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 memory.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 H1, H2, H3, and H4. Among the histaminergic receptor subtypes, histamine H3 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 H3 receptors in 1983,7 tremendous advances have been achieved in identification of H3-related ligands.6,7 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 H3 antagonists/inverse agonists. There are several lines of evidence demonstrating the effectiveness of H3 antagonists/inverse agonists in several neurological disorders such as narcolepsy, Alzheimer, attention deficit hyperactivity disorder (ADHD), epilepsy just to mention a few.8-11 The H3 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, piperazone. 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...
or without cataplexy. Currently, there are several non-imidazole containing compounds at different phases of clinical trials (see 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. 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 H3 antagonists are rationalized through QSAR and docking techniques. The results can be used for the design and discovery of novel non-imidazole based H3 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, in which the binding affinities H3 receptor stably expressed in CHO-K1 cells were determined by radioligand binding assay using [125I]-Iodoproxyfan. The binding affinities (Ki) used in QSAR analysis as uniformly being transformed as pK (-log K). 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 methods was used for further fully optimization of the generated structures. 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 mean of zero and variance of unity, 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 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 Q2, SDEP, R2_m, R2_p for internal validation and R2_test, Q2_F1, Q2_F2, Q2_F3, CCC, and MAE for external validation. Leave-one-out (LOO) cross validation method is used for internally validating 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:

\[
Q^2 = 1 - \frac{\sum (Y_{obs} - Y_{pred})^2}{\sum (Y_{obs} - \bar{Y})^2}
\]

Y_obs, Y_pred, and \(\bar{Y}\) are observed, predicted and average of trained activity values.

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

\[
SDEP = \sqrt{\frac{\sum (Y_{obs} - Y_{pred})^2}{n}}
\]

where n refers to the number of data points.

The other parameter used for internally evaluation of the generated model is \(R^2_m\) suggested by Roy et. al. The following equations demonstrate the calculation of this parameter:

\[
\Delta r_m^2 = \frac{r_m^2 + r_{-m}^2}{2}
\]

\[
r_m^2 = r^2 \times \left(1 - \sqrt{r^2 - r_0^2}\right), r_{-m}^2 = r^2 \times \left(1 - \sqrt{r^2 - r_0^2}\right)
\]

\[
r_0^2 = 1 - \frac{\sum (Y_{obs} - k \times Y_{pred})^2}{\sum (Y_{obs} - \bar{Y})^2}
\]

\[
k = \frac{\sum (Y_{obs} \times Y_{pred})}{\sum (Y_{pred})^2}
\]
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 $r_p^2$ are the squared correlation coefficients while the line is passed from the origin. In the case of $r_p^2$, $X$ and $Y$ axes are reversed. The slopes of the squared correlation coefficients are denoted by $k$ and $k'$, respectively.

The $s^2 R_p^2$ is the other parameter for assessing the robustness of the QSAR model. 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 $s^2 R_p^2$ as below:

$$s^2 R_p^2 = R \sqrt{R^2 - R_p^2}$$

where $R^2$ and $R_p^2$ 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:

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

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

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

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

$$\left| R_0^2 - R_{pred}^2 \right| < 0.3$$

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

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

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

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

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

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

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

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. The following formula indicates the calculation of CCC parameter:

$$\text{CCC} = \frac{2 \sum_{i=1}^{n_{EXT}} (Y_{obs(test)} - \bar{Y}_{obs(test)}) \cdot (Y_{pred(test)} - \bar{Y}_{pred(test)})}{\sum_{i=1}^{n_{EXT}} (Y_{obs(test)} - \bar{Y}_{obs(test)})^2 + \sum_{i=1}^{n_{EXT}} (Y_{pred(test)} - \bar{Y}_{pred(test)})^2 + n \cdot (Y_{obs(test)} - \bar{Y}_{pred(test)})^2}$$

The applicability domain for the studied non-imidazole based H$_2$ antagonists was determined Roy’s MLR plus validation tool.

