Pharmacophore mapping studies on pyrazoles as anti-proliferative agents

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Abstract: Pharmacophore mapping investigations were carried out on a dataset of 36 pyrazole derivatives that demonstrate antiproliferative activity in human ovarian adenocarcinoma A2780 cells. Pharmacophore modeling is a powerful tool for activity prediction of the ligands. Three point pharmacophore model was developed with two hydrophobic groups, one hydrogen bond acceptor, as pharmacophoric features. The pharmacophore hypothesis AHH.14 with R^2 value 0.909 yields a 3D-QSAR model which is statistically significant and best pharmacophore hypothesis. External validation of AHH.14 was performed by activity prediction of test set molecules with squared predictive correlation coefficient of 0.875 was observed between experimental and predicted activity values of test set molecules. AHH.14 pharmacophore model may offer potential for the design of antiproliferative agents via development of lead structures.

Keywords: Antiproliferative activity, Pyrazole, 3D-QSAR, Pharmacophore mapping, Computational studies.

INTRODUCTION

Cancer involves the abnormal growth of cells wherein the cells exhibit aggressiveness and invasiveness characterized by "unlimited growth and division" and "invasion of adjacent tissues", respectively (Lemon *et al*., 1997). The above two properties along with the metastatic nature forms the inherent part of the malignant cancer (MC) and makes MC significantly different with respect to benign tumors characterized by self-limited growth, non-invasiveness and non-metastatic nature (Pae *et al*., 2003). Although there are many underlying reasons of this unregulated growth, genetic abnormalities of transformed cells are considered as its major cause (Bhalla *et al*., 2003). Nitrogen heterocycles such as indole, pyrrole, piperazines, imidazole, pyrazole etc. exhibits variety of biological activities (Sharma *et al*., 2010). Pyrazole derivatives have been reported to possess antiproliferative activities against various cancer cell lines (Balbi *et al*., 2011; Lv *et al*., 2009).

Pharmacophore modeling (PM) is a three dimensional computational approach, mainly to rationalize distribution of activities within groups of molecules and supposed to be recognized by the same site of a target protein (Tafi *et al*., 2009). Hence, the structural or chemical features of a drug which are essential for optimum binding to the protein are identified by this process (Bharatham *et al*., 2007). PM is of special significance in drug design and development particularly in case of unavailable structural data of the desired receptor. Although, PM was initially used for lead molecules discovery, it is now an established protocol for lead optimization (Khedkar *et al*., 2007).

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The present research report was envisaged to develop a pharmacophore model using PHASE protocol i.e. pharmacophore alignment and scoring engine of Schrödinger suite for pyrazole derivatives as antiproliferative agents. The pharmacophore points were aligned to derive the pharmacophore-based 3D-QSAR model (Hariprasad *et al*., 1996). The developed hypothesis identifies the most important structural properties for the antitumor activity of pyrazole rings.

MATERIALS AND METHODS

Dataset

Briefly, 36 pyrazole derivatives with potential antiproliferative activity were employed to generate the *in vitro* biological data employing human ovarian adenocarcinoma A2780 cells (Balbi *et al*., 2011). The concentration (μm) of pyrazole derivatives required for 50% inhibition of cell proliferation (IC_{50}) was calculated and finally expressed as pIC_{50} (-log IC_{50}) for PHASE data input. The dataset was equally and randomly divided into test and training set with 50% molecules (18) per set. Fig. 1 and table 1 list the basic structure and respective substituents of pyrazole derivatives used in the study. The training set was used for the generation of pharmacophore model while the prediction of test set activity was employed for the validation of proposed models.

Pharmacophore Modeling

For the generation of pharmacophore models, PHASE software was used. Maestro algorithm was used to draw the chemical structures of the molecules followed by the application of LigPrep program to produce perfectly chiral and high quality all-atom 3D structures. The structures so obtained were subjected to ionization at pH

7. Conformers of the training set molecules were generated with Monte Carlo method by OPLS-2005 force field as provided in Macro Model 9.6 2010, then these were minimized using Truncated Newton Conjugate Gradient (Phase, 2008; Maestro, 2008).

