QSAR and Molecular Docking Studies on Non-Imidazole-Based Histamine H_3 Receptor Antagonists

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Abstract

Background: In the recent years, histamine H_3 receptor (H_3R) has been receiving increasing attention in pharmacotherapy of neurological disorders. The aim of the current study was to investigate structural requirements for the prediction of H_3 antagonistic activity using quantitative structure-activity relationship (QSAR) and molecular docking techniques.

Methods: To this end, genetic algorithm coupled partial least square and stepwise multiple linear regression methods were employed for developing a QSAR model. The obtained QSAR model was stringently assessed using different validation criteria.

Results: The generated model indicated that connectivity information and mean absolute charge are two important descriptors for the prediction of H_3 antagonistic activity of the studied compounds. To gain insight into the mechanism of interaction between studied molecules and H_3R, molecular docking was performed. The most important residues involved in the ligand-receptor interactions were identified.

Conclusion: The result of current study can be used for designing of new H_3 antagonist and proposing structural modifications to improve H_3 inhibitory potency.

Introduction

Histamine as multifunctional small biogenic amine contributes to a broad range of physiological functions, including allergic reactions, inflammatory responses, gastric acid secretion. Moreover, owing to its neurotransmission property, it is responsible for regulation of sleep-wake cycle, cognition, arousal, and memor.[1-4] Although the major source of histamine is mast cells and basophils, it is also found in stomach enterochromaffin-like (ECL) cells as well as neurons of central/peripheral nervous system. Histamine elicits its biological functions through four distinct G-protein coupled receptor (GPCR) designated as H_1R, H_2R, H_3R, and H_4R. Among the histaminergic receptor subtypes, histamine H_3 receptor is prominently expressed as auto- and heteroreceptor in the central nervous system (CNS) responsible for modulation of synthesis and release of histamine and other neurotransmitters such as norepinephrine, dopamine, serotonin, and acetylcholine through a negative feedback mechanism.[5,6] Since discovery of histamine H_3 receptors in 1983,[7] tremendous advances have been achieved in identification of H_3-related ligands.[8,9] The pharmacological importance of this receptor in the pathophysiology of many diseases such as neurological disorders has motivated the great interest in the development of novel

H_3 antagonists/inverse agonists. There are several lines of evidence demonstrating the effectiveness of H_3 antagonists/inverse agonists in several neurological disorders such as narcolepsy, Alzheimer, attention deficit hyperactivity disorder (ADHD), epilepsy just to mention a few.[5,10,11] The H_3 antagonists are mainly divided into two classes of compounds: imidazole- and non-imidazole-based compounds. At the beginning, the initial studies were focused on imidazole-containing compounds through structural modification of endogenous ligand (i.e. histamine). However, this type of compounds was not successful in the context of drug design and development. Poor brain penetration, rapid metabolism, CYP450 enzyme inhibition, off-target activity are the main obstacles associated with imidazole-containing compounds preventing them to be entered the pharmaceutical market.[12-18] These undesirable features were the driving force for designing the non-imidazole based compounds. This was made available by substitution of imidazole ring to its bioisosteres such as piperidine, pyrrolidine, piperazine. The only successful example of therapeutic agent belonging to non-imidazole based compounds is pitolisant (Wakix®) approved by the European Medicines Agency for the treatment of narcolepsy with

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or without cataplexy.[19,20] Currently, there are several non-imidazole containing compounds at different phases of clinical trials (see[9] for comprehensive details). The biological activities of the compounds are attributed to their chemical structures, and hence, there are a variety of approaches where therapeutic agents are rationally designed and developed based on their structural features. Among which computational studies accelerate the identification of "lead compounds" through significant reduction of the time and costs associated with drug design processes. Quantitative structure-activity relationship (QSAR) is one of the useful techniques in computational studies.[21,22] It relies on mathematical relationship between biological endpoints and the structural features of the studied compounds. However, the development of a reliable QSAR model is not a trivial task and needs appropriate validation by performing rigorous statistical tests. In this study, binding affinities of a set of non-imidazole based H_3 antagonists are rationalized through QSAR and docking techniques. The results can be used for the design and discovery of novel non-imidazole based H_3 antagonists with improved biological activities.

