy remains as a difficult and costly process. The number of possible folds protein in nature appears to be limited and the 3D structure of proteins is better conserved than their sequences, it is often possible to identify a homologous protein with a known structure (template) for a given protein sequence (target) (Chothia, 1992). Homology modeling had proven to be the chosen method to generate a reliable 3D model of a protein structure from its amino acid sequence (Tramontano and Morea, 2003). Two main approaches to computational structure determination are *de novo* prediction and comparative modeling. Similar project performed by Werner and colleagues chose the crystal structure of human β 2-adrenergic G-protein-coupled receptor in its inactive state (PDB 2rh1) as template to build the homology model of H4R by MODELLER 9v3 (Werner and Tanrikulu, 2009).

MATERIALS AND METHODS

The H4R sequence (Gene ID: 59340) was downloaded from www.ncbi.nlm.nih.gov. The H4R sequence was submitted to <http://tookit.tubingen.mpg.de/hhpred> to identify the structural template. The web server searches the template automatically based on homology. Automatic sequence alignment was carried out by ClustalX2 v2.0.11. Homology models of H4R were built by using SWISS-MODEL (<http://swissmodel.expasy.org>) and the windows version of MODELLER (<http://salilab.org/modeller>). SWISS-MODEL and MODELLER are the softwares in comparative protein modeling that were used in this study.

SWISS-MODEL is the oldest homology modeling technique in which sections from aligned regions of the template are connected together or separately constructed non-conserved region to form the backbone model. Suitable template structure with similar sequences to the query was identified by BLAST search of the SWISS-MODEL template library ExpDB, to generate the protein. The selected templates were then superposed using an interactive least squares algorithm, the backbone atom positions averaged and the query sequence fitted to the template to optimize placement of insertion and deletion regions (Guex and Peitsch, 1997; Schwede *et al.*, 2003; Arnold *et al.*, 2006).

MODELLER has become the model building program of choice for several homology servers because of its relative speed and reliability. MODELLER generates a 3D model starting from protein sequence. The sequence is converted into a profile, which is searched against a MODELLER-specific database of PDB sequences. Homologous sequences are submitted to a multiple sequence alignment, which in turn is used to construct a multiple structure alignment to identify the optimal template structures. These template structures are finally used to calculate the spatial restraints (Sali and Blundell, 1993; Sali *et al.*, 1995; Eswar *et al.*, 2007).

The models were validated by DOPE, QMEAN, and Ramachandran plot, respectively as followed: the one with the best DOPE and/or QMEAN scores was selected for further analysis by Ramachandran plot (Habeeb *et al.*, 2011).

Finally, the best model was used to dock its agonist (histamine) by AutoDock v1.4.4. Active sites determination was carried out by Q-SiteFinder (<http://www.modelling.leeds.ac.uk/qsitefinder/>).

RESULTS AND DISCUSSION

The H4R sequence from *Homo sapiens* consists of 390 amino acids (Oda *et al.*, 2000). This sequence was submitted to <http://tookit.tubingen.mpg.de/hhpred> to identify the 3D structure's template. The web server automatically searched templates from crystallized protein data bank based on H4R amino acid sequence homology. The hits showed two templates of protein codes (Table 1), which are beta-2-adrenergic receptor (symbolized as 2rh1 A) and human adenosine A2A receptor (symbolized as 3em1 A).

Probability showed the true positive correlation of the templates with H4R sequence based on score and SS (secondary structure) parameters. Higher score indicates closer relation to H4R.

The E-value (energy value) shows total average of false positive. The E-value (=0) of this study confirmed that beta-2-adrenergic receptor (2rh1 A) and human adenosine A2A receptor (3em1 A) were the best template candidates for H4R modeling.