**Molecular docking**

In order to predict the interactions between studied H$_2$ antagonists and histamine H$_2$ receptor, each compound was docked on the modeled H$_2$ model 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. Briefly, the binding site was determined based on the known amino acids (i.e. Asp$^{114}$, Thr$^{118}$, Tyr$^{199}$, Phe$^{198}$, Glu$^{206}$, Trp$^{371}$, and Tyr$^{379}$) involved for the interactions in the binding site of H$_2$ 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$^{199}$ side chain of protein.
| No | Structure | pKi | Ref. | No | Structure | pKi | Ref. |
|----|-----------|-----|------|----|-----------|-----|------|
| 1* | ![Structure 1](image1) | 6.15 | 17   | 29 | ![Structure 29](image2) | 7.4 | 21   |
| 2  | ![Structure 2](image3) | 6.13 | 17   | 30 | ![Structure 30](image4) | 6.6 | 21   |
| 3  | ![Structure 3](image5) | 6.14 | 17   | 31 | ![Structure 31](image6) | 7.1 | 21   |
| 4* | ![Structure 4](image7) | 6.15 | 17   | 32 | ![Structure 32](image8) | 6.7 | 21   |
| 5  | ![Structure 5](image9) | 6.14 | 17   | 33 | ![Structure 33](image10) | 6.3 | 21   |
| 6  | ![Structure 6](image11) | 6.25 | 17   | 34 | ![Structure 34](image12) | 6.6 | 21   |
| 7  | ![Structure 7](image13) | 6.47 | 17   | 35 | ![Structure 35](image14) | 6   | 21   |
| 8  | ![Structure 8](image15) | 6.47 | 17   | 36*| ![Structure 36](image16) | 8.8 | 21   |
| 9  | ![Structure 9](image17) | 6.51 | 17   | 37 | ![Structure 37](image18) | 6.6 | 12   |
| 10*| ![Structure 10](image19) | 6.42 | 17   | 38*| ![Structure 38](image20) | 6.56| 12   |
Table 1 Continued.

| 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 |
Results and Discussion
In the past decades, H₃ antagonists have been the topic of many researches in pharmaceutical sciences. The effectiveness of H₃ 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.
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.

\[
pK_i = 6.88172(\pm0.05381) -0.3405(\pm0.10581) \text{BEH}_{m5} -0.30056(\pm0.09403) \text{BEHe}_{5} +0.7095(\pm0.08032) \text{BEH}_{p4} +0.37588(\pm0.06164) \text{Qmean}
\]

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. 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. BEH_{m5}, BEHe_{5}, and BEH_{p4} are the selected BCUT parameters in the QSAR model. According to the model coefficients, BEH_{m5} and BEHe_{5} are inversely correlated with H_{3} binding affinities while BEH_{p4} 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 BEH_{p4} 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 1. Plot of experimental vs predicted values of biological activities expressed as pKi for non-imidazole based H_{3} antagonists. Training set compounds are depicted as filled circles while test sets are illustrated as open diamonds.

Figure 2. 3D representation of the docked compound 49 into binding site of the modeled H_{3}R 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 H₁ binding affinities for non-imidazole based H₁ antagonists.

In the case of applicability domain analysis, the descriptive-qualitative results obtained from MLR plus validation tool 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 H₁R 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 H₁R for docking experiment using GOLD program. The analysis of the results demonstrated that Tyr₁¹⁵, Tyr₁⁸⁹, Phe₁⁹², Leu₁⁹⁹, Glu₂⁰₆, Trp₁⁷⁷, Tyr₁⁷⁸, Met₁⁷⁹, Tyr₁₇⁶, and Phe₁⁸⁸ from H₁R are important amino acids in the interactions with the compounds. A two dimensional illustration for the complex of compound 49 (as a representative) with H₁R is shown in Figure 2. As shown in the figure, the main interactions observed for docked compound 49 into the H₁R include: a stacking, two hydrophobic interactions, and two hydrogen bonds. The interaction was formed between phenyl ring of ligand and Tyr₁⁸⁹. Residues Glu₂⁰₆ and Tyr₁⁷⁴ are engaged in the hydrogen bonds with nitrogen of piperidine and linker oxygen atom of compound 49, respectively. Moreover, Tyr₁¹⁵ and Trp₁⁷¹ 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. Particularly, site directed mutagenesis study by Uveges et al. revealed the importance of Leu₁⁹⁹ and Glu₂⁰₆ located in transmembrane helix five (TMHS) for the binding of ligands to H₃ receptors.

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 H₁ 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 H₁R, 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.

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Conflict of Interests

The authors declare that there is no conflict of interest.

