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RESEARCH ARTICLE

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Molecular Modelling Studies on Chemometric Properties of Protein Tyrosine Kinase Inhibitors for Indolinone Derivatives

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Abstract

Protein tyrosine kinases (PTKs) are important components for signal pathways that control cell proliferation, differentiation and various regulatory mechanisms. Selective inhibition of the PTKs is now recognized as an attractive strategy for the development of new cancer therapeutics. Indolinone derivatives are already proved as potential PTK inhibitors of Fibroblast Growth Factor Receptor (FGFR) and anti cancer agent. In the present work, a set of indolinone derivatives are considered for chemometric designing for anti-cancer agents using ligand-based 3D QSAR and pharmacophore modelling studies. The 3D-QSAR models permit an understanding of steric, electrostatic, and hydrophobic along with HB acceptor and donor requirements for ligand interactions at the active site. Pharmacophore is the molecular framework that carries the essential features that can visualize the potential interaction between the ligand and the receptor and it is based on the kind of interactions observed in molecular recognition. In the present study, molecular field and similarity analyses (CoMFA: R^2=0.997, Q^2=0.714, *se*=0.037, R^2_{pred}=0.602, s_p=0.351; CoMSIA: R^2=0.999, Q^2=0.747, *se*=0.016, R^2_{pred}=0.712, s_p =0.541) explained that both steric and electrostatic are crucial for inhibitory activity. Additionally, HB acceptor and donor are obtained as important for HB bonding interactions. The pharmacophore modelling study (R^2 = 0.924, Q^2=0.752, *se*=0.047, R^2_{pred}=0.771, s_p =0.421) revealed the importance of HB acceptor and donor for the binding interaction at the active site cavity along with ring aromatic feature is found to be prime factor for inhibitory activity of the molecules. The study explained that rings 'C' and 'D' along with carbon chain between them are found to be essential for inhibitory activity. The amine and oxo group present in the ring 'C' also found critical for bonding interactions in the active site cavity of tyrosin kinase.

Keywords: Protein kinase, Indolinone Derivatives, QSAR, CoMFA, CoMSIA, Pharmacophore

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INTRODUCTION

In cancer, abnormal division of cells takes place mostly because of the enzyme, protein kinase (TK) and it exerts a variety of effects in human being and cytokines mediate cellular signalling through activating TK. In human disease, TKs are associated as potential therapeutic targets for different diseases including cancers, cardiovascular, inflammatory and fibrotic diseases [1]. It is elucidated that several synthetic compounds are identified those can block the function of specific TK and preclinical data of these compounds are emerging to support the use in clinical studies [1]. In the area of cancer, receptor TK plays important roles in the process of tumours development and spread. The receptor tyrosine kinases (RTKs) are involved in tumours growth, survival, metastasis, and angiogenesis. It is reported that the 3-substituated indolin-2-ones containing tetrahydroindolin moity have been designed and synthesized as novel class of TK inhibitors [2,3] such that it inhibit fibroblast growth factor receptor (FGFR) [4] and also act as competitive inhibitors with respect to ATP for binding to the intracellular catalytic domain.[2,3]