Two pharmacophoric features namely hydrogen bond acceptor (A) and hydrophobic groups (H) were used to generate pharmacophore sites to map all essential chemical features of dataset molecules. Three to six sites for all the features were fixed. Pharmacophore hypotheses and common pharmacophore were created by Phase, 2008. The regression was carried out on hypotheses with stepwise increasing number of PLS factors. 3D-QSAR models were generated using 18 molecules of training set with 1 to 3 PLS factors and by fixing grid spacing of 1 A◦. Further, common pharmacophore hypotheses were evaluated using survival score to find the best alignment of the active molecules using root mean square deviation value of 1.2 Å. The scoring of hypotheses were performed using default parameters. Survival score secured by each hypothesis, is the measure of the quality of alignment for a particular hypotheses (Kaushik *et al*., 2012).

Validation of Pharmacophore Model

For the assessment of the best hypotheses, validation needs to be performed wherein the pharmacophore model can be validated for its ability to accurately predict the biological activity or for the identification of the active molecules from the dataset. Among the above two options; the activity prediction of test set was chosen as the validation criteria for the developed pharmacophore model. Briefly, the test molecules were processed using the same development protocol as was used for the training set molecules followed by activity prediction of test molecules employing the pharmacophore model developed in the study. Finally, determination of a prospective correlation between experimental and predicted activities of the test molecules was envisaged.

Table 1: Antiproliferative activity data for pyrazole derivatives

Comp. No	R	R	R	R	R	R_5	R_6	pIC_{50}	Set
1a	2-pyridinyl	H	H	methoxy	H	methyl	H	4.0	Test
1b	2-pyridinyl	H	H	methoxy	H	phenyl	H	4.0	Training
1c	2-pyridinyl	H	H	methoxy	H	H	phenyl	4.0	Training
1d	2-pyridinyl	H	H	methoxy	H	H	methyl	4.170	Training
1e	2- pyridinyl	H	H	methoxy	H	-	-	5.539	Test
1f	4-chlorophenyl	hydroxy	H	H	methyl	-	-	4.293	Test
1g	2-pyridinyl	hydroxy	H	H	chloro	-	-	4.0	Test
1h	2-pyridinyl	hydroxy	H	methoxy	H	-	-	5.913	Training
1i	2-pyridinyl	H	H	acetamidyl	H	-	-	4.0	Training
1j	2-pyridinyl	hydroxy	H	H	H	-	-	4.0	Test
1k	2-pyridinyl	H	methoxy	H	H	-	-	4.0	Training
1l	2-pyridinyl	H	H	morpholino	H	-	-	4.0	Test
1m	2-pyridinyl	methoxy	H	methoxy	H	-	-	4.070	Training
1n	2-pyridinyl	methoxy	H	H	H	-	-	4.0	Test
1o	4-chlorophenyl	H	H	methoxy	H	-	-	5.250	Training
1p	4-chlorophenyl	hydroxy	H	H	chloro	-	-	4.277	Test
1q	4-chlorophenyl	hydroxy	H	methoxy	H	-	-	5.628	Test
1r	4-chlorophenyl	hydroxy	H	H	H	-	-	4.201	Test
2a	2- pyridinyl	H	H	methoxy	H	-	-	4.0	Test
2b	2-pyridinyl	hydroxy	H	H	chloro	-	-	4.195	Training
2c	2-pyridinyl	hydroxy	H	methoxy	H	-	-	4.195	Test
2d	2-pyridinyl	hydroxy	H	H	H	-	-	4.160	Training
2e	2-pyridinyl	methoxy	H	H	H	-	-	4.0	Training
3a	3-chlorophenyl	H	-	-	-	-	-	4.276	Training
3b	ethylethanoate	H	-	-	-	-	-	4.134	Test
3c	thiocarboxamide	H	-	-	-	-	-	4.298	Training
3d	4-methoxyphenyl	H	-	-	-	-	-	4.290	Training
3e	benzyl	H	-	-	-	-	-	4.317	Training
3f	3,4-dimethylphenyl	H	-	-	-	-	-	4.301	Test
3g	2-pyridinyl	H	-	-	-	-	-	4.339	Test
3h	2-pyridinyl	methyl	-	-	-	-	-	4.075	Training
4a	phenyl	-	-	-	-	-	-	4.0	Test
4b	2-furyl	-	-	-	-	-	-	4.0	Training
4c	4- chlorophenyl	-	-	-	-	-	-	4.0	Test
4d	3-chlorophenyl	-	-	-	-	-	-	4.0	Training
4e	3,4-dimethoxyphenyl	-	-	-	-	-	-	4.0	Test

