ORIGINAL ARTICLE

3D QSAR Studies of 2-Arylpyrimidines and S-Triazines as Selective PDE4B Inhibitors

ANAND GAURAV, DHARMENDRA KUMAR

ABSTRACT

Background: Phosphodiesterase 4B (PDE4B) has emerged as important target for design of anti-inflammatory drugs for respiratory tract. Several selective PDE4B inhibitors are under various stages of development, among them 2-arylpyrimidines and s-triazines have been identified as inhibitors with high degree of selectivity for PDE4B. However, the structural features responsible for the PDE4B selectivity of these molecules have not been identified and explored so far.

Method: 3D QSAR studies were performed for the series of 2-arylpyrimidines and s-triazines using Accelrys Discovery Studio 3.5. The IC_{50} values were transformed to PDE4B selectivity by taking the ratio of IC_{50} values i.e. PDE4D(IC_{50})/PDE4B(IC_{50}) for all the molecules in the series, and used as the dependent variable. The dataset was divided into training and test set of 45 and 10 compounds respectively and 3D QSAR was performed using the default parameters. Test set prediction and Fischer statistic was used for validation of the developed model.

Results: Statistically robust and predictive 3D QSAR models with high r^2_{cv} value of 0.9794 were obtained. The contour maps revealed the sterically and electronically favourable and unfavourable regions around the 2-arylpyrimidines and s-triazines scaffolds.

Conclusion: 3D QSAR model for 2-arylpyrimidines and s-triazines as selective PDE4B inhibitors were developed and validated. The models were highly predictive and provided vital structural information for the design of newer and more selective PDE4B inhibitors having the 2-arylpyrimidine and s-triazines scaffold. The results of the present study will be followed up by the design, synthesis and experimental evaluation of newer selective PDE4B inhibitors.

Keywords: Cyclic Nucleotide Phosphodiesterases, Type 4B; 3D Quantitative Structure-Activity Relationship; Fischer statistic; 2-arylpyrimidines; s-triazines

INTRODUCTION

Prevalence of Inflammatory diseases of respiratory tract i.e., asthma and COPD has increased in recent years, with more than 200 million people affected by it worldwide. Most of the mortality related to these inflammatory disorders occurs in low- and low middle income countries[1].

Phosphodiesterase 4 (PDE4) is a major family of enzymes that selectively hydrolyze 3',5'-cyclic adenosine monophosphate (cAMP) and are involved in regulating the release of anti-inflammatory and pro-inflammatory cytokines within cells[2,3,4]. Even though PDE4s are widely expressed in immune and inflammatory cells, levels of different PDE4 subtypes (PDE4A, PDE4B, PDE4C and PDE4D) vary in a specific cell. PDE4B is abundant in monotypes and neutrophils, while PDE4A is expressed to very low levels and PDE4C is absent in inflammatory cells[5,6,7,8,9]. This makes PDE4B an interesting and promising targets for anti-inflammatory drugs meant to be used in respiratory inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Inhibition of PDE4 has been shown to suppress a diverse spectrum of inflammatory responses *invitro* and *in vivo.*[10-13] More importantly, many PDE4 inhibitors in development are efficacious in animal models of various inflammatory disorders, such as asthma, COPD, psoriasis, inflammatory bowel diseases, and rheumatoid arthritis[11,14,15], as well as in clinical trials for asthma and COPD[16,17,18]. However the development of PDE4 inhibitors has been slowed down due to narrow therapeutic window of most of the compounds. A major reason for their poor clinical results is the consequence of dosing limitation caused by side effects such as nausea and emesis.[19] Recent findings in PDE4 knockout mice suggest that an inhibitor with PDE4B selectivity should retain many beneficial anti-inflammatory effects without the unwanted side effects[20,21].

The highly conserved catalytic domain of PDE4 isozymes makes the generation of inhibitors with PDE4 subtype selectivity a challenging task. However, residues in regulatory domain such as control region 3 (CR3) vary among subfamilies, which has proved to be responsible for PDE4B selectivity.[22]

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, UCSI University, Taman Connaught, Cheras, 56000, Kuala Lumpur, Malaysia
Correspondence to Dr. Anand Gaurav Email: anand.pharma@gmail.com.Tel. +60176894547; Fax: +6039102 2614

CR3 forms weak interaction with catalytic domain and its length is variable in different PDE4 structure[23,24]. Besides, mutation studies have proved that LEU in PDE4B vs GLN in PDE4D are major contributors to PDE4B or PDE4D selectivity[22]. Thus, subtype selective PDE4 inhibitors have recently been described[14,21,15]. Biological evaluation of selective PDE4B inhibitors revealed their potent anti-inflammatory effects *in vitro* and *in vivo*. Investigation in ferrets also showed a significantly less emesis with the compound compared with the non-selective PDE4 inhibitor cilomilast[21].

