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RESEARCH PAPER

INDOLIZINE DERIVATIVES AS PHOSPHODIESTERASE IV INHIBITORS: DEVELOPMENT AND VALIDATION OF PHARMACOPHORE MODELS

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The challenges of drug discovery are largely overcome by computer aided designing and among various drug designing techniques, ligand based drug designing proves to be an effective one. Looking at the usefulness, in the present investigation ligand-based pharmacophore models has been developed by analyzing common chemical features of phosphodiesterase IV (PDE4) inhibitors. A dataset of 38 indolizine derivatives was selected in order to built pharmacophore models which were developed by using pharmacophoric features *viz.* hydrogen bond acceptor (A) and aromatic ring (R). In order to build up a statistically significant model, ARRRR.30 hypothesis was selected among different developed hypothesis with a R^2 value 0.880. The selected hypothesis ARRRR.30 was further validated by performing external validation on a test set where R^2 was found to be 0.804 (between experimental and predicted activity). The developed model could be an efficient tool to develop new PDE4 inhibitors.

Key words: Indolizine; Phosphodiesterase IV inhibitors; QSAR; Rational Drug Design; Pharmacophore.

INTRODUCTION

Recognition of cyclic adenosine monophosphate (cAMP) as second messenger (Sutherland *et al* 1968), can be considered as an important discovery as cAMP mediates a wide range of cellular processes (Gloerich and Bos, 2010; Hofer and Lefkimmiatis, 2007) *viz*. inflammation (Moore and Willoughby, 1995), T-cell proliferation (Muñoz *et al* 1990), cardiac actions (Haikala *et al* 1997), CNS activity (Han *et al* 2006) etc. The control over cAMP is an important area where research is under progress in order to have potent drug against different ailments.

Phosphodiesterases (PDEs) regulates cAMP and cyclic guanosine monophosphate (cGMP) levels in cells and further catalyze their degradation into AMP and GMP, respectively (Chen *et al* 2011). Role of PDE4 isozyme in cAMP degradation which in turn mediates key inflammatory cytokines is widely recognized (Bäumer *et al* 2007; Oger *et al* 2005) and considering the fact that PDE4 inhibitors played an important role in ailments like inflammation, Alzheimer's disease, Parkinson's, asthma etc. (Castro *et al* 2005; Banner and Trevethick, 2004; Jeffery, 2005; O'Donnell and Zhang, 2004; Spina, 2004; Houslay *et al* 2005). Chen and coworkers (Chen *et al* 2011) designed and synthesized a series of novel indolizine-2-oxoacetamides as PDE4 inhibitors.

Different heterocyclic derivatives like pyrazole, quinoline, furan, indolizine etc. were designed in recent past as PDE4 inhibitors and availability of

a 3D-QSAR model on PDE4 inhibitors will be an added advantage as it can screen different heterocyclic derivatives for their PDE4 inhibitory potential. However, some 3D-Pharmacophore models are available on PDE4 inhibitors like quinoline (Gaurav and Singh, 2014), benzodiazepine (Ducrot *et al* 2001) but to the best of our knowledge a 3D-model on indolizine derivative against PDE4 isozyme is not available. Considering the fact, we thought it is worthwhile to generate pharmacophore models on the indolizine derivatives against PDE4 inhibitor. A pharmacophore model with a decent R^2 could be an effective way for designing of novel indolizine derivatives with potent PDE4 inhibitory potential.

In the present study, pharmacophoric features were aligned *via* PHASE module (Schrödinger) in order to develop 3D-QSAR models (PHASE, 2008). Generated hypothesis was then be used to find important structural features vital to judge PDE4 inhibitory potential of indolizine derivatives.

EXPERIMENTAL

Dataset

A dataset of 38 indolizine-2-oxoacetamides designed as PDE4 inhibitors (Chen *et al* 2011) was selected to develop a pharmacophore model. The chosen dataset was then randomly divided into training (17) and test set (21). The IC_{50} value of each derivative was converted into pIC_{50} (negative logarithm of IC_{50}). The basic structures with their respective substituents are shown in **Figure 1** and **Table 1**, respectively.