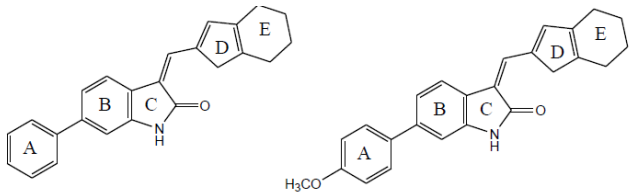


Fig. 1. Structures of most active indolinone derivative

Traditional drug discovery is a complex and resource consuming along with expensive process. Use of information technology in the field of drug development is well known as chemometric technique and rapidly gaining popularity, implementation and appreciation [5]. This technique can develop molecules with optimistic efficacy and low toxicity. Researchers from academic and industry are devoted to optimize drug like molecules by implementing chemometric techniques in the wide range of diseases. In the present study, indolinone derivatives have been explored to obtain pharmacophore/structural requirement for inhibiting kinase activity with the help of quantitative structure activity relationship (QSAR), and pharmacophore modelling studies. The well known Comparative Molecular Field Analysis (CoMFA) [6,7] and Comparative Molecular Similarity Indices Analysis (CoMSIA) [8,9] methods of 3D-QSAR study involved in the generation of a common 3D lattice around a set of compound. The steric and electrostatic energies are calculated at the lattice points for CoMFA whereas CoMSIA uses similarity function represented as Gaussian. The molecular information are transferred into numerical data using partial least square (PLS) [10] method that reduces the dimensionality of data by generating components that can correlate with the biological activity. The pharmacophore model is a set of functional group in a spatial arrangement that represent the interaction made in a common scaffold by a set of small molecular ligands with the protein receptor.

MATERIAL AND METHODS

Molecular models are derived using dataset of indolinone derivatives [2,3], for exploring structure activity relationship (SAR) of selective PTK inhibitory activities. The dataset containing 27 indolinone derivatives (Table 1) is divided into training set (n_{tr}=19) and test set (n_{ts}=8). The training set containing most and least active compounds are considered for model generation; while test set is used for the validation of the model. Different statistical parameters of model are calculated including R^2 (correlation coefficient) which signifies the presence of variance in the dependant variable that can be explained by all of the independent variables taken together, the Q^2 (cross validated correlation coefficient) value that shows the predictive performance of model, s_e (standard error of estimation), R^2_{pred} (Correlation coefficient of test compounds) and s_p (standard error of prediction). The best models are selected based on high R^2, Q^2, R^2_{pred} and low s_e and s_p values.

3D QSAR Study

In QSAR, use of descriptors of physical properties allow for the application of mathematical models to analyse and predict drug activity. In the present work, 3D QSAR studies are performed on the dataset which involves the techniques such as CoMFA and CoMSIA to correlate biological activity with the 3D structure of compounds. The molecular alignment is an important step in 3D QSAR to align molecules against each other to maximize the overlap of the pharmacophores to generate molecular fields correctly [11]. If the crystal structure of target proteins is available then molecular docking is the solution of alignment of molecules for development of CoMFA/CoMSIA models. It is reported that docking conformers are appropriately aligning the ligands and developing reliable QSAR models [12,13]

In this purpose, individual molecules of dataset are docked with crystal structure of PTK (PDB ID: 2C7W) [14] obtained from Protein Data Bank, and best docked conformer of each compound has been considered for 3D QSAR studies. CoMFA and CoMSIA studies are used to derive the 3D contour map for binding affinity and the models obtained from it give the understanding of electrochemical properties essential for ligand binding. As a consequence, the structure variation in the compound that gives rise to variation in the molecular fields at a particular region of the space is correlated to the biological properties.

