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Ligand-Based CoMFA Study on Pyridylpyrazolopyridine Derivatives as PKCθ Kinase Inhibitors

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Abstract

Protein kinase C theta (PKC-θ) is a serine/threonine specific protein kinase. It is largely expressed in the T-cells and CD28 signaling. PKC-θ phosphorylates diverse proteins that are involved in the various cellular signaling pathways. Activated PKC-θ in turn activates other transcription factors that control the proliferation and differentiation of T- cells. PKC-θ is considered to be an interesting therapeutic target due to its crucial role in the proliferation, differentiation and survival of T-cells. In the present study, we have performed ligand-based CoMFA study on a series of pyridylpyrazolopyridine derivatives as PKC-θ inhibitors. An acceptable CoMFA model (q^2=0.544; ONC=4; r^2=0.876) was developed and validated by Bootsrapping and progressive sampling. The CoMFA contour map suggested the regions to increase the activity. Bulky substitutions in R^2 position of the piperizine ring could increase the activity. Similarly positive, small substitution in the R^1 position of the Pyridine ring could considerably increase the activity. Our work could assist in designing more potent PKC-θ inhibitors of pyridylpyrazolopyridine derivatives.

Key words: PKC-θ, CoMFA, Pyridylpyrazolopyridine Derviatives, Kinase, Inhibitors

1. Introduction

Protein kinase C (PKC) belongs to the family of serine/threonine kinases. PKCs play an important role in the signal transduction pathway in various cells[1]. Based on the sequence and domain similarities, they are divided into three subfamilies[2], a conventional, novel and atypical PKCs. The conventional PKCs include PKC α, β, γ whereas the novel and atypical PKCs are PKC δ, θ, η, ε and PKC ζ, λ respectively[3]. Each isoforms has unique roles in the regulation of cellular functions [2]. PKC-θ is a member of novel PKCs which are $Ca2^+$-independent PKC subfamily. PKC-θ is primarily expressed in T lymphocytes and muscle cells[4-6]. Many studies have indicated that PKC plays a critical role in mature T cell activation[7]. Activation of PKC-θ in turn activate a range of transcription factors in the nuclei of T-cells such as NF-κB, NFAT, c-Jun, c-Fos and AP-1[8].

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Due to the role it plays in proliferation, differentiation and survival of the T-cells [9], PKC θ is considered to be an attractive target for a diverse immunological and T-cell mediated diseases. New PKC-θ inhibitors are being developed and tested for their potency against various T-cells mediated diseases such as rheumatoid arthritis, transplantation and multiple sclerosis. Among the many designed inhibitors, staurosporines and related bisindolylmaleimides show good inhibitory activity against the PKC isoforms [10]. But, meticulous study on these compounds revealed that, these inhibitors can inhibit other kinase families more potently than the targeted PKC isotypes. Hence, the need to design a potent and selective inhibitor for PKC-θ becomes highly essential. Our group has reported several research and review articles on various *insilico* techniques such as application of partial charges, molecular docking, and 3D-QSAR studies[11-15]. In this study, we have performed a ligand-based CoMFA study on series of pyridylpyrazolopyridine derivatives have carried out.

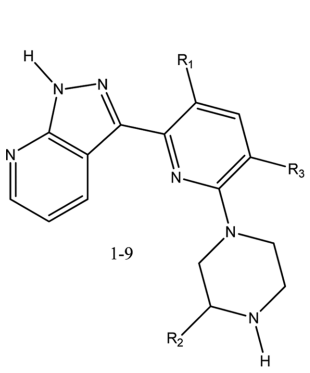
2. Methodology

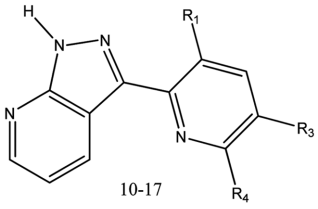
2.1. Data Set

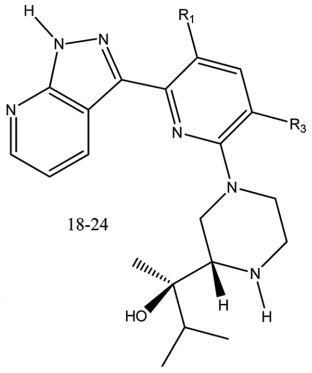
A series of 24 pyridylpyrazolopyridine derivatives

were reported by Jimenez *et al*., was taken for this study[16]. All the reported IC_{50} values were converted into pIC_{50} values ((-logIC_{50}). All the structures of the dataset were drawn using sketch program of SybylX2.1[17]. The structure of the most active compound **24** was drawn and geometry of the molecule was optimized using sybyl Tripos force field after which Gasteiger charges were applied as partial charge. The energy optimized conformation of compound **24**, was taken as the active conformation to draw the rest of the molecules in the dataset. The molecules taken for the study are shown in Table 1.