Materials and Methods
QSAR study
Multiple linear regression based model
A dataset of non-imidazole containing compounds were collected from those synthesized by Stark's group[12,17,18,23,24] in which the binding affinities H_3 receptor stably expressed in CHO-K1 cells were determined by radioligand binding assay using [^{125}I]-Iodoproxyfan. The binding affinities (K_i) used in QSAR analysis as uniformly being transformed as pK_i (-log K_i). The 3D structures of the studied compounds were generated using Hyperchem software (version 8.0.8) followed by energy minimization using MM+ force field. Then, AM1 level of theory implemented in semiempirical method was used for further fully optimization of the generated structures.[25,26] Hyperchem, Dragon (version 5.0) and ACDlabs suite of programs (version 2015.2.5) were employed to calculate molecular descriptors. After calculation of molecular descriptors, pretreatment procedure on the calculated descriptors was performed through standard normalization (i.e., auto-scaling). To do this, the entire values of a given descriptor were transformed to auto-scaled values of which they have a mean of zero and variance of unity.

By applying Kennard-Stone, Euclidean distance, and activity-property algorithms, the training and test sets were generated with a 3:1 ratio, respectively.[27,28] Following the dataset division, in order to choose top ranking parameters, the selection was carried out on training set using PLS coupled genetic algorithm (GA-PLS) tool[29,30] implemented in the MATLAB programming environment repeated for ten times. The default parameters for GA-PLS tool were population size, 30; probability of mutation, 0.01; probability of cross-over, 0.5; number of runs, 100. The selected parameters (independent variables) were subjected to multiple linear regression (MLR) modeling for finding the best predictive QSAR model considering the biological activities of studied compounds as dependent variables. The final descriptors in the QSAR model were selected based on their significant p-values (less than 0.05) of their corresponding coefficient values.

Statistical validation criteria
Following the generation of the model, different approaches were utilized for further validation of the QSAR model including internal and external assessment criteria. These include leave-one-out Q^2, SDEP, r_m^2, $^cR_p^2$ for internal validation and R_{Test}^2, Q_{F1}^2, Q_{F2}^2, Q_{F3}^2, CCC, and MAE for external validation .

Leave-one-out (LOO) cross validation method is used for internally validating of a generated model. In this approach compounds are excluded one-by-one in order to predict their endpoint activities based on the model trained using the remaining compounds. The metric showing the predictive capability of the LOO method is determined using the following equation:

$$Q^2 = 1 - \frac{\sum \left(Y_{obs(train)} - Y_{pred(train)} \right)^2}{\sum \left(Y_{obs(train)} - \overline{Y}_{train} \right)^2}$$

Y_{obs}, Y_{pred}, $\overline{Y}_{train}$ and   are observed, predicted and average of trained activity values.

For calculation of standard deviation error of prediction (SDEP) the following formula was used:

$$SDEP = \sqrt{\frac{\sum \left(Y_{obs(train)} - Y_{pred(train)} \right)^2}{n}}$$

where n refers to the number of data points.

The other parameter used for internally evaluation of the generated model is r_m^2 suggested by Roy et. al.[31] The following equations demonstrate the calculation of this parameter:

$$\overline{r_m^2} = \frac{(r_m^2 + r'^2_m)}{2}$$

$$\Delta r_m^2 = \left| r_m^2 - r'^2_m \right|$$

$$r_m^2 = r^2 \times \left(1 - \sqrt{r^2 - r_0^2} \right), r'^2_m = r^2 \times \left(1 - \sqrt{r^2 - r'^2_0} \right)$$

$$r_0^2 = 1 - \frac{\sum \left(Y_{obs} - k \times Y_{pred} \right)^2}{\sum \left(Y_{obs} - \overline{Y}_{obs} \right)^2}$$

$$r'^2_0 = 1 - \frac{\sum \left(Y_{pred} - k' \times Y_{obs} \right)^2}{\sum \left(Y_{pred} - \overline{Y}_{pred} \right)^2}$$

$$k = \frac{\sum \left(Y_{obs} \times Y_{pred} \right)}{\sum \left(Y_{pred} \right)^2}$$

$$k' = \frac{\sum \left(Y_{obs} \times Y_{pred} \right)}{\sum \left(Y_{obs} \right)^2}$$