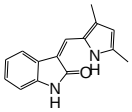
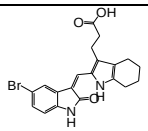
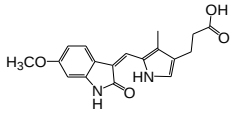
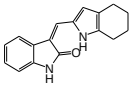
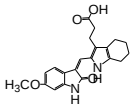
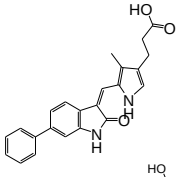
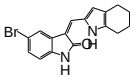
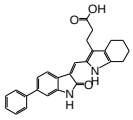
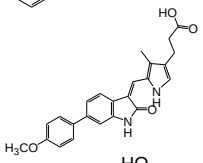
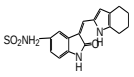
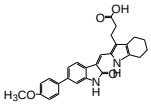
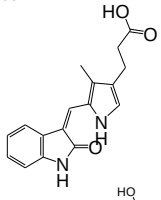
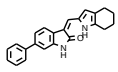
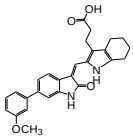
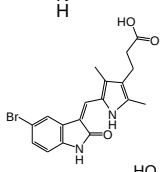
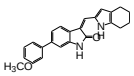
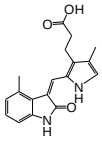
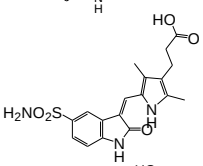
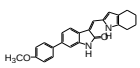
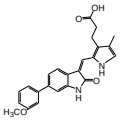
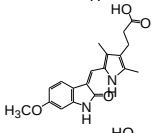
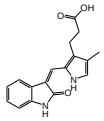
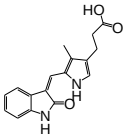
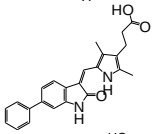
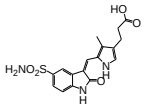
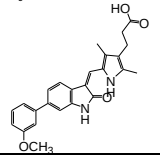
S. No.	Structure	pIC_{50}	S. No.	Structure	pIC_{50}	S. No.	Structure	pIC_{50}
1		5.15	**10**		6.57	**19***#$		5.41
2		4.98	**11**$		5.97	**20**		5.98
3		4.88	**12***		5.90	**21**		5.81
4*#		6.66	**13**		5.87	**22***#$		5.52
5		4.70	**14**		6.06	**23**		5.69
6		4.76	**15***#$		7.52	**24**		6.55
7		4.70	**16**#$		5.92	**25***#$		5.27
8*#$		7.52	**17***		5.43	**26**		5.64
9#$		6.57	**18**		6.34	**27**		5.85

Table 1. Structure and observed activity of indolinone derivatives [2,3].

pIC_{50} = log(10000/IC_{50}); Test compounds: *CoMFA, #CoMSIA, $Pharmacophore modelling

In both cases, molecules are minimized by simulated annealing technique. The molecules are heated at 700 K for 1000 fs and annealing is done at 200 K for 1000 fs. In case of CoMFA fields are generated using steric ('s') and electrostatic ('e') interactions, and calculated on a regular space grid of 3Å. The energy values of the field are truncated at 30.0Kcal/mol. Gasteiger-Huckel method is proved useful for the calculation of partial atomic charges. Furthermore tripos force field method is used to perform the energy minimization. In case of CoMSIA 's', 'e' and hydrophobic ('p') parameters are considered with hydrogen bond (HB) donor ('HBD') and acceptor ('HBA') factors. The partial least-squares (PLS) analysis algorithm is used in conjugation with the cross-validation (leave-one-out) option to obtain an optimum number of components, which were used to generate the final CoMFA and CoMSIA models without cross validation. The result from a cross validation analysis was expressed as Q^2.

Pharmacophore modelling

The pharmacophore concept is based on the kind of interaction observed in molecular recognition i.e., hydrophobic, hydrogen bonding and electronic interactions. To generate the hypothesis,

conformational models are developed for each ligand to ensure good coverage of conformational space within a minimal number of conformers using Catalyst [15]. The chemical features optimized for exploring the spatial pharmacophore maps of this group of compounds are 'HBA', 'HBD', 'p', and 'r' (aromatic ring). To generate optimal pharmacophore hypothesis, different control parameters employed are spacing, uncertainty, and weight variation [16]. The parameter spacing represents the minimum inter-features distance that may be allowed in the resulting hypothesis. In the present study, it is varied from 150 to 300 pm. In the generated hypothesis, each feature signifies some degree of magnitude of the compound's activity. The level to which this magnitude is explored by the hypothesis generator is controlled by the weight variation parameter. This is varied in some cases from 1 to 2. In other cases, the default value of 0.3 is generally considered. The uncertainty parameter reflects the error of prediction and denotes the standard deviation of a prediction error factor called the error cost. In the present work, values of 1.5 to 3.0 are considered as the uncertainty parameter. The hypothesis is validated to nullify over prediction of bioactivity for inactive compounds through a process known as hyporefine, which considers the steric influence of the compound portrayed in the validated (refined) hypothesis. The quality of the hypothesis is adjudged thorough cross-validated technique.