Furthermore, the identification of structural features which can differentiate between the two receptor subtypes will allow the design of selective PDE4B inhibitors. 2-arylpyrimidine and s-triazine based compounds have been recently identified potent selective PDE4B inhibitors[21,16]. These compounds can be further optimized to enhance their potency as well as selectivity. Quantitative structure activity relationships have been widely used as a tool in past for optimisation of activity of several different classes of compounds. In the present study 3D QSAR approach has been used to study the potency and selectivity of 2-arylpyrimidines and s-triazines as PDE4B inhibitors. Structural features responsible for PDE4B selectivity have been identified. The information obtained from 3DQSAR studies will be used to propose new selective PDE4B inhibitors based on the structure of 2-arylpyrimidines and s-triazines.

MATERIALS AND METHODS

A dataset comprising of 55compounds (2-arylpyrimidines and s-triazines) with available PDE4B and PDE4D inhibition IC_{50} (nM) data was taken from literature for the development of the atom-based 3D QSAR model for PDE4B inhibition (Table 1 in supplementary data).The IC_{50} values were transformed to PDE4B selectivity by taking the ratio of IC_{50} values i.e. PDE4D(IC_{50})/PDE4B(IC_{50}) (Table 2 and 3)[21,26]. The 3D QSAR studies were performed using Discovery studio 3.1. The PDE4B selectivity of the compoundswasused as the independent variable to develop the 3D QSAR model of PDE4B selectivity. The training set was composed of 45 compounds while the remaining 10 compounds were used as the test set. The training and test selection module implemented in Discovery studio 3.1 (based on the PDE4B selectivity values) was used for this purpose. The compounds in the test set have a range of PDE4B selectivity values similar to that of the training set. The ligands were pre-aligned using substructure basedmolecular overlay method and placed in a 3D grid space. The grid spacing was 1.5 Å. The energy potentials on every grid point were then calculated using a CHARMm force field which used the electrostatic potential and the Van der Waals potential and treated as separate terms. A +1e point charge is used as the electrostatic potential probe and distance-dependent dielectric constant is used to mimic the solvation effect. For the van der Waals potential a carbon atom with a 1.73 Å radius is used as a probe. The energy grid potentials can be used as independent variables to create partial least-squares (PLS). Furthermore, the best 3D QSAR model was validated by predicting activities of the test set compounds. Validation was also performed using leave one out cross validation method (5 folds). The 3D QSAR was evaluated by using $r^2$and cross validatedr^2.

RESULTS AND DISCUSSION

The atom-based 3D QSAR models were developed from the training set of 45 inhibitors and the test set of 10 inhibitors (Table 1 supplementary data), using substructure based molecular overlay alignment. The atom-based3D QSAR model was built using the built 3D QSAR model module of Discovery Studio 3.1. Default setting were used for model development. Validation of the developed model was performed based on the internal predictions of the training set and the external predictions of the test set as well as leave one out cross validation method (5 folds). PLS analyses of the training sets showed a high cross-validated r^2_{cv}value of 0.6983 using three principal components,RMS residual error (cross-validation) of 3.4139,non-cross-validated r^2 value of 0.9794,RMS residual error of 2.2421, r^2_{adj}of0.9650 (Table 2 supplementary data). The r^2 value for the test compounds was 0.9553 suggesting good predictive ability of the model. The predicted PDE4B selectivity at 3rd PLS factor for the training and test set are tabulated in Table 1. From Table 1, it is quite evident that almost all compounds in the test set and training set yielded a good predicted value.The graphical plot of actual vs predicted PDE4B selectivity for both the training set as well as the test set is shown in Fig. 1.

All the parameters of the QSAR models confirmed their reliability and predictability which can be used in the design of new and high selectivity PDE4B inhibitors.

The van der Waals and electrostatic contour plots obtained using the atom based 3D QSAR methods are shown in Figure 2A and Figure 2B respectively. The van der Waalsplot shows green and yellow contours indicating regions favoring bulky and lighter groups respectively. The mostly yellow colored contours and few green contours surrounding the most selective molecule 2-arylpyridine 34 suggests

that a steric bulk in general in not favorable for PDE4B selectivity however there are specific positions where steric bulk is well tolerated (near the green contours).Bulky group i.e., ethyl, cyclopropyl will be favored at these positions.