In these equations, r^2 shows the squared correlation coefficient between observed and predicted values considering the intercept of the model. The r_0^2 and r'^2_0 are the squared correlation coefficients while the line is passed from the origin. In the case of r'^2_0, X and Y axes are reversed. The slopes of the squared correlation coefficients are denoted by k and k', respectively.

The $^c R_p^2$ is the other parameter for assessing the robustness of the QSAR model.[32] One of the methods for assessing the quality of the generated QSAR model is Y-scrambling method where the models are produced using randomization of biological activities. The determined criterion for this method is calculated by $^c R_p^2$ as below.

$$^c R_p^2 = R\sqrt{R^2 - R_r^2}$$

where R^2 and R_r^2 are the calculated correlation coefficients of the models prior and after randomization of the endpoints values.

Moreover, for externally validating the developed trained QSAR model, the endpoint values for test dataset are predicted. A correlation coefficient (R_{pred}^2) is defined to demonstrate the predictive capability of the model using the following equation:

$$R_{pred}^2 = 1 - \frac{\sum (Y_{obs(test)} - Y_{pred(test)})^2}{\sum (Y_{obs(test)} - \overline{Y}_{(training)})^2}$$

In this equation, observed and predicted biological values for the test compounds are shown by by $Y_{obs(test)}$ and $Y_{pred(test)}$, respectively, whereas the average values of biological activities for training set are depicted by $\overline{Y}_{training}$. The other considered conditional parameters known as Golbraikh and Tropsha's criteria are shown as below:[33]

$$\frac{\left(R^2 - R_0^2 \right)}{R^2} < 0.1 \, and \, 0.9 \leq k \leq 1.1$$

$$\frac{\left(R^2 - R_0'^2 \right)}{R^2} < 0.1 \, and \, 0.9 \leq k' \leq 1.1$$

$$\left| R_0^2 - R_0'^2 \right| < 0.3$$

Root mean square error of prediction (RMSEP)[34] and mean absolute error (MAE)[35] are another external validation parameters depicting the differences of biological activity between observed and predicted values of the test set calculated as:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - Y_{pred(test)} \right)^2}{n_{EXT}}}$$

$$MAE = \frac{\sum_{i=1}^{n_{EXT}} \left| \left(Y_{obs(test)} - Y_{pred(test)} \right) \right|}{n_{EXT}}$$

The other parameters known as Q^2 family functions originally derived from Q^2 leave-one-out internal cross validation are defined as followings:[36-39]

$$Q_{F1}^2 = 1 - \frac{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - Y_{pred(test)} \right)^2}{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - \overline{Y}_{train} \right)^2}$$

$$Q_{F2}^2 = 1 - \frac{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - Y_{pred(test)} \right)^2}{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - \overline{Y}_{test} \right)^2}$$

$$Q_{F3}^2 = 1 - \frac{\sum_{i=1}^{n_{EXT}} \left(Y_{obs(test)} - Y_{pred(test)} \right)^2 / n_{EXT}}{\sum_{i=1}^{n_{train}} \left(Y_{obs(train)} - \overline{Y}_{train} \right)^2 / n_{train}}$$

Concordance correlation coefficient (CCC) is the other measure to illustrate an inverse relationship between scatteredness of the observed *vs* predicted test set endpoints and accuracy of the model.[40] The following formula indicates the calculation of CCC parameter:

$$CCC = \frac{2\sum_{i=1}^{n}(Y_{obs(test)} - \overline{Y}_{obs(test)}) \left(Y_{pred(test)} - \overline{Y}_{pred(test)} \right)}{\sum_{i=1}^{n} \left(Y_{obs(test)} - \overline{Y}_{obs(test)} \right)^2 + \sum_{i=1}^{n} \left(Y_{pred(test)} - \overline{Y}_{pred(test)} \right)^2 + n \left(Y_{obs(test)} - \overline{Y}_{pred(test)} \right)}$$