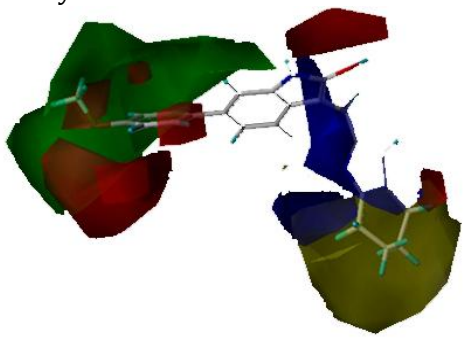
RESULTS AND DISCUSSION

3D QSAR study

CoMFA and CoMSIA studies are performed on the dataset to explore the field and similarity analysis and results of 3D-QSAR studies are shown in Table 2. The observed and predicted activity of training and test compounds are given in Table 2 and Fig 5. The CoMFA, CoMSIA and Pharmacophore models yielded a consensus binding properties and not depend to any other model for prediction of biological activity against TK enzyme. That indicates no one model is superior to any other.

CoMFA study

The individual 's' and 'e' factors does not produce any significant contour maps but it is found that combination of 's' and 'e' factors developed a validated model (Fig. 2) with cross validated correlation coefficient (Q^2) of 0.714, correlation coefficient (R^2) of 0.997 with standard error of estimation (se) of 0.037, and boot strapping correlation coefficient (R^2_{bs}) of 0.997 with error of bootstrapping (s_b) is 0.035. The contributions of 's' and 'e' are 41.60, and 58.40% respectively, indicating the essentiality of both 's' and 'e' fields for potential interaction to target. Further test compounds are fitted to the model to calculate predicted activity. The high correlation value between observed and predicted activities and low error of estimation (R^2_{pred} = 0.602, s_p = 0.351) of the model justify the robustness of the selected model.



Steric: Green – Favourable
Yellow – Unfavourable
Electrostatic: Blue – Favourable
Red – Unfavourable

Figure. 2. Best CoMFA model of indolinone derivatives

S.No.	Activity			
	Observed	Predicted (pIC_{50})		
		CoMFA	CoMSIA	Pharmacophore
1.	5.15	5.12	5.21	5.22
2.	4.98	4.96	4.82	4.75
3.	4.88	4.91	4.75	4.65
4.	6.66	6.02*	6.64*	6.85
5.	4.70	4.73	4.50	4.20
6.	4.76	4.76	4.70	4.77
7.	4.70	4.63	4.62	4.58
8.	7.52	7.78*	7.57*	7.21*
9.	6.57	6.54	5.78*	5.52*
10.	6.57	6.57	6.55	6.87
11.	5.97	5.99	5.37	5.59*
12.	5.90	5.73*	5.85	6.51
13.	5.87	5.89	5.82	5.98
14.	6.06	6.05	6.24	6.02
15.	7.52	7.15*	7.16*	7.82*
16.	5.92	5.95	6.18*	5.42*
17.	5.43	5.58*	5.33	5.45
18.	6.34	6.33	6.32	6.24
19.	5.41	6.04*	6.06*	5.12*
20.	5.98	5.95	5.81	6.25
21.	5.81	5.79	5.85	5.98
22.	5.52	5.43*	5.96*	5.20*
23.	5.69	5.70	6.01	5.62
24.	6.55	6.51	6.50	6.25
25.	5.27	6.42*	6.00*	5.21*
26.	5.64	5.64	5.80	5.35
27.	5.85	5.93	5.80	5.98

Table 2. Observed and predicted activities as per CoMFA, CoMSIA and Pharmacophore studies

