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Original Article

3D QSAR STUDIES ON PYRROLOPYRIMIDINES AS SELECTIVE P-GLYCOPROTEIN ANTAGONIST

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ABSTRACT

Objective: The present study is wrought to design the 3D QSAR models on pyrrolopyrimidines as p-glycoprotein inhibitors for the treatment of a wide variety of diseases.

Methods: A dataset comprising of 33 pyrrolopyrimidine derivatives have been divided into training set and test set. A three dimensional pharmacophore hypotheses were built from training set (24 compounds) using hydrogen bond acceptor, hydrophobic and aromatic features.

Results: The proposed model possesses high value of regression coefficient (0.9334) and was validated by using test set predictions. The squared predictive correlation coefficient of 0.985 was observed between experimental and predicted activity values of test set molecules.

Conclusion: The nature of fitness and the distance between pharmacophoric features explain the inhibitory activity of pyrollopyrimidines. The proposed pharmacophoric models possess as potential for the design of novel p-glycoprotein inhibitors.

Keywords- P-glycoprotein, MDR, 3D-QSAR, Pharmacophore hypothesis, Regression coefficient, Squared predictive correlation coefficient.

INTRODUCTION

The development of pharmacological agents able to counteract the mechanisms of drug resistance in oncology has remained a major goal for the past 10 years and this is the most ascertained cause of failure in cancer therapy.[1,2] Multidrug resistance (MDR) is the term that is used to describe the broad pattern of cross resistance appears after prolonged exposure of cells to a single drug, and it is characterized by resistance to a series of structurally unrelated compounds with different subcellular targets.(3,4) High level of P-gp results in a lower intracellular accumulation of drug and an increase in efflux and MDR is caused by the overexpression of efflux transporters such as ABCB1 and ABCG2, located in the plasma membrane of cancer cells, actively extruding a vast number of structurally unrelated compounds, including many commonly used anticancer drugs. (5,6)

The main role of P-gp is likely to protect the susceptible organs from toxic compounds and preventing them to enter the cytosol. [7] P-glycoprotein (P-gp) is a 170-kDa membrane protein and member of the ATP binding cassette (ABC) super family of secretory transport proteins[8]. Human ATP binding cassette (ABC) transporters belong to a family of 49 genes classified into seven subfamilies: ABC-A, ABC-B, ABC-C, ABC-D, ABC-E, ABC-F and ABC-G among theseABC-B1; better known as P-glycoprotein (P-gp) (9).

The structural core of ABC transporters consists of two membrane domains composed of six trans-membrane helical segments each and two nucleotide binding domains located at the cytoplasmic surface of the membrane.[10]. P-gp confers resistance to drugs by preventing their accumulation within the cell. P-gp's efflux capabilities appear to reflect its ability to bind substrates within the inner leaflet of the plasma membrane. Subsequently, and in a potentially ATP-dependent manner, substrates are expelled from the cell.[11] The typical multidrug resistance in tumor cells is associated with a decreased cellular drug accumulation achieved through ATP-dependent transport of the drugs out of cells by P-glycoprotein.[12] Researchers have demonstrated that drug does not cross the endothelial lining of the in vivo BBB due to the presence of P-gp localized on the blood-facing surface. [13-15] P-gp activity inhibition is a way of reversing MDR, and it has been extensively studied for more than 2 decades and some second-generation P-gp modulators have been developed, including (+)-verapamil (dexverapamil) and biricodar and these agents are more potent and less toxic than the first generation modulators.[16]

An obvious starting point to circumvent MDR in P-gp expressing cells is to design the novel chemotherapeutic agents that are not recognized/transported by the pump[17] A series of substituted pyrrolopyrimidines inhibit the function of p-glycoprotein without modulating multidrug resistance-related protein (MRP).These compounds have their toxicity towards drug sensitive tumor cells and have their ability to antagonize the p-glycoprotein mediated resistant cells and MRP-1 mediated resistant cells.[18]