RESULTS

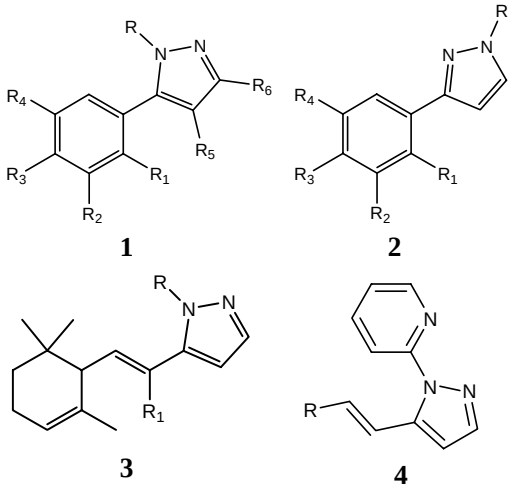


Fig. 1: Basic structures of pyrazole derivatives (**1-4**)

Table 2 depicts the results of three featured pharmacophore hypotheses, labeled AHH.14, AHH.16, AHH.30 and AHH.55. AHH.14 represented the best hypothesis (fig. 2) with the highest survival score of 3.647, highest R^2 value of 0.9096, and Pearson R value of 0.7871 featuring a hydrogen bond acceptor (A) and hydrophobic groups (H). Table 3 and table 4 represent the distances and angles forming the molecular attributes between different AHH.14 sites. Table 5 shows the fitness score of training set while fig. 3 shows the alignment of AHH.14 with molecule 1h (pIC_{50}=5.913) of the training set.

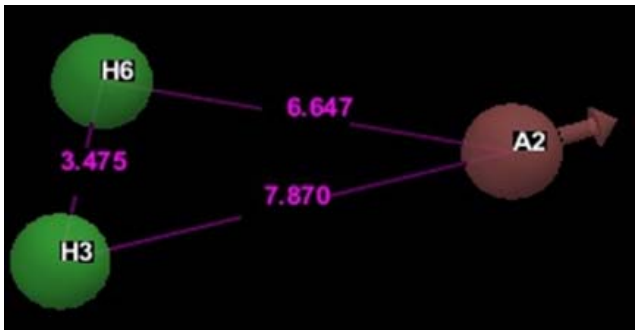


Fig. 2: PHASE generated pharmacophore model AHH.14 illustrating hydrogen bond acceptor (A2; pink), and hydrophobic group (H6, H3; green) features showing distances (in Å) between different sites of AHH.14.