References

1. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. Physiol Rev. 2008;88(3):1183-241. doi:10.1152/physrev.00043.2007
2. Hu W, Chen Z. The roles of histamine and its receptor ligands in central nervous system disorders: An update. Pharmacol Ther. 2017;175:116-32. doi:10.1016/j.pharmthera.2017.02.039
3. Lin JS, Sergeeva OA, Haas HL. Histamine H3 receptors and sleep-wake regulation. J Pharmacol Exp Ther. 2011;336(1):17-23. doi:10.1124/jpet.110.170134
4. Tiligada E, Kyriakidis K, Chazot PL, Passani MB. Histamine pharmacology and new CNS drug targets. CNS Neurosci Ther. 2011;17(6):620-8. doi:10.1111/j.1755-5949.2010.00212.x
5. Gemkow MJ, Davenport AJ, Harich S, Ellenbroek BA, Cesura A, Hallett D. The histamine H3 receptor as a therapeutic drug target for CNS disorders. Drug Discov Today. 2009;14(9-10):509-15. doi:10.1016/j.drudis.2009.02.011
6. Berlin M, Boyce CW, Ruiz Mde L. Histamine H3 receptor as a drug discovery target. J Med Chem. 2011;54(1):26-53. doi:10.1021/jm100064d
7. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (h3) of histamine receptor. Nature 1983;302(5911):832-7. doi:10.1038/302832a0
8. Sadek B, Łażewska D, Hagenow S, Kiec-Kononowicz K, Stark H. Histamine h₃r antagonists: From scaffold hopping to clinical candidates. In: Blandina P, Passani MB, editors. Histamine receptors: Preclinical and clinical aspects. Cham: Springer International Publishing; 2016. p. 109-55.
9. Ghamari N, Zarei O, Arias-Montano JA, Reiner D, Dastmalchi S, Stark H, et al. Histamine H3 receptor antagonists/inverse agonists: Where do they go? Pharmacol Ther. 2019;200:69-84. doi:10.1016/j.pharmthera.2019.04.007
10. Celanire S, Wijtjmans M, Talaga P, Leurs R, de Esch IJ. Histamine H3 receptor antagonists reach out for the clinic. Drug Discov Today. 2005;10(23–24):1613–27. doi:10.1016/s1359-6464(05)03625-1

11. Leurs R, Bakker RA, Timmerman H, de Esch IJ. The histamine H3 receptor: From gene cloning to H3 receptor drugs. Nat Rev Drug Discov. 2005;4(2):107-20. doi:10.1038/nrd1631

12. Lazewska D, Kuder K, Ligneau X, Schwartz JC, Schunack W, Stark H, et al. Piperidine variations in search for non-imidazole histamine H3 receptor ligands. Bioorg Med Chem. 2008;16(18):8729-36. doi:10.1016/j.bmc.2008.07.071

13. Sadek B, Stark H. Cherry-picked ligands at histamine receptor subtypes. Neuropharmacology. 2016;106:56-73. doi:10.1016/j.neuropharm.2015.11.005

14. Brioni JD, Ebsenshade TA, Garrison TR, Bittner SR, Cowart MD. Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. J Pharmaco Exp Ther. 2011;336(1):38-46. doi:10.1124/jpet.110.166876

15. Sander K, Kottke T, Stark H. Histamine H3 receptor antagonists go to clinics. Biol Pharm Bull. 2008;31(12):2163-81. doi:10.1248/bpb.31.2163

16. Lazewska D, Wiecek M, Ligneau X, Kottke T, Weizel L, Seifert R, et al. Histamine H3 and h4 receptor affinity of branched 3-(1h-imidazol-4-yl) propyl n-alkylcarbamates. Bioorg Med Chem Lett. 2009;19(23):6682-5. doi:10.1016/j.bmcl.2009.10.005

17. Lazewska D, Ligneau X, Schwartz JC, Schunack W, Stark H, Kiec-Kononowicz K. Ether derivatives of 3-piperidinopropan-1-ol as non-imidazole histamine h3 receptor antagonists. Bioorg Med Chem. 2006;14(10):3522-9. doi:10.1016/j.bmc.2006.01.013