The concept of pharmacophore was first introduced in 1909 by Ehrlich, who defined the pharmacophore as 'a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity. [19] A pharmacophore refers to the three dimensional (3D) arrangement of atoms or functional groups in a molecule necessary for the compound to bind to a specific enzyme or receptor [20]. The computational models can be generated and validated utilizing either the 3D structure of the target or a set of active analogues specific to the target or by combining information from structure of the drug target and a set of active analogues specific to the target. [21] 3D database pharmacophore searching attempts to identify molecules in a database on the basis of their possessing a particular pharmacophore in their structure, expressed necessarily in three dimensions [22]. Pharmacophore modeling and 3D database searching are integral components of lead discovery and lead optimization, and the continuing need for improved pharmacophore based tools has driven the development of PHASE [23] The discovery of an innovative new chemical entity requires three key steps: the discovery of relevant biological targets, the generation of "lead" compounds, and the optimization of these leads.[24] Mukesh C. Sharma used the the molecular modeling software package VLife Molecular Design Suite version 3.5_3D for the study of substituted benzimidazole derivatives as dipeptidylpeptidase IV inhibitors.[25] Simone Brogi wield a Software Phase to derive a 3D-QSAR model based, as alignment rule, on a pharmacophore built on three compounds highly active against MCF-7 cell line[26]. Vivek K. Vyas et al established the Pharmacophore models of PKB _ inhibitors using the DISCOtech.[27] Maria L. Lopez-Rodriguez optimized a pharmacophore model for 5-HT7R

antagonism, with the incorporation of recently reported ligands and using an efficient procedure with the CATALYST program.[28] Pharmacophore models from the ligand based approach are very useful for analyzing receptor ligand interactions. The generated pharmacophore model can be useful for rational design and identifying novel active compounds through a database search. The purpose of pharmacophore is to perform in silico screening searches in a 3 dimensional database of a virtual or real compound library to find diverse structures with desired binding activity and selectivity. The purpose of present work is to develop a rapid and robust, chemical feature based 3-dimensional pharmacophore model to predict the p-glycoprotein inhibitory activity. In addition, we aim to gain more information that could be used in further development and optimization of such p-glycoprotein inhibitors. To reach our objective, we used a series of substituted pyrrolopyrimidines that have their toxicity towards drug sensitive tumor cells and antagonize p-glycoprotein.

MATERIALS AND METHODS

Dataset

In the present investigation, 33 pyrrolopyrimidine derivatives having p-glycoprotein inhibitory activity were considered [18]. Data set of compounds considered were divided randomly into training set and test set by considering the 75% of the total molecules in the training set and 25% in the test set.

These compounds were evaluated in drug accumulation assays using Pg-p expressing cell line. The p-glycoprotein inhibitory activity was expressed as IC_{50} i.e., concentration in μM required for 50% inhibition of activity Twenty four molecules forming the training set were used to generate pharmacophore models and prediction of the activity of test set (08 analogues) molecules was used as a method to validate the proposed models.. The basic structure for these analogues is shown in Fig.1 and various substituents are enlisted in Table 1.