*Test compounds, $pIC_{50} = \log(10000/IC_{50})$

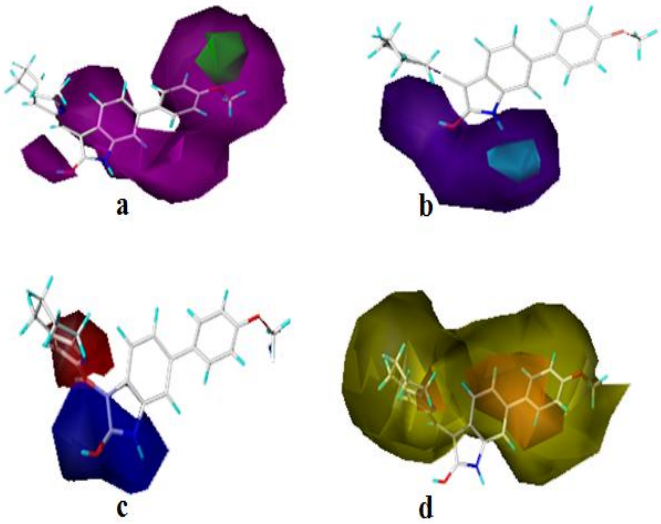
The field analysis model suggest that the region around phenyl ring 'A' (Fig. 1) is crucial for steric interaction whereas, the non-aromatic ring 'E' gives negative impact for steric factor. Rings 'C' and 'D' along with carbon link between rings 'C' and 'D' are found to be important for electrostatic interaction at the active site cavity. Regions around rings 'A' and 'C' and methoxy group attached to ring 'A' show negative impact for inhibitory activity. Contribution of steric and

electrostatic fields suggests that both factors are almost equally important for inhibitory activity.

CoMSIA study

Similarity study failed to obtained validated model individually by 's', 'p', 'HBD', 'HBA', and 'e' but best model obtained with contribution of 'e', 'p', 'HBA' and 'HBD' factors for the inhibitory activity. The best model explained Q^2 of 0.747, R^2 of 0.999 with *se* of 0.037, and R^2_{bs} of 0.997 with error of bootstrapping (s_b) as 0.035. The contribution of 'e', 'p', 'HBA' and 'HBD' factors are 26.00, 24.60, 24.50 and 24.90% respectively in model for the inhibiting activity indicating all factors are essential for potential interaction to target site. The contour map of CoMSIA model is depicted in Fig. 3

Methoxy group attached to ring 'A' gives positive contribution to the inhibitory activity but rest of the surface area show the negative contribution. Amine group present at 'C' ring behaves as hydrogen bond donor but region around rings 'C' and 'D' show negative influence of hydrogen bond donor factor. Region around 'C' found as crucial for electrostatic interaction whereas ring 'D' is found as unfavorable for the interaction. Regions around rings 'A', 'B' and 'D' found as favorable for hydrophobicity but rest of the surface area shows negative for imparting hydrophobicity. High correlation and low error of estimation (R^2_{pred} = 0.712, s_p = 0.009) of the selected model explains robustness of the model.



a) Hydrogen acceptor: Green – Favourable; Magenta– Unfavourable

b) Hydrogen donor: Cyan – Favourable; Purple – Unfavourable

c) Electrostatic: Blue – Favourable; Red – Unfavourable

d) Hydrophobic: Orange – favourable; Yellow – Unfavourable

Figure. 3. Best CoMSIA model of indolinone derivative

Parameters	CoMFA	CoMSIA	Pharmacophore
R^2	0.997	0.999	0.924
se	0.037	0.016	0.047
F(df)	1800.006 (3.15)	5807.329 (5,13)	--
Q^2	0.714	0.747	0.752
R^2_{bs}	0.997	1	--
S_b	0.035	0.009	--
R^2_{pred}	0.602	0.712	0.771
s_p	0.351	0.541	0.421
Contribution (%)			
s	41.60	--	--
e	58.40	26.00	--
p	--	24.60	--
HBA	--	24.50	--
HBD	--	24.90	--