18. Amon M, Ligneau X, Schwartz JC, Stark H. Fluorescent non-imidazole histamine H3 receptor ligands with nanomolar affinities. Bioorg Med Chem Lett. 2006;16(7):1938-40. doi:10.1016/j.bmcl.2005.12.084

19. Syed YY. Pitolisant: First global approval. Drugs. 2016;76(13):1313-8. doi:10.1348/00401706.131206-1

20. Kolb-Sielecka M, Demolis P, Emmerich J, Markey G, Salmonson T, Haas M. The european medicines agency review of pitolisant for treatment of narcolepsy: Summary of the scientific assessment by the committee for medicinal products for human use. Sleep Med. 2017;33:125-9. doi:10.1016/j.sleep.2017.01.002

21. Dearden JC. Whither qsar? Pharm Sci. 2017;23(2):82-3. doi:10.15171/ps.2017.13

22. Cherkasov A, Muratov EN, Fourches D, Varnek A, Baskin II, Cronin M, et al. Qsar modeling: Where have you been? Where are you going? J Med Chem. 2014;57(12):4977-5010. doi:10.1021/jm4004285

23. Miko T, Ligneau X, Pertz HH, Arrang JM, Ganellin CR, Schwartz JC, et al. Structural variations of 1-4-(phenoxymethyl)benzyl)piperidines as nonimidazole histamine H3 receptor antagonists. Bioorg Med Chem. 2004;12(10):2727-36. doi:10.1016/j/bmcl.2004.03.009

24. Ligneau X, Garbarg M, Vizuete ML, Diaz J, Purand K, Stark H, et al. [125i]iodoproxyfan, a new antagonist to label and visualize cerebral histamine h3 receptors. J Pharmacol Exp Ther. 1994;271(1):452-9.

25. Dewar MJS, Thiel W. Ground states of molecules. 39. Mndo results for molecules containing hydrogen, carbon, nitrogen, and oxygen. J Am Chem Soc. 1977;99(15):4907-17. doi:10.1021/ja00457a005

26. Allinger NL. Conformational analysis. 130. Mm2. A hydrocarbon force field utilizing v1 and v2 torsional terms. J Am Chem Soc. 1977;99(25):8127-34. doi:10.1021/ja00467a001

27. Martin TM, Harten P, Young DM, Muratov EN, Golbraikh A, Zhu H, et al. Does rational selection of training and test sets improve the outcome of qsar modeling? J Chem Inf Model. 2012;52(10):2570-8. doi:10.1021/ci300338w

28. Kennard RW, Stone LA. Computer aided design of experiments. Technometrics. 1969;11(1):137-48. doi:10.1080/00401706.1969.10490666

29. Leardi R. Application of genetic algorithms for feature selection in spectral data sets. J Chemom. 2000;14(5-6):643-55. doi:10.1021/ja1004285

30. Leardi R, Lupiáñez González A. Genetic algorithms applied to feature selection in pls regression: How and when to use them. Chemom Intell Lab Syst. 1998;41(2):195-207. doi:10.1016/s0169-7439(98)00051-3

31. Roy K, Chakraborty P, Mitra I, Ojha PK, Kar S, Das RN. Some case studies on application of “rm2” metrics for judging quality of quantitative structure–activity relationship predictions: Emphasis on scaling of response data. J Comput Chem. 2013;34(12):1071-82. doi:10.1002/jcc.23231

32. Roy K, Kar S, Das RN. Chapter 7 - validation of qsar models. Understanding the basics of qsar for applications in pharmaceutical sciences and risk assessment. Boston: Academic Press; 2015. p. 231-89.

33. Golbraikh A, Troshina A. Beware of q2! J Mol Graph Model. 2002;20(4):269-76. doi:10.1016/s1099-8084(01)00123-1

34. Chirico N, Gramatica P. Real external predictivity of qsar models: How to evaluate it? Comparison of different validation criteria and proposal of using the discordance correlation coefficient. J Chem Inf Model. 2011;51(9):2320-35. doi:10.1021/ci200211n

35. Chirico N, Gramatica P. Real external predictivity of qsar models. Part 2. New intercomparable thresholds for different validation criteria and the need for scatter plot inspection. J Chem Inf Model. 2012;52(8):2044-58. doi:10.1021/ci300084j