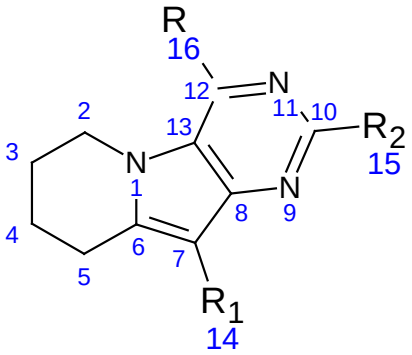
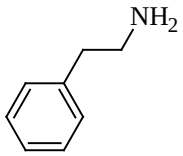
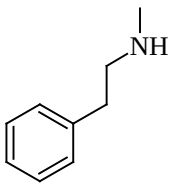
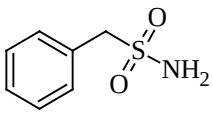
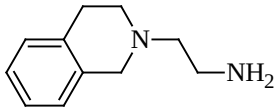
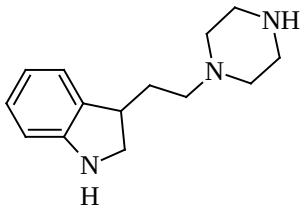
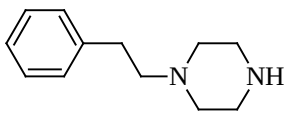
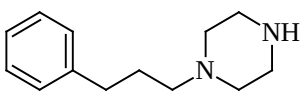
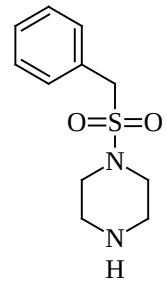
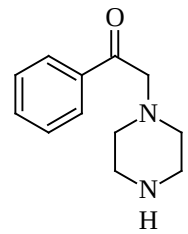
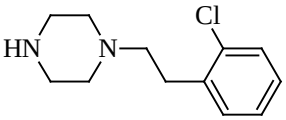
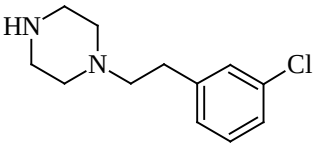
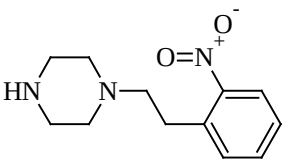
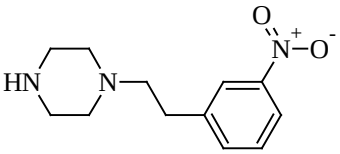
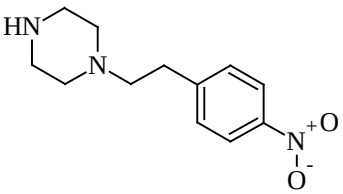
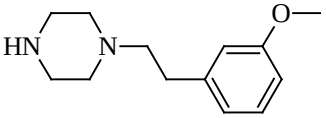
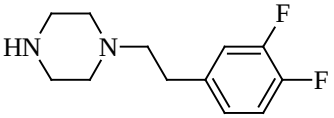
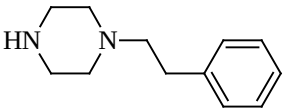
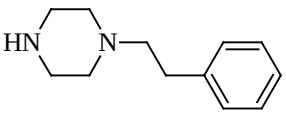
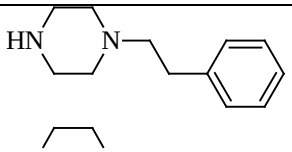
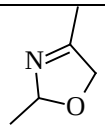
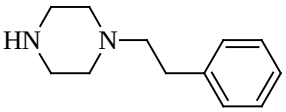
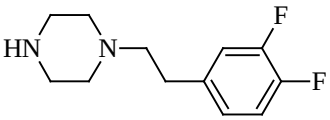
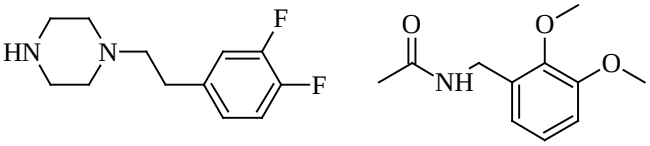
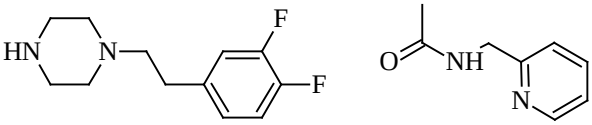
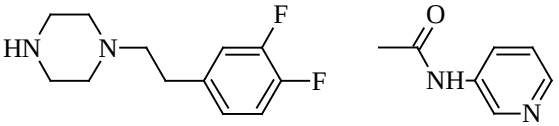


Fig. 1: Basic structure of Pyrrolopyrimidines

Table 1: Experimental inhibitory activity of compounds towards P-glycoprotein

Compound no	R	R_1	R_2	p-gp Antagonism IC_{50}	Fitness Score
Training Set					
1)		CN	H	16.0	2.43
2)		CN	H	33.0	2.47
3)		CN	H	5.4	1.94
4)		CN	H	50	2.32
5)		CN	H	5.82	2.59
6)		CN	H	9.63	2.89
7)		CN	H	13.	2.63

8)		CN	H	14.0	2.29
9)		CN	H	23	2.78
10.		CN	H	10.63	2.91
11.		CN	H	17.9	2.89
12.		CN	H	50	2.72
13.		CN	H	9.665	1.45
14.		CN	H	12.94	2.89
15.		CN	H	16.2	2.90
16.		CN	H	10.52	3.00
17.		CN	I	13.05	2.35
18.		CN	Ph	5.0	2.75