The applicability domain for the studied non-imidazole based H₃ antagonists was determined Roy's MLR plus validation tool.[41]

Molecular docking

In order to predict the interactions between studied H₃ antagonists and histamine H₃ receptor, each compound was docked on the modeled H₃ model [42] using the GOLD program (version 5.0, CCDC, Cambridge, UK), running under LINUX operating system. The docking procedure was carried out based on the procedure reported previously.[42] Briefly, the binding site was determined based on the known amino acids (i.e. Asp[114], Thr[119], Tyr[189], Phe[198], Glu[206], Trp[371], and Tyr[374]) involved for the interactions in the binding site of H₃ receptor. Flexible docking of the compounds were performed using two constraints: one of them between nitrogen atom of piperidine ring of the compounds and oxygen atom of Glu[206] side chain from protein, and the other one between phenyl rings from the studied compounds and Tyr[189] side chain of protein.

Table 1. List of non-imidazole based compounds for QSAR-based analysis. Test set data have been characterized by asterisk *.
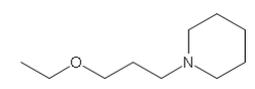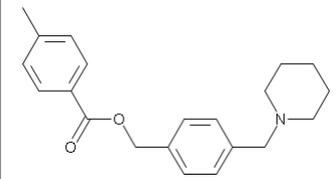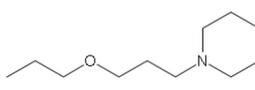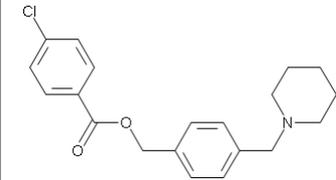
No	Structure	pKi	Ref.	No	Structure	pKi	Ref.
1*		6.15	17	29		7.4	21
2		6.13	17	30		6.6	21
3		6.14	17	31		7.1	21
4*		6.15	17	32		6.7	21
5		6.14	17	33		6.3	21
6		6.25	17	34		6.6	21
7		6.47	17	35		6	21
8		6.47	17	36*		8.8	21
9		6.51	17	37		6.6	12
10*		6.42	17	38*		6.56	12
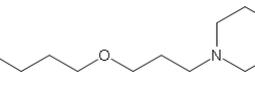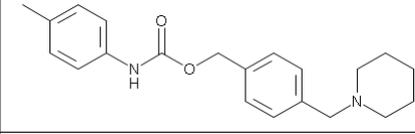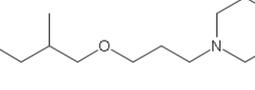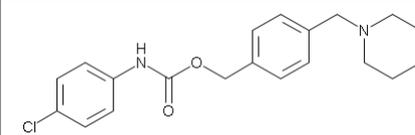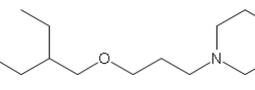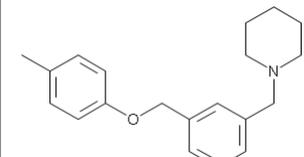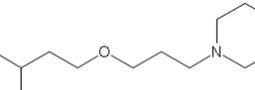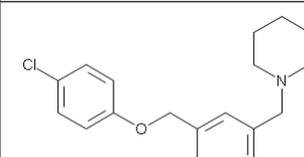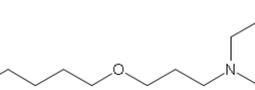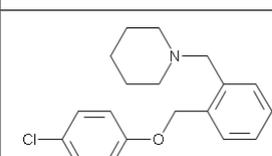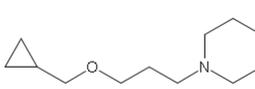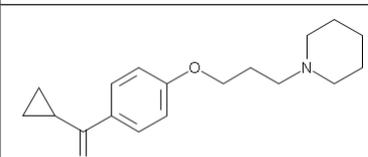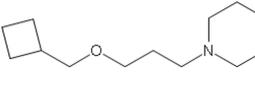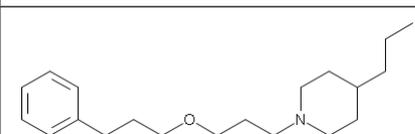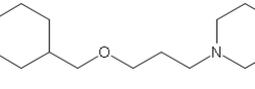
Table 1 Continued.