The electrostaticplot shows red and blue contours indicating regions favoring electronegative and electropositive groups respectively.Most of the contours surrounding the most selective molecule 2-arylpyridine 34 are red in color indicating that electronegative substituents will be favorable for PDE4B selectivity, while the positions surrounded by the few blue contours can be substituted by electropositive groups for better PDE4B selectivity.The electronegative substitutions are likely to be responsible for electrostatic interactions with the positively charged residues of the active site of PDE4B.

The above findings will be used to design new more selective PDE4B inhibitors based on the 2-arylpyrimidine and s-triazine scaffold. The designed compounds will be synthesized and evaluated for PDE4B selectivity in a wet lab.

CONCLUSION

3D QSAR model for a series of 2-arylpyrimidines and s-triazinesas selective PDE4B inhibitors were developed. The best 3D QSAR modelwas validated using different methods to evaluate their predictive power over the test set compounds. Less bulky and electronegative substitutions were identified to be favorable for PDE4B selectivity in general however bulky as well as electropositive substitutions were tolerated at some positions. The structural information derived using the developed model will be useful in design of more selective PDE4B inhibitors based on 2-arylpyrimidines and s-triazines scaffold. In future studies the design, synthesis and pharmacological evaluation of such molecules will be performed.

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Fig. 1: Plot of actual vs predicted PDE4B selectivity for training (top) and test sets (bottom).

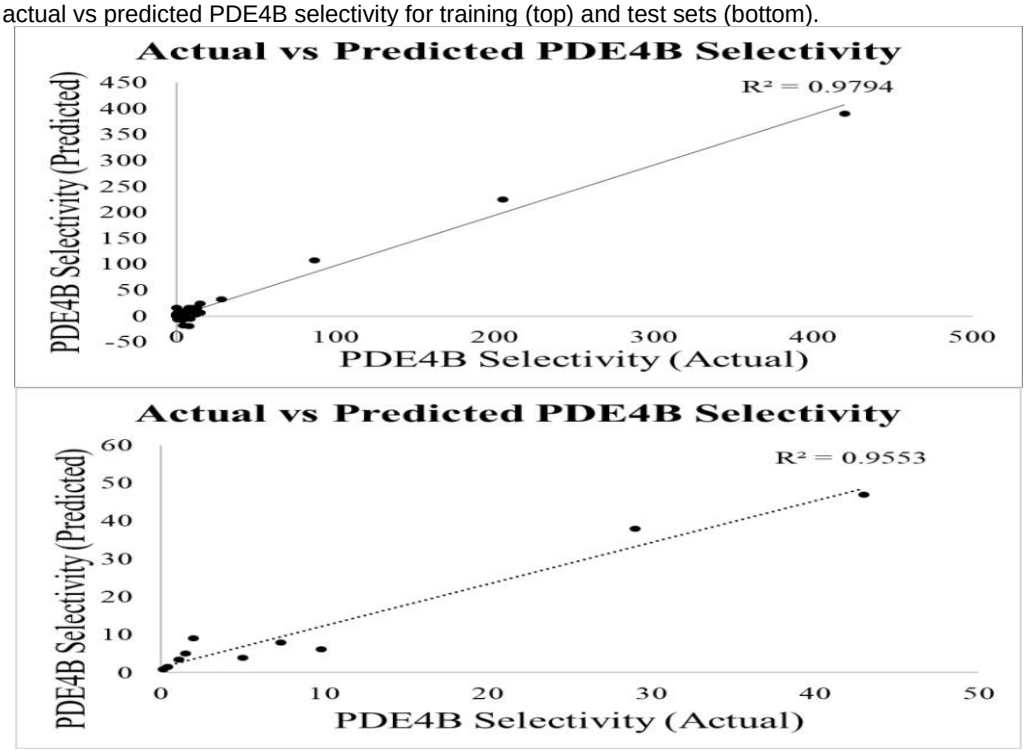

Fig. 2: The contour plots with the most selective compound (2arylpyrimidine 34) **A**. van der Waals contour plot; Yellow: steric unfavorable, Green: steric favorable **B**. Electrostatic contour plot; Red: electronegative favorable, Blue: electropositive favorable.