Table 1. Structure and Biological values of pyridylpyrazolopyridine derivatives as pkcθ kinase inhibitors







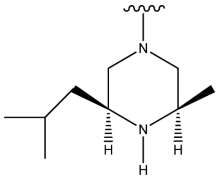
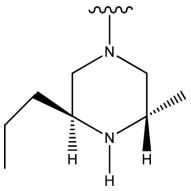
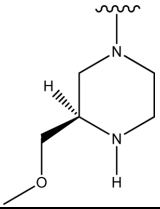
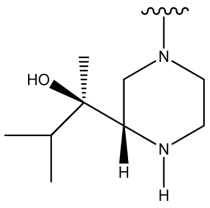
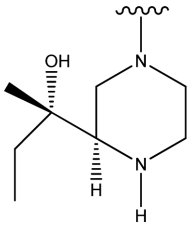
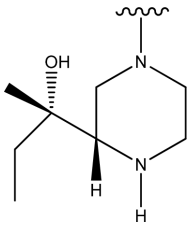
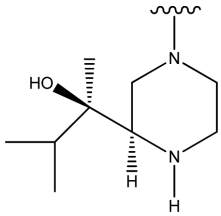
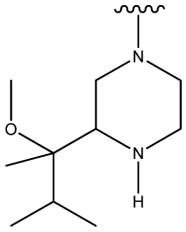
Compound	R^1	R^2	R^3	R^4	pIC_{50}
1	H	H	-	-	7.301
2	H	(R)-Me	-	-	7.168
3	H	(R)-Et	-	-	7.921
4	H	(S)^{i}Pr	-	-	7.824
5	H	(R)- ^{i}Bu	-	-	8.301
6	H	(S)- ^{i}Bu	-	-	8.699
7	H	(R)- Ph	-	-	8.301
8	F	(R)- ^{i}Bu	-	-	8.046
9	F	(S)- ^{i}Bu	-	-	8.398
10	F	-	-		8.301
11	F	-	-		6.155
12	F	-	-		7.292

Table 1. Continued

Compound	R^1	R^2	R^3	R^4	pIC_{50}
13	F	-	-		9.699
14	F	-	-		6.876
15	F	-	-		9.000
16	F	-	-		7.678
17	F	-	-		8.000
18	Cl	-	H	-	7.678
19	CF_3	-	H	-	7.699
20	F	-	OH	-	10.000
21	F	-	CN	-	9.000
22	F	-	CH_2OH	-	10.000
23	F	-	F	-	10.000
24	F	-	Cl	-	10.097

2.2. CoMFA

3D- QSAR technique helps in understanding of the biological properties based on the steric (van der Waals interactions) and electrostatic (Coulombic interactions) fields surrounding the compounds of the dataset. CoMFA was developed by Cramer *et al* [18]. Aligned molecules were placed in the 3D cubic lattice with the grid spacing of 2.0 Å. Electrostatic and steric fields in CoMFA were calculated from Coulomb and Lennard-Jones potentials, respectively. The fields were generated using sp^3 carbon probe atom carrying +1 charge and van der Waals radius of 1.50 Å. The energy cut off of 30.0 kcal/mol was set to 30.0 kcal/mol to reduce the distortion due to extreme energy in the model.

A leave-one-out (LOO) PLS was performed to determine the cross-validated r2 (q2) and the optimum number of components and minimum standard error of prediction (SEP) in the model. CoMFA descriptors were used as independent variables and pIC_{50} values were used as dependent variables in the PLS analysis. The cross-validated correlation coefficient (q^2) that was obtained was considered for further analysis. The non-cross-validated analysis was performed to determine conventional Pearson correlation coefficient (r^2), standard error of estimate (SEE) and Fischer's ratio (F) using the ONC previously obtained from the cross-validation method. The developed model was validated to check its predictability using Bootstrapping and Progressive sampling. Bootstrapping of 100 runs and progressive sampling of 100 samplings with 2 to 100 bins was performed to validate the models.