Table 2: Parameters of the featured pharmacophore hypotheses

S. No	Hypothesis	Survival Score	R^2	Pearson-R
1	AHH.14	3.647	0.9096	0.7871
2	AHH.16	3.647	0.8203	0.4434
3	AHH.30	3.626	0.7464	0.4835
4	AHH.55	3.557	0.9313	0.3387

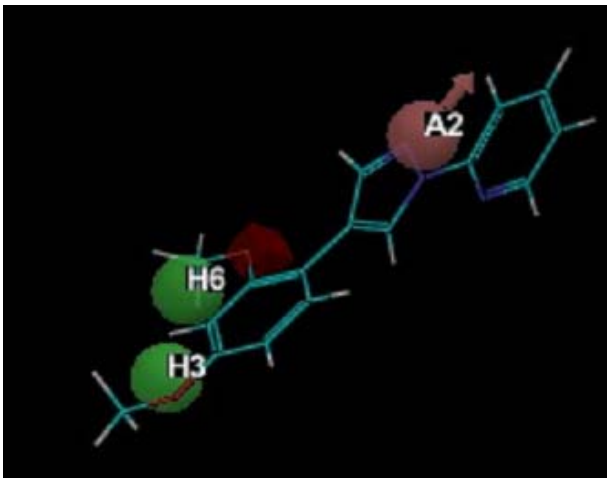
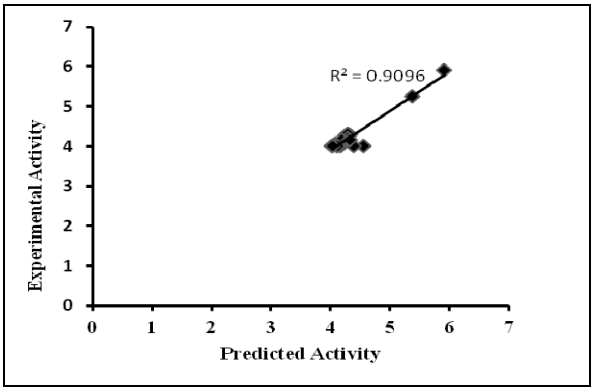
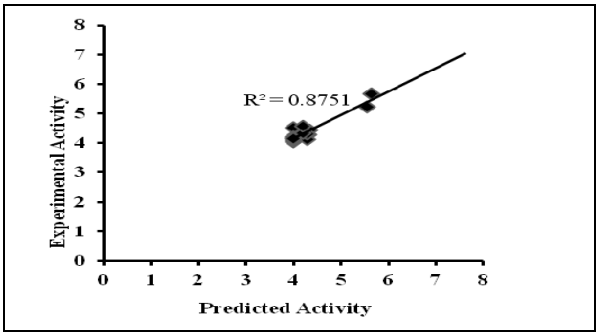


Fig. 3: Best pharmacophore model AHH.14 aligned with molecule 1h. Pharmacophoric features are color coded: hydrogen bond acceptor (A2; pink), Hydrophobic groups (H3, H6; Green).



(A)



(B)

Fig. 4: Relationship between experimental and predicted antiproliferative activity of training set (A) molecules and test set (B) molecules.

Table 3: Distances between different sites of model AHH.14

Entry	Site-1	Site-2	Distance (A)
AHH.14	A_2	H_6	6.647
AHH.14	A_2	H_3	7.870
AHH.14	H_6	H_6	3.475

Table 6 represents the experimental and predicted activity (pIC_{50}) values of the molecules from the test set. The predicted/reported antiproliferative activity of the test set exhibited a close correlation value of 0.8751 using the best model from the study (AHH.14) as depicted in fig. 4B.

The fitness score for the ligands was calculated by superimposing each ligand on AHH.14.

Table 4: Angles between different sites of model AHH.14

Entry	Site 1	Site 2	Site 3	Angle (˚)
AHH.14	H6	A2	H3	26.0
AHH.14	A2	H6	H3	97.1
AHH.14	A2	H3	H6	57.0

DISCUSSION

Pyrazoles are known to possess antiproliferative activity as they can inhibit topoisomerase II (Diana *et al*., 2007), interfere with the pyrimidine synthesis (Pae *et al*., 2003) and microtubule assembly (Balbi *et al*., 2011) etc. Guner, 2000, proposed that the ligand-based drug design is based on the molecular knowledge that can assist in the development of a pharmacophore capable of defining the minimum structural requirement essential for molecule-target binding.