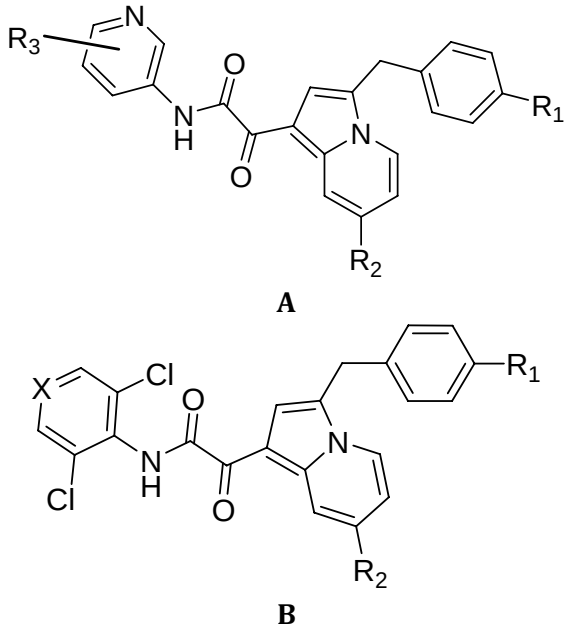


Fig. 1. Basic structures of selected indolizine derivatives

Table 1. PDE4 inhibitory activity data of indolizine derivatives

Ligand No.	Series	R_1; R_2; R_3	X	Exp. Activity	PLS Factors	Predicted Activity	Fitness score	Dataset
1a	A	H; H; H	-	6.458	1, 2, 3	6.25; 6.38; 6.03	2.19	Test
1b	A	F; H; H	-	6.467	1, 2, 3	5.75; 5.93; 6.25	2.1	Test
1c	A	Cl; H; H	-	6.782	1, 2, 3	5.72; 5.90; 6.25	2.1	Test
1d	A	MeO; H; H	-	6.349	1, 2, 3	6.85; 6.44; 6.35	2.96	Test
1e	A	OH; H; H	-	6.485	1, 2, 3	5.87; 6.07; 6.40	2.1	Test
1f	A	NO_2; H; H	-	6.244	1, 2, 3	5.83; 5.98; 6.22	2.08	Test
1g	A	NH_2; H; H	-	6.084	1, 2, 3	6.40; 6.19; 6.42	2.61	Test
1h	A	CN; H; H	-	6.143	1, 2, 3	6.58; 6.08; 5.87	2.96	Test
1i	A	CF_3; H; H	-	6.429	1, 2, 3	5.75; 5.92; 6.24	2.06	Test
1j	A	*i*-Pr; H; H	-	6.441	1, 2, 3	5.75; 5.92; 6.20	2.09	Test
1k	A	F; OH; H	-	6.383	1, 2, 3	6.69; 6.27; 6.18	2.99	Test
1L	A	F; Cl; H	-	6.621	1, 2, 3	6.86; 6.49; 6.49	3	Test
1ad	B	CN; Cl; NA	N	7.086	1, 2, 3	7.28; 7.77; 7.39	2.15	Test
1ae	B	F; Cl; NA	N^+-O^-	7.267	1, 2, 3	7.27; 7.72; 7.44	2.18	Test
1af	B	F; F; NA	N^+-O^-	7.744	1, 2, 3	6.78; 6.96; 7.66	2.44	Test
1ag	B	Cl; H; NA	N^+-O^-	7.721	1, 2, 3	7.11; 7.50; 7.10	2.16	Test
1ah	B	MeO; H; NA	N^+-O^-	7.096	1, 2, 3	6.82; 7.01; 7.70	2.4	Test
1ai	B	OH; H; NA	N^+-O^-	7.537	1, 2, 3	7.31; 7.75; 7.43	2.17	Test