Table 3. Statistical results of CoMFA, CoMSIA and Pharmacophore studies

Pharmacophore mapping study

The best pharmacophore model (R^2 = 0.924, *se* = 0.016, Q^2 = 0.752) developed using hypogen technique with importance of hydrophobicity along with HB acceptor and donor and depicted in Fig. 4, whereas the results are given in Table 3 and Fig. 5. The model is further used to produce the biological activity of test compounds and high correlation value between observed and predicted activity of test compounds and low error of estimation (R^2_{pred} = 0.771 and s_p = 0.421) confirm the suitability of the model selection. Estimated activity of training and test set compounds are delineated in Table 2. Oxo group attached to ring 'C' (Fig. 1) found to be crucial for hydrogen bond acceptor. It is observed that rings 'B' and 'D' are crucial for imparting the hydrophobicity of the molecules. The critical inter-features distances in 3D space are critical for inhibition at the active site.

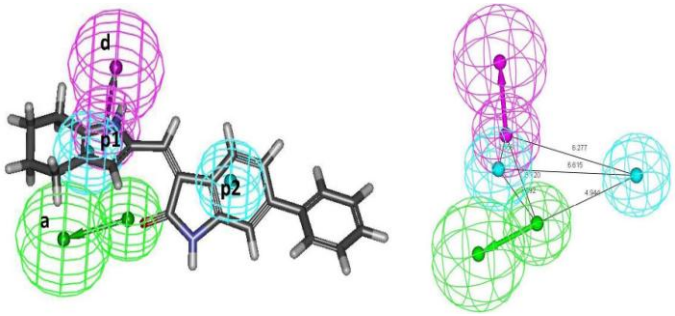


Figure 4. Pharmacophore model of indolinone derivatives (a: HBA, d: HBD and p: hydrophobicity)

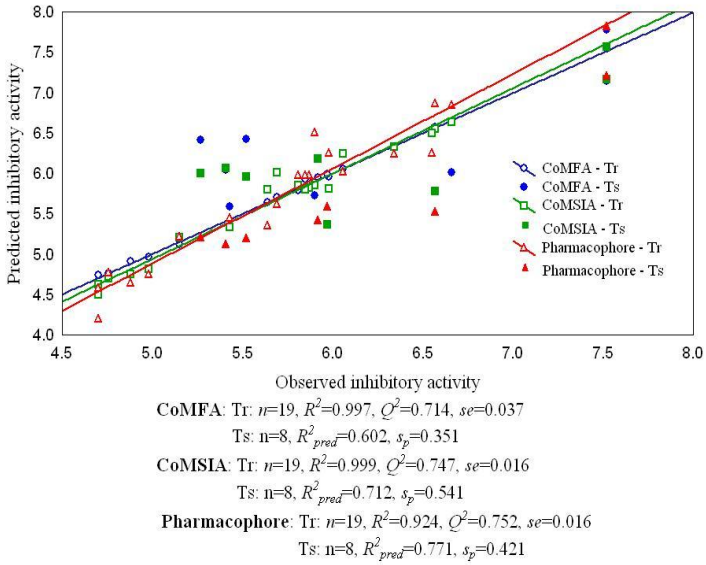


Figure. 5 Observed and predicted inhibitory activity of indolinone derivatives

CONCLUSION

In this study, 3D QSAR models using CoMFA and CoMSIA, and pharmacophore modelling approaches are adopted to rationalize the inhibitory activity of the compounds belonging to indolinone derivatives. Both CoMFA and CoMSIA studies depict well predictive models with high correlation with inhibitory activity activity. A high R^2 value and small standard deviation indicate a similar relationship exists in all compounds. 3D QSAR contour maps show good compatibility with the receptor properties. Similarly pharmacophore modelling study confirms the QSAR findings and indicates the critical distances between explored features in 3D geometric space are important for inhibitory activity. The study established that the substitution of electropositive group in ring 'A' and electronegative groups in rings 'C' and 'D' might be important to optimize the indolinone derivative. Further, addition of phenyl and non-aromatics rings present in the molecular scaffold is crucial for inhibitory activity. Oxo group attached to ring 'C' found to be crucial for hydrogen bond acceptor. The critical inter-features distances in 3D space are critical factors for inhibition at the active site.

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