36. Shi LM, Fang H, Tong W, Wu J, Perkins R, Blair RM, et al. Qsar models using a large diverse set of estrogens. J Chem Inf Comput Sci. 2001;41(1):186-95. doi:10.1021/ci000066d
37. Schüürmann G, Ebert RU, Chen J, Wang B, Kühne R. External validation and prediction employing the predictive squared correlation coefficient — test set activity mean vs training set activity mean. J Chem Inf Model. 2008;48(11):2140-5. doi:10.1021/ci800253u
38. Consonni V, Ballabio D, Todeschini R. Evaluation of model predictive ability by external validation techniques. J Chemom. 2010;24(3-4):194-201. doi:10.1002/cem.1290
39. Consonni V, Ballabio D, Todeschini R. Comments on the definition of the q2 parameter for qsar validation. J Chem Inf Model. 2009;49(7):1669-78. doi:10.1021/ci900115y
40. Lin LI. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 1989;45(1):255-68. doi:10.2307/2532051
41. Ambure P, Aher RB, Gajewicz A, Puzyn T, Roy K. “Nanobridges” software: Open access tools to perform qsar and nano-qsar modeling. Chemometr Intell Lab Syst. 2015;147:1-13. doi:10.1016/j.chemolab.2015.07.007
42. Ghamari N, Zarei O, Reiner D, Dastmalchi S, Stark H, Hamzeh-Mivehroud M. Histamine H3 receptor ligands by hybrid virtual screening, docking, molecular dynamics simulations, and investigation of their biological effects. Chem Biol Drug Des. 2019;93(5):832-43. doi:10.1111/cbdd.13471
43. Dearden JC, Cronin MT, Kaiser KL. How not to develop a quantitative structure-activity or structure-property relationship (QSPR/QSAR). SAR QSAR Environ Res. 2009;20(3-4):241-66. doi:10.1080/10629360902949567
44. Stanton DT. Evaluation and use of BCUT descriptors in qsar and QSAR studies. J Chem Inf Comput Sci. 1999;39(1):11-20. doi:10.1021/ci980102x
45. Sheng R, Tang L, Jiang L, Hong L, Shi Y, Zhou N, et al. Novel 1-phenyl-3-hydroxy-4-pyridinone derivatives as multifunctional agents for the therapy of alzheimer’s disease. ACS Chem Neurosci. 2016;7(1):69-81. doi:10.1021/acschemneuro.5b00224
46. Bajda M, Kuder KJ, Łazewska D, Kieć-Kononowicz K, Więckowska A, Ignasik M, et al. Dual-acting diether derivatives of piperidine and homopiperidine with histamine H3 receptor antagonistic and anticholinesterase activity. Arch Pharm (Weinheim). 2012;345(8):591-7. doi:10.1002/ardp.201200018
47. Levoin N, Labeeuw O, Krief S, Calmels T, Poupardin-Olivier O, Berrebi-Bertrand I, et al. Determination of the binding mode and interacting amino-acids for dibasic H3 receptor antagonists. Bioorg Med Chem. 2013;21(15):4526-9. doi:10.1016/j.bmc.2013.05.035
48. Morini G, Comini M, Rivara M, Rivara S, Lorenzi S, Bordi F, et al. Dibasic non-imidazole histamine h3 receptor antagonists with a rigid biphenyl scaffold. Bioorg Med Chem Lett. 2006;16(15):4063-7. doi:10.1016/j.bmcl.2006.04.092
49. Łazewska D, Jończyk J, Bajda M, Szalaj N, Więckowska A, Panek D, et al. Cholinesterase inhibitory activity of chlorophenoxy derivatives—histamine H3 receptor ligands. Bioorg Med Chem Lett. 2016;26(16):4140-5. doi:10.1016/j.bmcl.2016.04.054
50. Kuder K, Łazewska D, Łatacz G, Schwed JS, Karcz T, Stark H, et al. Chlorophenoxy aminoalkyl derivatives as histamine H(3)r ligands and antiseizure agents. Bioorg Med Chem. 2016;24(2):53-72. doi:10.1016/j.bmc.2015.11.021
51. Uveges AJ, Kowal D, Zhang Y, Spangler TB, Dunlop J, Semus S, et al. The role of transmembrane helix 5 in agonist binding to the human H3 receptor. J Pharmacol Exp Ther. 2002;301(2):451-8. doi:10.1124/jpet.301.2.451