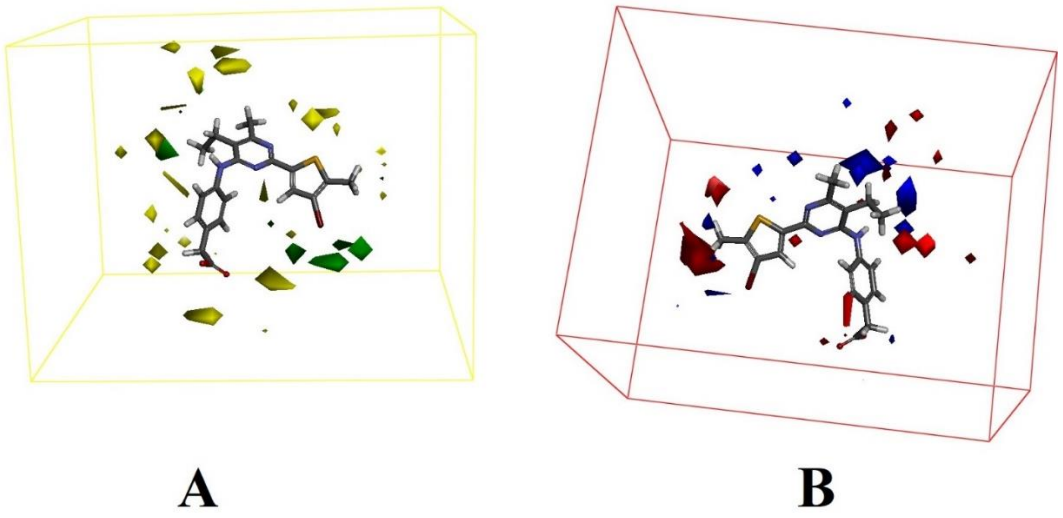

Table 1: Training and test set molecules with their actual and predicted PDE4B selectivity and residuals.

	PDE4B Selectivity (Actual)	PDE4B Selectivity (Predicted)	Residuals
Training set			
2-arylpyridine 1	9.70	13.99	-4.29
2-arylpyridine 2	7.90	9.90	-2.00
2-arylpyridine 3	15.00	24.16	-9.16
2-arylpyridine 4	5.60	9.77	-4.17
2-arylpyridine 8	12.00	2.68	9.32
2-arylpyridine 10	5.20	5.85	-0.65
2-arylpyridine 11	15.00	6.66	8.34
2-arylpyridine 12	2.40	9.12	-6.72
2-arylpyridine 14	3.40	-5.61	9.01
2-arylpyridine 15	11.00	15.98	-4.98
2-arylpyridine 16	15.00	6.62	8.38
2-arylpyridine 17	4.20	6.51	-2.31
2-arylpyridine 18	4.10	-17.61	21.71
2-arylpyridine 19	3.50	-8.21	11.71
2-arylpyridine 20	13.00	15.36	-2.36
2-arylpyridine 21	6.90	-3.74	10.64
2-arylpyridine 22	8.90	-4.85	13.75
2-arylpyridine 23	8.60	11.97	-3.37
2-arylpyridine 26	12.00	9.12	2.88
2-arylpyridine 27	8.00	-19.65	27.65
2-arylpyridine 28	8.00	16.04	-8.04
2-arylpyridine 29	28.00	32.37	-4.37
2-arylpyridine 32	87.00	107.02	-20.02
2-arylpyridine 34	420.00	390.49	29.51
2-arylpyridine 35	205.00	224.72	-19.72
s-triazine 1	0.34	2.86	-2.52
s-triazine 4	1.80	-4.14	5.94
s-triazine 5	2.30	1.98	0.32
s-triazine 7	5.90	4.81	1.09
s-triazine 10	1.20	2.10	-0.90
s-triazine 12	0.50	-1.45	1.95
s-triazine 14	0.38	1.53	-1.15
s-triazine 15	0.86	-6.25	7.11
s-triazine 16	1.20	3.21	-2.01
s-triazine 18	1.00	6.01	-5.01
s-triazine 20	1.00	1.93	-0.93
s-triazine 24	2.00	-0.45	2.45
s-triazine 26	0.90	1.00	-0.10
s-triazine 27	0.90	3.61	-2.71
s-triazine 29	0.04	-0.58	0.62
s-triazine 30	1.00	-1.58	2.58
s-triazine 31	0.90	-0.20	1.10
s-triazine 32	0.40	0.80	-0.40
s-triazine 33	0.09	16.05	-15.96
s-triazine 34	0.40	3.67	-3.27
Test Set			
2-arylpyridine 24	9.80	6.20	3.60
2-arylpyridine 30	29.00	38.00	-9.00
2-arylpyridine 31	43.00	47.00	-4.00
s-triazine 8	5.00	3.90	1.10
s-triazine 9	7.30	7.90	-0.60
s-triazine 11	1.50	5.00	-3.50
s-triazine 19	0.15	0.90	-0.75
s-triazine 22	0.40	1.50	-1.10
s-triazine 23	2.00	9.00	-7.00
s-triazine 28	1.10	3.50	-2.40