The pharmacophore model was developed by using eighteen molecules wherein hydrogen bond acceptor (A) and hydrophobic groups (H) were employed as pharmacophore features for creating interaction sites. Initially, 3 to 6 featured models were generated.

Pharmacophore hypotheses with low value of survival score were rejected as a complete binding space of a molecule cannot be defined in this case. Therefore, 4, 5 and 6 featured pharmacophore hypotheses were rejected and only 3 featured one was selected and further scoring function analysis was carried out. The fitness score not only measures the distance between the feature on the ligand and centre of the hypothesis feature but also evaluate if the feature is mapped or not (Rani *et al*., 2011). The prediction profiling of the test set was employed to assess the validity and predictive character of AHH.14 wherein the 18 molecule test set was analyzed. Likewise training set; the test set was sequentially built and further minimized before carrying out the conformational analysis. Thereafter, the biological activities of the test set were predicted employing AHH.14 model and additionally compared with the experimental antiproliferative activity. For the construction of a reliable model, Goyal and co-workers, 2011, proposed that the value for squared predictive correlation coefficient should be >0.60. In view of this; the AHH.14 model could be used for the prediction of

Table 5: Experimental and predicted pIC_{50} values of training set molecules based on hypothesis AHH.14

Comp. No	Experimental pIC_{50}	Predicted pIC_{50}	Fitness Score	Comp. No.	Experimental pIC_{50}	Predicted pIC_{50}	Fitness Score
1b	4.0	4.120	2.23	2d	4.160	4.180	2.15
1c	4.0	4.040	1.97	2e	4.0	4.100	2.11
1d	4.170	4.320	2.00	3a	4.276	4.260	2.74
1h	5.913	5.910	2.88	3c	4.298	4.270	2.66
1i	4.0	4.020	2.12	3d	4.290	4.320	2.71
1k	4.0	4.380	2.02	3e	4.317	4.290	2.58
1m	4.070	4.080	1.54	3h	4.075	4.110	2.31
1o	5.250	5.370	2.56	4b	4.0	4.170	2.11
2b	4.195	4.280	1.48	4d	4.0	4.560	2.10

Table 6: Experimental and predicted pIC_{50} values of test set molecules based on hypothesis AHH.14

Comp. No	Experimental pIC_{50}	Predicted pIC_{50}	Fitness Score	Comp No	Experimental pIC_{50}	Predicted pIC_{50}	Fitness Score
1a	4.0	4.050	2.05	1r	4.201	4.340	2.15
1e	5.539	5.230	2.11	2a	4.0	4.150	2.23
1f	4.293	4.120	2.02	2c	4.195	4.560	2.11
1g	4.0	4.050	2.56	3b	4.134	4.250	2.74
1j	4.0	4.110	2.11	3f	4.301	4.280	2.66
1l	4.0	4.190	1.92	3g	4.339	4.440	2.71
1n	4.0	4.180	1.91	4a	4.0	4.520	2.58
1p	4.277	4.320	1.54	4c	4.0	4.150	2.31
1q	5.628	5.660	1.48	4e	4.0	4.160	2.10

antiproliferative activity.

CONCLUSION

In the present study, AHH.14 pharmacophore model was generated using pyrazole derivatives acting as antiproliferative agents. Hypothesis AHH.14 represents the best pharmacophore model having regression coefficient 0.9096, survival score 3.647 and Pearson *R* 0.7871. It consisted of two hydrophobic groups and one hydrogen bond acceptor. A strong correlation between predicted and experimental activity of the test (R^2=0.8751) and training (R^2=0.9096) set molecules was exhibited by AHH.14 model. In view of the above results, antiproliferative activity was accurately predicted by AHH.14 model. Validation results further increase the suitability of the proposed pharmacophore model. In the end, we concluded that AHH.14 model can be a suitable tool for the design of new bioactive ligands.

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