1aj	B	F; H; NA	CH	5	1, 2, 3	4.94; 5.48; 5.72	2.44	Test
1ak	B	F; H; NA	CCF_3	5	1, 2, 3	4.48; 4.86; 5.10	2.37	Test
2b	B	F; H; NA	N^+-O^-	7.537	1, 2, 3	6.73; 6.90; 7.58	2.43	Test
1m	A	Cl; MeO; H	-	6	1, 2, 3	6.18; 5.92; 6.11	2.6	Training
1n	A	CN; H; 2-Cl	-	5.327	1, 2, 3	5.57; 5.68; 5.97	1.89	Training
1o	A	Cl; H; 2,4-$(Cl)_2$	-	7.075	1, 2, 3	5.67; 5.97; 6.67	1.99	Training
1p	A	CN; H; 2,4-$(Cl)_2$	-	6.602	1, 2, 3	6.90; 6.41; 6.26	2.91	Training
1q	A	F; H; 2,4-$(Br)_2$	-	5	1, 2, 3	6.53; 5.96; 5.38	2.75	Training
1r	A	CN; H; 2,4-$(MeO)_2$	-	5	1, 2, 3	6.02; 5.98; 4.85	2.09	Training
1s	A	CN; H; 6-(OMe)	-	5.823	1, 2, 3	6.06; 5.63; 5.64	2.58	Training
1t	B	CN; H; NA	N	6.935	1, 2, 3	7.15; 7.57; 7.10	2.14	Training
1u	B	F; H; NA	N	7.886	1, 2, 3	6.93; 7.17; 7.91	2.44	Training
1v	B	Cl; H; NA	N	7.795	1, 2, 3	7.77; 7.45; 7.64	2.84	Training
1w	B	MeO; H; NA	N	7.397	1, 2, 3	7.30; 7.79; 7.54	2.17	Training
1x	B	OH; H; NA	N	7.823	1, 2, 3	7.50; 8.03; 7.78	2.17	Training
1y	B	Cl; Cl; NA	N	7.585	1, 2, 3	6.08; 6.56; 7.59	1.98	Training
1z	B	F; Cl; NA	N	7.552	1, 2, 3	7.47; 8.03; 7.85	2.19	Training
1aa	B	F; F; NA	N	7.886	1, 2, 3	7.84; 7.55; 7.78	2.85	Training
1ab	B	OH; Cl; NA	N	8.698	1, 2, 3	8.10; 7.88; 8.21	2.85	Training
1ac	B	MeO; Cl; NA	N	7.92	1, 2, 3	7.57; 8.14; 7.93	2.13	Training

Pharmacophore modeling

In initial stage, Maestro algorithm and LigPrep program (PHASE software) were used to draw high quality 3D-structures with perfect chirality which were then subjected to ionization at pH 7. The conformers of the training set molecules were generated with Monte Carlo method by OPLS-2005 force field as provided in MacroModel 9.6 2008 and were subsequently minimized using Truncated Newton Conjugate Gradient (PHASE, 2008; Maestro, 2008).

During generation of hypothesis, two pharmacophoric features *i.e.* aromatic ring (R) and hydrogen bond acceptor (A) were appeared frequently. These pharmacophoric features were used to generate pharmacophore sites to map all essential chemical features of dataset molecules. Pharmacophoric sites (3-6) were fixed for all the chemical features and a common pharmacophore was generated which in turn leads to creation of various pharmacophore hypotheses by using PHASE, 2008. The regression was carried out on hypotheses with stepwise increasing number of PLS factors. Later on, by employing training set molecules (after setting a grid spacing of 1 A°) 3D-QSAR models were created. In the next step, the common pharmacophore hypotheses were assessed on the basis of their survival score in order to find finest configuration of the active molecules using root mean square deviation value of 1.2 A°. The scoring of hypotheses were performed using default parameters.

The stepwise process of QSAR model development is presented in **Figure 2** while detailed methodology has already been explained (Kaushik *et al* 2012; Kaur *et al* 2012; Bansal *et al* 2011; Rani and Kumar, 2011).