19.			H	3.7	2.24
20.		$CONH_2$	H	50	2.24
21.		CONHEt	H	4.79	2.28
22.			H	3.7	2.13
23.			H	3.4	2.16
24.			H	4.95	2.07
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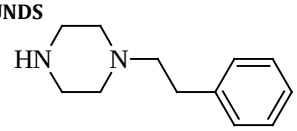
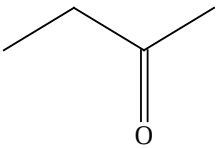
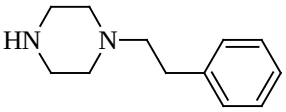
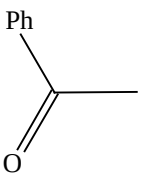
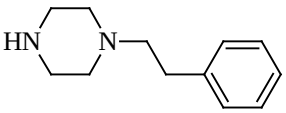
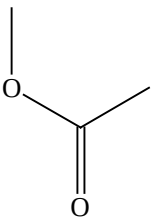
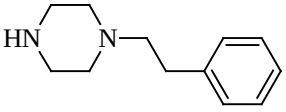
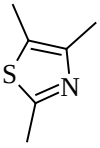
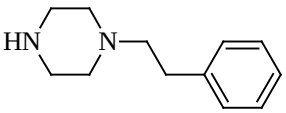
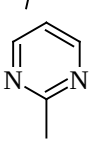
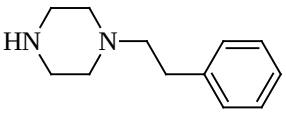
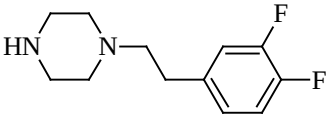
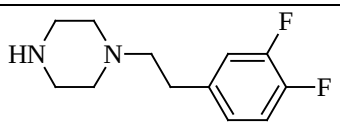
1			H	50	1.99
2			H	10.7	1.30
3			H	16.5	2.39
4			H	6.0	1.44
5			H	50	2.24
6		CN	4-$CH_3CONHPh$	23.7	2.66
7		CN	4-Pyr	1.31	2.74

8		CN	2-CH_3-Ph	17.99	2.28

Pharmacophore modeling

The pharmacophore modeling studies were performed using 'PHASE': a module of Schrödinger's software program 'MAESTRO. [29] The continuing need for improved pharmacophore based tools has driven the development of 'PHASE'. To reach our research objectives we used 'PHASE': a module of Schrödinger's software program 'MAESTRO'[30]

Preparation of ligands

The first step for pharmacophore mapping was ligand preparation. The chemical structures of all the compounds were drawn in maestro and geometrically refined using the option 'clean structures' and 'generate confermers', produces a single, low-energy, 3D structure with correct chiralities for each successfully proposed input structure. While performing this step, chiralities were determined from 3D structure and original states of ionization were retained. Tautomers were generated using MacroModel method discarding current conformers. The conformations were generated by the Monte Carlo (MCMM) method as implemented in MacroModel version 9.6 using a maximum of 2,000 steps with a distance-dependent dielectric solvent model and an OPLS-2005 force field. All the conformers were subsequently minimized using truncated Newton conjugate gradient (TNCG) minimization up to 500 iterations. For each molecule, a set of conformers with a maximum energy difference of 30 kcal/mol relative to the global energy minimum conformer was retained.

Pharmacophore modeling

A common pharmacophore hypothesis is a spatial arrangement of chemical features common to two or more active ligands,which is proposed to explain the key interactions involved in ligand binding. Phase uses chemical features that was not an atomistic description of the compounds but a description of chemical properties(e.g.,hydrogen bond donor or acceptor, etc.) to build up the pharmacophores. A pharmacophore model or hypothesis is a collection of chemical features placed in 3D space that represent the most important characterstics of a ligand to bind to its receptor or have a certain biological affinity. Once a pharmacophore model is established, a beneficial use of it is 3D database searching to retrieve novel compounds that would match the pharmacophore. Common features hypothesis generated by phase are designed specifically for finding chemical features shared by a set of selective p-gp inhibitors and for providing the compounds relative alignment with a particular common features hypothesis. At the beginning of the hypothesis generation every conformer of each molecule was examined for the presence of chemical functions which will be selected and used to build the parmacophore hypothesis. Each hypothesis is scored according to how well the active ligands superimpose when they are aligned on the features associated with that hypothesis. A set of seven hypothesis were generated with 28 compounds.