No	Structure	Value	Ref	No	Structure	Value	Ref
11		7.40	17	39		6.49	12
12		6.41	17	40		6.41	12
13*		6.28	17	41		6.25	12
14		6.47	17	42*		6.63	12
15		8.25	17	43		6.88	12
16		7.51	17	44*		6.79	12
17		7.66	17	45*		6.52	12
18		8.08	17	46		6.75	12
19		6.9	21	47*		6.78	12
20*		6.9	21	48		7	12
21*		7.4	21	49		8.51	12
22		7.1	21	50*		6.45	18

Table 1 Continued.

23		7.1	21	51		6.75	18
24		6.9	21	52		7.51	18
25		6.7	21	53		7.51	18
26		6.6	21	54		6.77	18
27		6.8	21	55		7.96	18
28		6.7	21	56*		7.72	8

Results and Discussion

In the past decades, H_3 antagonists have been the topic of many researches in pharmaceutical sciences. The effectiveness of H_3 antagonists has been evidenced in many neurological disorders in preclinical and clinical studies. In the current study, a set of non-imidazole based compounds were selected for QSAR and molecular docking studies. The structures used for this study are illustrated in Table 1. To perform the QSAR analysis, the structures of the compounds were generated and optimized energetically, and then, almost close to 1500 molecular descriptors including constitutional, thermodynamic, topological,

geometrical, and electronic descriptors were calculated. Following the normalization of the data (i.e. SD=1 and mean=0), the curated descriptors were subjected to train (%75) and test (%25) sets division using Kennard-Stone, Euclidean distance, and activity-property algorithms. The descriptor reduction was performed on the basis of GA-PLS technique followed by MLR-based model generation. The significance of variables in the final QSAR model was decided according to the p-values of the coefficient values obtained for the variables (i.e. values less than 0.05). The selected variables did not show any significant intercorrelation.

Table 2. The summarized statistics for QSAR models along with their threshold values using non-imidazole based compounds.

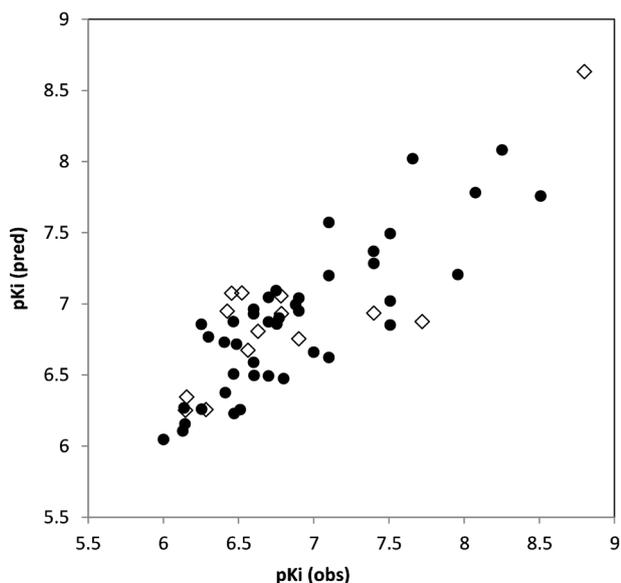|	R^2	R^2_{adj}	Q^2_{LOO}	R^2_{pred}	$\overline{r^2_{(m)}}LOO$	$\Delta r^2_m LOO$	$\overline{r^2_{(m)}}test$	$\Delta r^2_m test$	$^c R^2_p$
Model	0.7	0.67	0.58	0.69	0.47	0.15	0.53	0.2	0.66
Threshold			>0.5	>0.6	>0.5	<0.2	>0.5	<0.2	
	RMSEP	Q^2_{F1}	Q^2_{F2}	Q^2_{F3}	CCC	MAE	K	K'	$|R^2_0 - R'^2_0|$
Model	0.39	0.69	0.68	0.57	0.81	0.27	0.99	1.01	0.18
Threshold					0.85		0.9≤K≤1.1	0.9≤K'≤1.1	<0.3