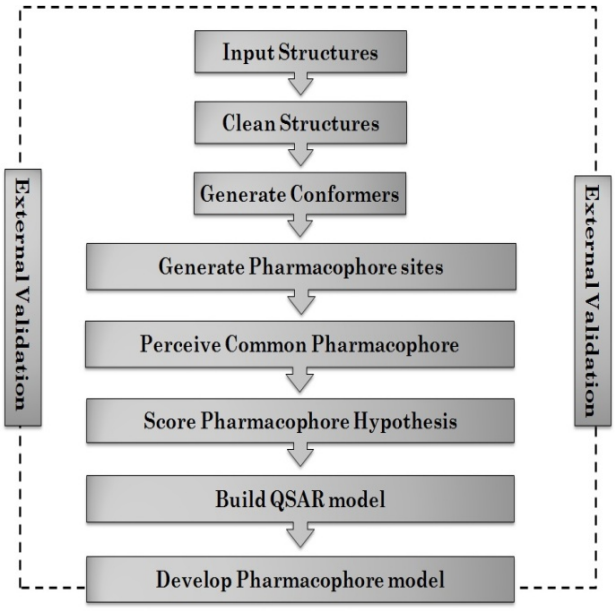


Fig. 2. Phase workflows

Validation of pharmacophore model

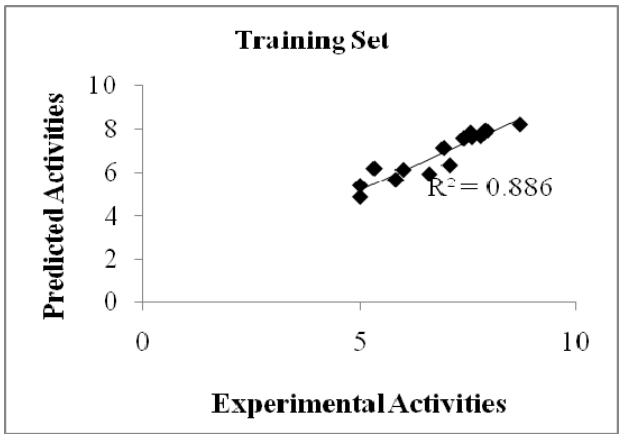
For the appraisal of the best hypotheses, validation used to be carried out to verify the strong correlation between the structures and their corresponding biological activity. In brief, both test and training set molecules were processed using similar protocol followed by activity prediction of test 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.

RESULTS AND DISCUSSION

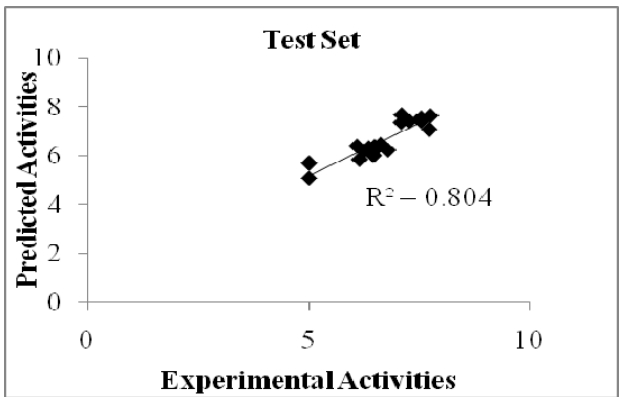
In our recent review, anti-tubercular, anti-proliferative, antimicrobial, anti-inflammatory potential of different indolizine derivatives was discussed (Sharma and Kumar, 2014). Furthermore, computational techniques like docking, molecular modeling play an important role in drug discovery (Kumar, 2011; Sharma *et al* 2011; Bansal *et al* 2011; Balasubramanian and Vijaya Gopal, 2012). Ligand-based drug design is such a significant technique by which a QSAR model may be developed that can portray vital structural requirements required for a particular biological activity.

The present study envisages the development of pharmacophore model by using 38 molecules in which aromatic rings (R) and hydrogen bond acceptor (A) were engaged as pharmacophoric features for creation of interaction sites. Different pharmacophore hypotheses (five features) were developed and each of them was further evaluated *via* stringent scoring function analysis. **Table 2** portrays the results of different pharmacophore hypotheses.

ARRRR.30 represented the best hypothesis (**Figure 3**) with highest R^2 value of 0.880 and good survival score of 3.396, featuring a hydrogen bond acceptor (A) and aromatic rings (R). However the specifications of ARRRR.30 model are presented in **Table 3** (distances) and **Table 4** (angles).



A



B

Fig. 3. Relationship between experimental and predicted PDE4 inhibitory activity of training set (A) molecules and test set (B) molecules

Table 2. Parameters of five featured pharmacophore hypothesis

Hypothesis	Survival	R-squared	F
ARRRR.21	3.523	0.637; 0.803; 0.862	61.5; 69.4; 69
ARRRR.22	3.481	0.609; 0.732; 0.872	54.5; 46.4; 75.1
ARRRR.30	**3.396**	**0.600; 0.727; 0.880**	**52.7; 45.3; 81**
AARRR.31	3.087	0.438; 0.646; 0.860	27.4; 31.1; 68
ARRRR.29	2.835	0.645; 0.736; 0.849	63.7; 47.4; 61.9
AARRR.2	2.417	0.448; 0.692; 0.821	28.4; 38.2; 50.5

Fitness score of a QSAR model could be useful in judging the correctness of a model as it was calculated by superimposing each ligand on the best available model *i.e.* ARRRR.30 in this study (**Table 2**). The superimposition measures the distance between the selected pharmacophoric features and the centre of the hypothesis feature which further evaluates its mapping potential. In order to validate the developed model, activity prediction of training set was performed besides survival score analysis. Therefore, the ARRRR.30 model was regressed against the training set. The PDE4 inhibitory activity data related to predicted and experimental activities of training

Table 3. Distances between different sites of 'model ARRRR.30'