Only the active ligands are normally considered when developing common pharmacophore hypothesis.Inactives can be used to eliminate hypothesis that do not provide a good explanation of activity on the basis of pharmacophore alone. The inactives scores may be used to assigned adjusted score that reflect the degree to which the models distinguish actives from inactives. For generating a phamacophore hypothesis a set of pharmacophore features were created using 'create sites' option.The chemical feature types are hydrogen-bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H) and aromatic ring (R),negative (N).In the present study three features donor (D),hydrophobic group(H), aromatic ring(R) could effectively map all critical chemical features of all the ligands. The minimum and maximum sites for all the features were kept 4 by "maximum and minimum columns" in 'features frequency' table. pharmacophore from all conformations of the active ligands were examined and those pharmacophore that contained identical set of features with very similar spatial arrangement were grouped together. These features were selected and used to build a series of hypothesis with the find common pharmacophore option in Phase.

Scoring Hypothesis

In the next step, common pharmacophores were examined and a scoring procedure were applied to identify the pharmacophore that yields the best alignment of the active ligands.This pharmacophore provided a hypothesis to explain how the active ligands bind to receptor. The quality of alignment were measured by survival score.[31] This scoring procedure provides a ranking of the different hypothesis to make rational choices about which hypothesis are the most appropriate for the model.

Build QSAR model

All the ligands were separated into training and test.In this partial least square (PLS) regression was applied to dataset to obtain a 3D QSAR model. From this it was encoded that ligand atoms or features occupied the various cubes. We can set the size of the cubes by changing the value in the Grid spacing text box. The regression is done by constructing a series of models with an increasing number of PLS factors. In present case, the pharmacophore based model were generated by keeping 1Å grid spacing and 3 as maximum number of PLS factors.

Validation

Validation is a crucial aspect of pharmacophore design, particularly when the model is built for the purpose of predicting activities of compounds in external test series. In the present case the good predicted power of this model is indicated by the high correlation coefficient between experimental and predicted activity values.

RESULTS AND DISCUSSIONS

P-glycoprotein (P-gp) a member of the ABC transporter superfamily confers resistance to drugs by preventing their accumulation within the cell or by pumping chemotherapeutic drugs from the cytoplasm. [32,33] When the mechanisms of multidrug resistance have been identified, the hope of identifying molecules able to reverse simultaneously the resistance to a number of unrelated drugs has stimulated research in this field.[34] Application of computational methodologies in the drug discovery process is well established. In virtual screening, computational models are used to predict the biological activity of compounds.

The ultimate goal of quantitative structure-activity relationship (QSAR) studies is the design of new, more-active, and selective compounds. [32] The interest in the idea of pharmacophores has been grown tremendously due to the availability of computer graphics, a number of computational methods to determine the pharmacophoric geometry and various softwares for 3D database mining using the concept of a pharmacophore pattern match.[35] In this current study, we have used the molecular structures of substituted pyrrolopyrimidines. These ligands were used to generate a common feature 3D pharamacophore. The pharmacophore alignment consisted of features two acceptors, one hydrophobic group (H) and one aromatic ring (R) which indicate that they are the important features for interaction. The purpose of pharmacophore modeling is to perform in silico screening searches in a 3 dimensional database of a virtual or real compound library to find diverse structures with desired binding activity and selectivity [36]. In the present study, a series of pyrrolopyrimidines were considered for molecular modeling studies. The studies were aimed at developing a ligand based pharmacophore model relating the P-

glycoprotein inhibitory activity of pyrrolopyrimidines to gain a greater understanding of the P-gp– ligand interactions. Twenty four molecules forming the training set were used to develop the pharmacophore models. The pharmacophoric features selected for creating sites were hydrogen bond acceptors, hydrophobic region (H) and aromatic ring (R). The three and five featured pharmacophore hypotheses were rejected due to low value of survival score, as they were unable to define the complete binding space of the selected molecules. Four featured pharmacophore hypotheses were selected and subjected to stringent scoring function analysis.

The results of four featured pharmacophore hypotheses, labeled AAHR1, AAHR2, AAHR3, AAHR4, AAHR5 and AAHR6 are presented in Table. 2. The hypothesis AAHR4 is the best hypothesis in this study, characterized by good survival score, highest F value and the best regression coefficient (0.9334). The AAHR4 pharmacophore hypothesis is presented in Fig.2. The features represented by this hypothesis are two hydrogen bond acceptors, one hydrophobic group (H) and one aromatic ring (R). The angles and distances between different sites of AAHR.6 are given in Table.3 and table.4 respectively.