Automatic sequence alignment was carried out by ClustalX2 v2.0.11 based on dynamic programming, i.e. algorithm to determine the best possibility to find the similarity between template and H4R sequence. The homology principle was based on similar genetic

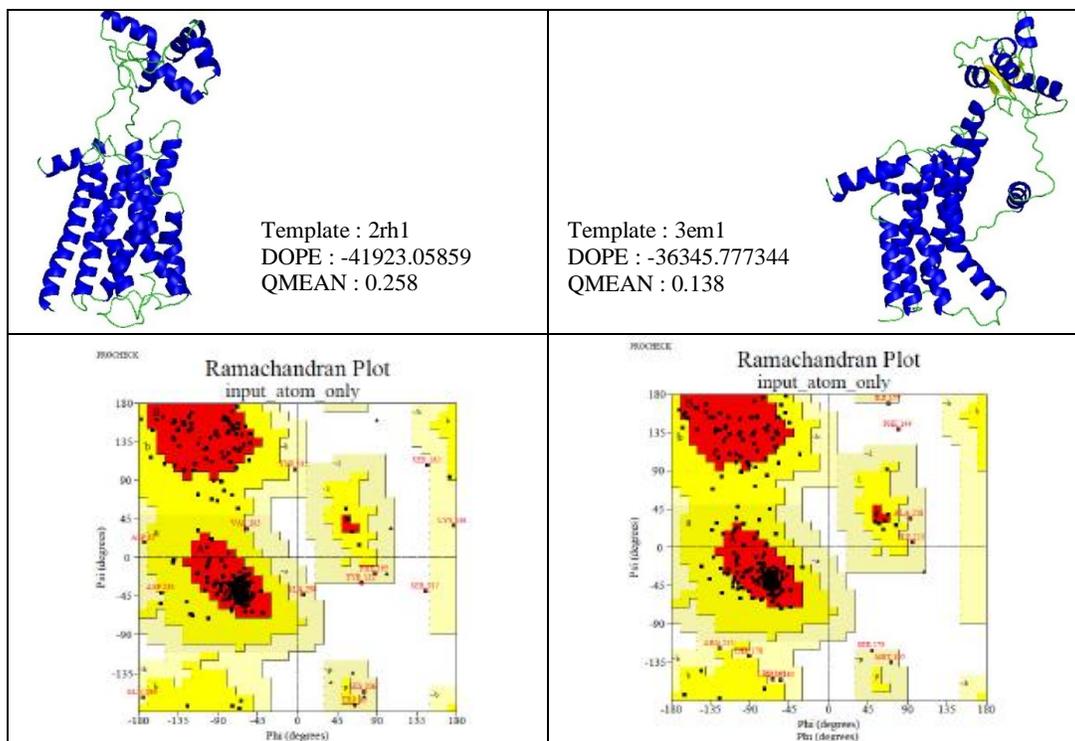


Figure 1. The H4R 3D structures generated by using SWISS-MODEL

relationship.

The two templates were further used to build H4R structure based on their similarities, while other parts were generated by SWISS-MODEL and MODELLER softwares. Results showed that similarity were only 21.54% for 2rh1 and 11.79% for 3em1, hence the models were categorized in the twilight zone. The twilight zone refers to less than 30% of amino acid sequences similarity. The output of this step was four 3D structure models of H4R written in pdb files. All four models were validated by DOPE, QMEAN, and Ramachandran plot. DOPE was applied to achieve the energy value of the distance of atoms that do not have interactions. QMEAN was used to calculate the scoring function to assess the accuracy of the models. Ramachandran plot defined the amino acid position probabilities in nature (Figure. 1 and 2).

The H4R model from 2rh1 template, which was built by using MODELLER, gave better DOPE value than that of SWISS-MODEL, on the contrary the same model built by using SWISS-MODEL showed better QMEAN value.

The H4R model from 3em1 template, which was built by using MODELLER, gave better DOPE and QMEAN values than those of SWISS-MODEL.

The H4R models that were built using SWISS-MODEL showed incomplete amino acids sequences (365 AA for 2rh1 and 363 AA for 3em1), because the amino acids were spliced at the beginning and at the end of

sequence but did not influence the result of the models.

The Ramachandran plot showed that H4R models from 2rh1 template that were built by using both MODELLER and SWISS-MODEL gave better result than the models from 3em1 template (showed by higher percentages in red region which means most favoured regions). The percentage of the H4R models from 2rh1 template was 90.9% for MODELLER (87.0% for SWISS-MODEL), while the H4R models from 3em1 template was 86.7% for MODELLER (82.2% for SWISS-MODEL).