Figure 1. Plot of experimental *vs* predicted values of biological activities expressed as pKi for non-imidazole based H₃ antagonists. Training set compounds are depicted as filled circles while test sets are illustrated as open diamonds.

For selection of the final model, the initial assessment was based on acceptable threshold for correlation coefficient of training and test sets. The analysis of the results showed that the following model derived based on activity-property division of the dataset performed well in comparison to the other dataset division methods named above.

$$pK_i = 6.88172(\pm0.05381) - 0.3405(\pm0.10581) \text{ BEHm5} - 0.30056(\pm0.09403) \text{ BEHe5} + 0.7095(\pm0.08032) \text{ BEHp4} + 0.37588(\pm0.06164) \text{ Qmean}$$

For further determining the reliability and predictivity of the QSAR model, all the internal and external cross validation methods described in Materials and Methods

section were calculated for the selected model. Table 2 indicates the different validation parameters calculated for the QSAR model along with their corresponding threshold values.[43]

In the developed QSAR model for non-imidazole containing compounds, totally four descriptors were selected. Three of them are classified as BCUT descriptors (so called eigenvalue-based descriptors) as indicators of proximity measurements whereas the last parameter is Qmean indicating the mean absolute charge. For calculation of BCUT descriptors, connectivity information and atomic features are considered for generation of square symmetric matrix depicted in the form of a molecular graph. Different weighting schemes such as mass, electronegativities, and polarizability are employed for scaling of BCUT parameters.[44] BEHm5, BEHe5, and BEHp4 are the selected BCUT parameters in the QSAR model. According to the model coefficients, BEHm5 and BEHe5 are inversely correlated with H₃ binding affinities while BEHp4 positively affects the binding affinities to the receptor. Additionally, the positive model constant for Qmean in QSAR model reveals a direct impact on the endpoint values. Such a positive relationship of Qmean may explain that the higher value of the mean absolute charge for given molecules, the greater the observed affinities which are in agreement with positive effect of BEHp4 parameter weighted by polarizability.

Table 2 demonstrates the statistical criteria obtained for the developed QSAR model. As it can be seen almost all the required validation criteria are satisfied based on the threshold considered for each parameter. The narrow distribution of error obtained for the model i.e. MAE and RMSEP of 0.27 and 0.39, respectively, implies a good predictive power for the model. The other internal and external criteria are also indicatives of good performance
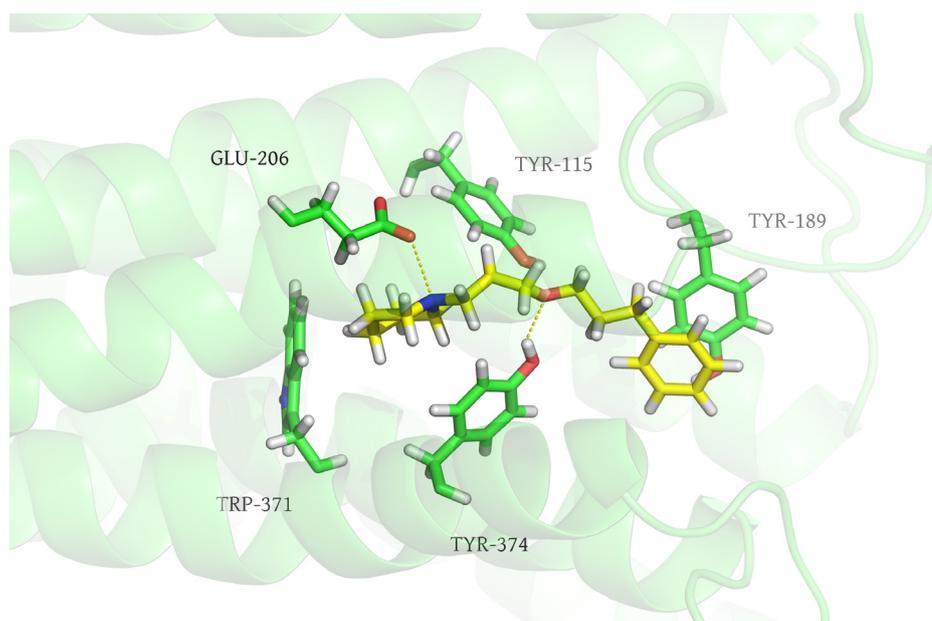