Table 2: Parameters of different three featured hypothesis

Hypothesis no	Hypothesis	Survival score	R^2	F
1.	AAHR.1	3.639	0.864	44.8
2.	AAHR2	3.639	0.791	26.5
3.	AAHR3	3.436	0.920	80.7
4.	AAHR4	3.436	0.933	98.1
5.	AAHR5	2.49	0.829	34.1
6.	AAHR6	2.49	0.688	15.5

Table 3: Distances between different sites

Site1	Site2	Distance
A1	A2	3.882
A1	H6	5.233
A1	R10	3.777
A2	H6	5.391
A2	R10	2.545
H6	R10	2.851

Table 4: Angle between different sites

Site1	Site2	Site3	Angle
A2	A1	H6	70.7
A2	A1	R10	38.8
H6	A1	R10	32.0
A1	A2	H6	66.4
A1	A2	R10	68.4
H6	A2	R10	2.7
A1	H6	A2	42.8
A1	H6	R10	2.7
A1	H6	A2	42.8
A1	H6	R10	44.6
A2	H6	R10	2.5
A1	R10	A2	72.8
A1	R10	H6	103.4
A2	R10	H6	174.8

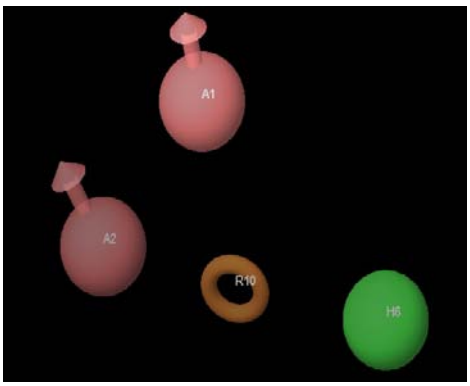


Fig. 2: PHASE generated pharmacophore model AAHR4 illustrates hydrogen bond acceptor (A1, A2; pink), aromatic ring (R10; orange) and hydrophobic ring (H6; green).

For each ligand, one aligned conformer based on the lowest RMSE of feature atom coordinates from those of the corresponding reference feature was superimposed on AAHR4 hypothesis.

Then fitness scores for all ligands were observed on the best scored pharmacophore model. The greater the fitness score, the greater the activity prediction of the compound.

The fit function does not only check if the feature is mapped or not, it also contains a distance term, which measures the distance that separates the feature on the molecule from the centroid of the hypothesis feature. Fig.3 shows the alignment of compound no 17 (antagonism at IC_{50} = 10.52) of the training set having max fitness score.

Besides this survival score analysis, the validity and predictive character of AAHR4 were further assessed by using test set molecules. A test set having eight molecules was analyzed. All the test set molecules were built and minimized as well as used in conformational analysis like all training set molecules.

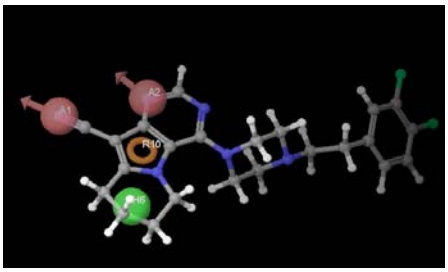


Fig. 3: Best pharmacophore model AAHR.6 aligned with the compound having max fitness score. Pharmacophore features are color coded: hydrogen bond acceptor (A1, A2; pink), aromatic ring (R10; orange) and hydrophobic ring (H6; green).

Then the activities of test set molecules were predicted using AAHR4 and compared with the actual activity. Actual and predicted activity values of test set molecules are given in Table 5. The predicted P-gp inhibitory activity exhibited a squared predictive correlation coefficient of 0.9835 with reported P-gp inhibitory activity using model AAHR4 (Fig.4). For a reliable model, the squared predictive correlation coefficient should be >0.60 [37,25]

The result of this study reveals that model AAHR4 can be used for the prediction of P-gp inhibitory activity.

Good and consistent external predictivity was observed for AAHR4 as compared to the others. AAHR4 showed a good r^2 value, i.e. 0.9308 and squared predictive correlation coefficient of 0.9835 was also observed between experimental and predicted activity values of test set molecules.