The H4R structure from 2rh1 template which was built using MODELLER was the best model, because it had the best in DOPE value and Ramachandran plot. This 3D structure of H4R was analyzed by submitting it to <http://www.modelling.leeds.ac.uk/qsitfinder/> and then further applied to dock histamine by using AutoDock v1.4.4 for defining the active site of the protein (Figures 3 and 4).

Q-SiteFinder calculates the active sites of the protein based on the most favorable binding energy. Predicted binding site selection is colour-coded according to the likelihood of being an actual binding site. Green is the most likely, followed by blue, purple and orange/brown (<http://www.modelling.leeds.ac.uk/qsitfinder/>).

The last step was docking of histamine, the agonist of histamine receptor, to the H4R structure (Table 2). This step was carried out to proof that the generated protein has its function as predicted. Histamine was docked into

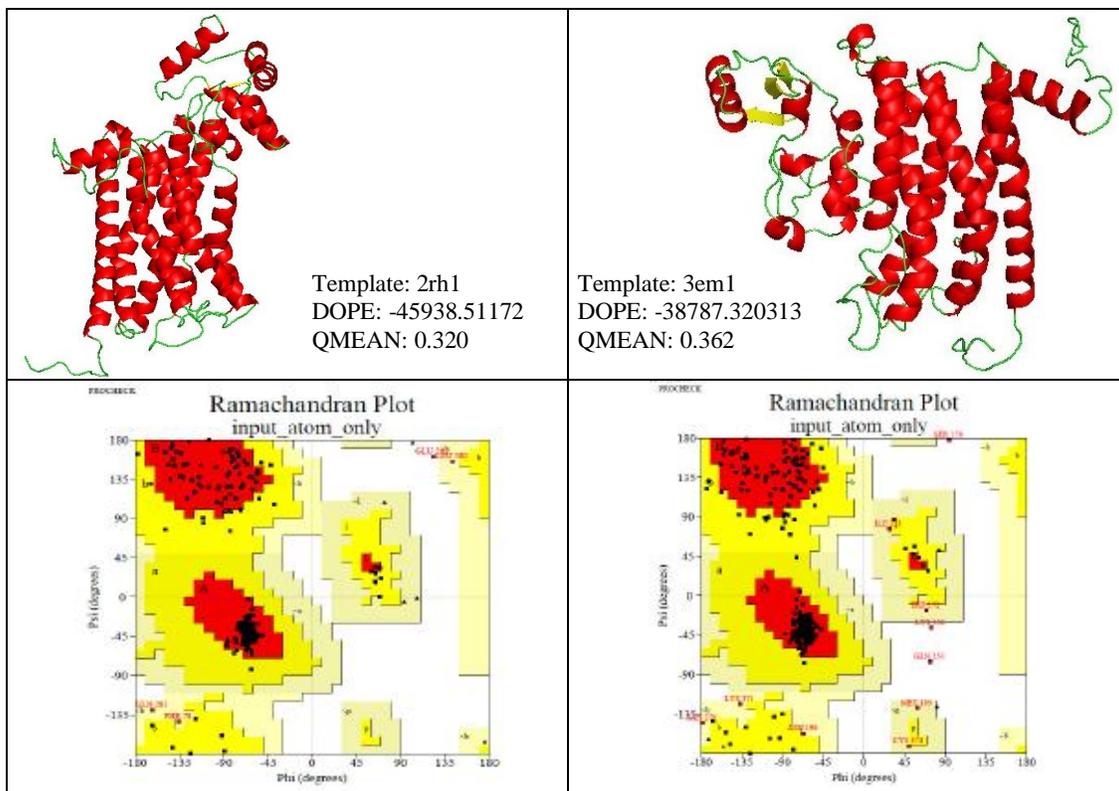


Figure 2. The H4R 3D structures generated by using MODELLER

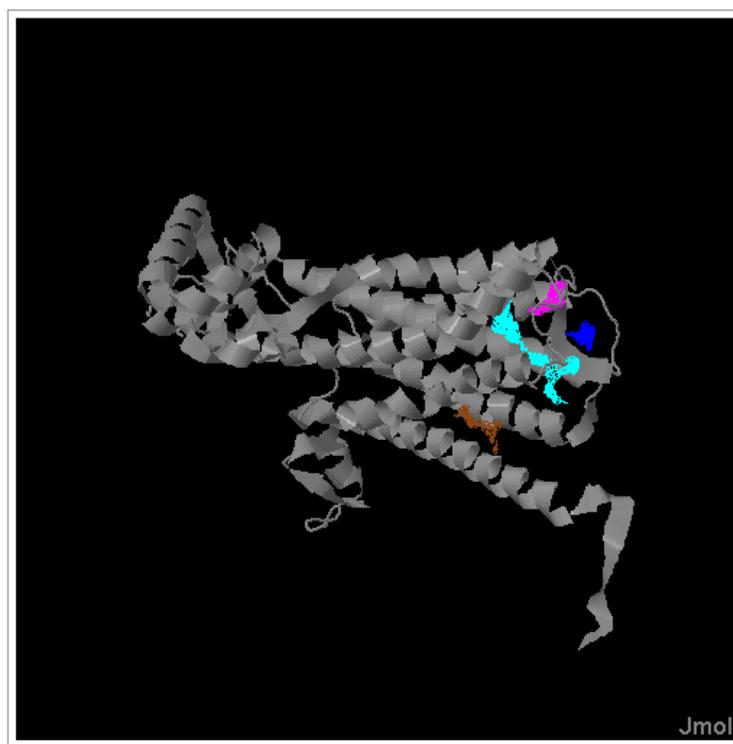
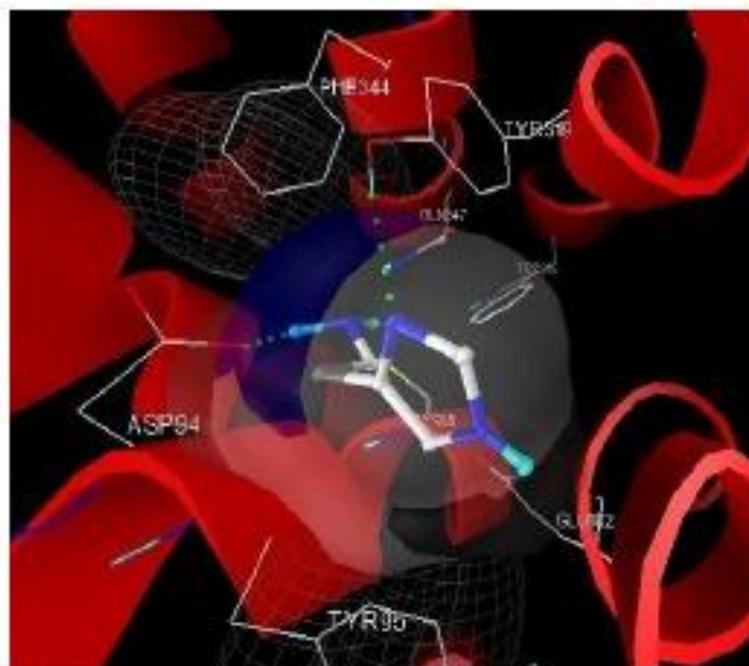


Figure 3. Active site prediction of H4R by Q-SiteFinder (<http://www.modelling.leeds.ac.uk/qsitefinder/>)

Table 2. Docking of histamine into H4R structure

Energy ^a (Kcal/mol)	K _i ^b (M)	ΣHB ^c	Amino acid residue
-6.22	7.14e-005	2	Asp94, Tyr95, Glu182, Trp316, Tyr319, Phe344

^a docking energy, ^b inhibition constant, ^c hydrogen bonding

**Figure 4.** Binding mode of histamine at the H4R binding site

the most likely predicted binding site of the protein (showed with green color in Figure 3).

Docking showed that histamine is able to be located in the binding site of generated-H4R structure (its binding energy is -6.22 kcal/mol). The active site of H4R consists of six amino acid residues which are Asp94, Tyr95, Glu182, Trp316, Tyr319, and Phe344. Histamine is bound in the binding site of this protein via the formation of two hydrogen bonds with Asp94 and Tyr319 (Figure 4).

Conclusion

3D structure of H4R was able to be generated and applied to dock histamine in its predicted active site. The active site consists of six amino acid residues which are Asp94, Tyr95, Glu182, Trp316, Tyr319, and Phe344. Its agonist, histamine, is bound in the binding site of this protein via the formation of two hydrogen bonds with Asp94 and Tyr319.

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