Figure 2. 3D representation of the docked compound 49 into binding site of the modeled H₃R generated by PyMol program (version 1.7.x). The ligand and the main interacting residues are illustrated as sticks. Only the side chains of the interacting residues from receptor are shown for further clarity.

of the model in terms of predictive capability. Figure 1 plots the correlation between the experimental *vs* predicted values of the H_3 binding affinities for non-imidazole based H_3 antagonists.

In the case of applicability domain analysis, the descriptivequalitative results obtained from MLR plus validation tool [41] identified one outlier in the dataset (i.e. compound 55) . However, this outlier was not excluded from the data set as its removal did not lead to the improvement of the model statistical parameters and predictive ability. The second reason is related to the range of endpoint values. Ignoring this data point narrows the binding affinities range which in turn debilitates the QSAR model quality. Furthermore, it was noted that compound 55 did not exhibit any distinguished behavior in terms of receptor binding studied by docking analysis described in detail below.

To further explore the structural features of both H_3R and antagonists involved in the formation of ligand-receptor complexes, molecular docking was performed. To do this, we used the previously homology-based modeled structure of H_3R for docking experiment using GOLD program. The analysis of the results demonstrated that Tyr[115], Tyr[189], Phe[193], Leu[199], Glu[206], Trp[371], Tyr[374], Met[378], Tyr[394], and Phe[398] from H_3R are important amino acids in the interactions with the compounds. A two dimensional illustration for the complex of compound 49 (as a representative) with H_3R is shown in Figure 2. As shown in the figure, the main interactions observed for docked compound 49 into the H_3R include: a stacking, two hydrophobic interactions, and two hydrogen bonds. The interaction was formed between phenyl ring of ligand and Tyr[189]. Residues Glu[206] and Tyr[374] are engaged in the hydrogen bonds with nitrogen of piperidine and linker oxygen atom of compound 49, respectively. Moreover, Tyr[115] and Trp[371] hydrophobically interact with linker alkyl moiety and piperidine ring, respectively. Similar interactions have been also observed for the other compounds. The results of the docking study are in close agreement with those reported previously.[45-50] Particularly, site directed mutagenesis study by Uveges et al. revealed the importance of Leu[199] and Glu[206] located in transmembrane helix five (TMH5) for the binding of ligands to H3 receptors.[51]

Taking all these information into consideration, it can be concluded that the developed QSAR model can be used for predicting the biological activity of the newly designed non-imidazole based compounds. Moreover, the predicted interactions may provide useful information for structural requirements needed for antagonistic activity of the novel compounds.

Conclusion

In the current work, we aimed to build a QSAR model for a set of non-imidazole based H_3 antagonists using GA-PLS and stepwise MLR methods. The generated model was evaluated using different internal and external assessment criteria. Analyses of the results demonstrated that the developed model has reasonable statistical parameters in

terms of predictivity. Moreover, it was shown that connectivity information (represented as BCUT descriptors) and mean absolute charge are important factors in predicting the biological activity of the studied compounds. To elucidate the mode of interaction between studied antagonists and H_3R, molecular docking was performed and the results indicated that numerous interactions such as H-bond, stacking, and hydrophobic interactions were established between ligands and the receptor.

Conflict of Interests

The authors declare that there is no conflict of interest.

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