Table 5: Experimental and predicted values of test set molecules based on hypothesis AAHR4

Comp No	Experimental activity IC_{50} (µM)	Predicted activity IC_{50} (Mm	Comp No	Experimental activity IC_{50} (µM)	Predicted activity IC_{50} (µM)
1.	33.00	33.86	5.	5.00	4.14
2.	13.00	12.13	6.	10.7	9.93
3.	23.00	23.27	7.	6.00	7.64
4.	12.940	13.86	8.	16.00	18.53

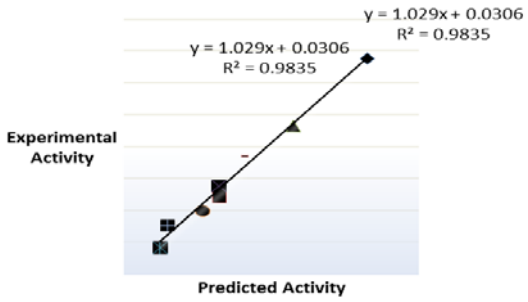


Fig. 4: Relation between experimental and predicted P-gp inhibitory activity values of test set molecules using model AAHR4

In order to gain additional insights about the nature of interactions of pyrrolopyrimidines with P-glycoprotein, we visualized and analyzed the 3-D QSAR models based on compound 17 using hydrogen bond acceptor and hydrophobicity features.

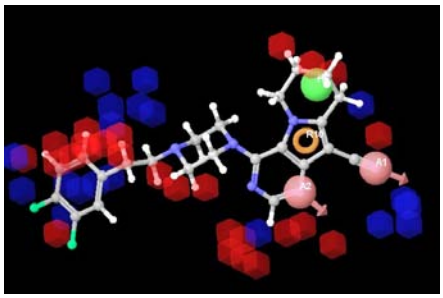


Fig. 5: 3-D QSAR model based on compound 17 illustrating hydrophobic feature

The 3-D QSAR model based on hydrophobic feature is shown in Figure 5. The blue region show hydrophobic substitution at 15 and 16 position will favour and increase the P-gp inhibitory activity and red region at 14 position shows that hydrophobic substitution at this position will decrease the activity. The substitutions on the ring attached to pyrollopyrimidine also affects the activity as blue regions at 3&4 position show that the activity is enhancing on substitution with hydrophobic groups and hydrophobic substitution at 5th position will decrease the activity as shown by red regions.

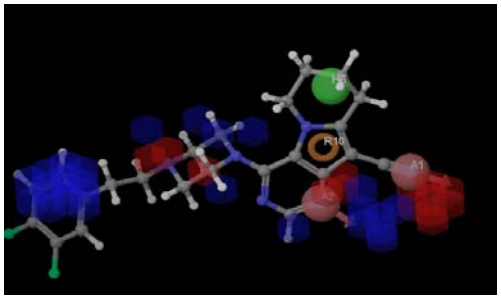


Fig. 6: 3-D QSAR model based on compound 17 illustrating electronwithdrawing feature.

In this fig.6 the compound no 17 is having intermediate activity. Blue regions show that any electron withdrawing groups at this position will increase the activity e.g the iodo group attached to R_2 in comp no 18 & compounds having 3,4-(F,F)-Ph at R_2 has more activity. Red region around nitirile group attached at R indicates that the substitutions at these positions by groups having less hydrogen bond acceptor property increase the p-gp inhibitory activity. Conversion of the nitrile into ketones and the corresponding methyl ester gave compounds 4, 6 of test set with increased p-gp activity as compared to analogue 17. Transformation of the nitrile into various heterocycles such as thiazole, oxadiazole, and pyrimidine 8 of test and 7 & 9 of training gave compounds with increasing activity. With the more active side chain, 3, 4-difluorophenethylpiperazine the simple amide analogue (comp no-22) appeared to be 2-fold more potent than the corresponding nitrile analogues at R.

CONCLUSION

The studies show the generation of a pharmacophore model AAHR4 for pyrrolopyrimidines compounds acting as P-gp inhibitors. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features. Hypothesis AAHR4 represents the best pharmacophore model for determining P-gp

inhibitory activity. AAHR4 consists of one hydrogen bond acceptor, one hydrophobic region and two aromatic ring features. This pharmacophore model was able to accurately predict P-gp inhibitory activity and the validation results also provide additional confidence in the proposed pharmacophore model. AAHR4 can provide us a way to rationally design new pyrrolopyrimidines compounds as P-gp inhibitors. Results suggested that the proposed 3-D QSAR model can be useful to identify new promising compounds as P-gp inhibitors in large 3-D database of molecules. These investigations may be useful for the design of more potent and selective p-gp inhibitors for the treatment of a wide variety of diseases.

CONFLICT OF INTERESTS

Declared None

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