Entry	Site1	Site2	Distance
ARRRR.30	A3	R7	3.48
ARRRR.30	A3	R9	4.136
ARRRR.30	A3	R8	5.558
ARRRR.30	A3	R10	7.437
ARRRR.30	R7	R9	2.153
ARRRR.30	R7	R8	7.239
ARRRR.30	R7	R10	4.744
ARRRR.30	R9	R8	8.907
ARRRR.30	R9	R10	6.533
ARRRR.30	R8	R10	8.23

Table 4. Angles between different sites of 'model ARRRR.30'

Entry	Site1	Site2	Site3	Angle
ARRRR.30	R7	A3	R9	31.4
ARRRR.30	R7	A3	R8	104
ARRRR.30	R7	A3	R10	29.8
ARRRR.30	R9	A3	R8	133
ARRRR.30	R9	A3	R10	61.1
ARRRR.30	R8	A3	R10	77.1
ARRRR.30	A3	R7	R9	91.4
ARRRR.30	A3	R7	R8	48.2
ARRRR.30	A3	R7	R10	128.8
ARRRR.30	R9	R7	R8	135.7
ARRRR.30	R9	R7	R10	139.5
ARRRR.30	R8	R7	R10	84
ARRRR.30	A3	R9	R7	57.3
ARRRR.30	A3	R9	R8	27.2
ARRRR.30	A3	R9	R10	85.3
ARRRR.30	R7	R9	R8	34.6
ARRRR.30	R7	R9	R10	28.2
ARRRR.30	R8	R9	R10	62.2
ARRRR.30	A3	R8	R7	27.8
ARRRR.30	A3	R8	R9	19.9
ARRRR.30	A3	R8	R10	61.7
ARRRR.30	R7	R8	R9	9.7
ARRRR.30	R7	R8	R10	35
ARRRR.30	R9	R8	R10	44.6
ARRRR.30	A3	R10	R7	21.4
ARRRR.30	A3	R10	R9	33.7
ARRRR.30	A3	R10	R8	41.2
ARRRR.30	R7	R10	R9	12.4
ARRRR.30	R7	R10	R8	61
ARRRR.30	R9	R10	R8	73.2

set molecules derived from ARRRR.30 hypothesis is presented in **Table 1**. **Figure 4** illustrates a good correlation of 0.886 between predicted and experimental PDE4 inhibitory activity of training set molecules using ARRRR.30 model. In the next step, predictive character or in other words validation of developed model was performed by assessing the PDE4 inhibitory activity of test molecules by using ARRRR.30 model which will in turn compared to experimental data.

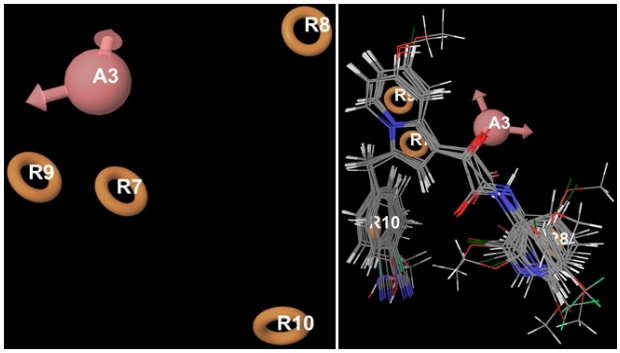


Fig. 4. (A) PHASE generated pharmacophore model ARRRR.30 illustrating one hydrogen bond acceptor (A3; pink), and four aromatic rings (R7; R8; R9; R10 orange) features. (B) All ligands overlapped on the generated model ARRRR.30.

In accordance with the observation of Goyal and co-workers, the squared predictive correlation coefficient of ARRRR.30 model is more than 0.60 (Goyal and Kumar, 2011), so ARRRR.30 model could be useful model in order to assess PDE4 inhibitory activity of indolizine derivatives. A correlation value of 0.804 was found between test and training set molecules (**Figure 3**).

CONCLUSION

In this paper, different pharmacophore models were generated by incorporating indolizine derivatives with PDE4 inhibitory activity. Among the developed models, hypothesis ARRRR.30 was selected on the basis of good regression coefficient value of 0.880 and survival score 3.396. The selected model constitutes one hydrogen bond acceptor (A) and four aromatic rings (R). ARRRR.30 model exhibits a correlation value of 0.886 in case of training set while on the other hand, ARRRR.30 model was effectively validated by assessing the activity of test molecules (R^2 = 0.804). Considering the correlation values and validation data it would be appropriate to mention ARRRR.30 as a good QSAR model which could be a valuable tool for designing of new PDE4 inhibitors.

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