3. Results and Discussion

3.1. CoMFA Model

Ligand-based CoMFA model was developed for a series of pyridylpyrazolopyridine derivatives possessing inhibitory activity against PKC-θ kinase. Lowest energy conformer of the most active compound 24 was considered as template. All the molecules were then aligned over the template using alignment method based on the common substructure. The common substructure of the compounds from template molecule 24 is shown in Fig. 1 and the alignments of the compounds are displayed in Fig. 2. A statistically reliable CoMFA model for the complete set of dataset compounds was developed (q^2=0.544, NOC=4, r^2=0.876) with Gasteiger charges as

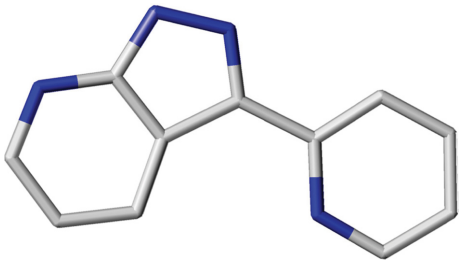


Fig. 1. Common Substructure from template compound **24**.

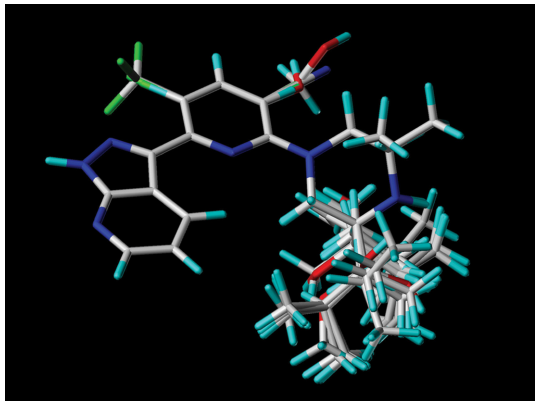


Fig. 2. Alignment of all the dataset molecules used for ligand-based CoMFA

Table 2. Statistical summary of the developed Ligand-based CoMFA model

Parameters	CoMFA MODEL
q^2	0.544
NOC	4
SEP	0.799
r^2	0.876
SEE	0.416
F value	33.617
BS r^2	0.976
BS SD	0.016
Q^2	0.457
Steric contribution	68%
Electrostatic contribution	32%

q^2: cross-validated correlation coefficient; NOC: Number of components; SEP: Standard Error of prediction; r^2: non-validated correlation coefficient; SEE: Standard Error of Estimation; F value: F-test value; BS-r^2: Bootstrapping r^2 mean; BS-SD: Bootstrapping Standard deviation; Q^2: Progressive sampling.

Table 3. Actual and predicted pIC_{50} with their residuals of the developed CoMFA model

Compound	Actual pIC50	CoMFA	
		Predicted	Residual
1	7.301	7.135	0.166
2	7.168	7.241	-0.074
3	7.921	8.035	-0.114
4	7.824	7.805	0.019
5	8.301	8.515	-0.214
6	8.699	8.181	0.518
7	8.301	8.348	-0.047
8	8.046	8.011	0.035
9	8.398	7.713	0.685
10	8.301	8.062	0.240
11	6.155	7.071	-0.916
12	7.292	7.273	0.020
13	9.699	9.007	0.692
14	6.876	7.275	-0.399
15	9.000	9.111	-0.111
16	7.678	7.713	-0.036
17	8.000	8.053	-0.053
18	7.678	7.902	-0.224
19	7.699	7.304	0.395
20	10.000	10.103	-0.103
21	9.000	9.681	-0.682
22	10.000	9.958	0.042
23	10.000	10.245	-0.245
24	10.097	9.691	0.406

partial charge. The model was found to be acceptable in terms of q^2 and r^2 values. Since the total number of compounds are less than 30, the data set was not divided into training and test set. The SEE and F values of the developed model were found to be 0.416 and 33.617 respectively. The model was then subjected to validation using bootstrapping and progressive sampling methods. The bootstrapping r^2 mean (BS-r^2) and BS- standard deviation (BS-SD) was 0.976 and 0.016 respectively. The progressive sampling (Q2) of 100 runs gave the value of 0.457. The model exhibited overall satisfactory statistical values. The detailed statistical values for the final Ligand-based CoMFA model are shown in Table 2. The experimental and predicted activity values of the molecules obtained for the ligand-based CoMFA model is tabulated in Table 3. The scatter plot and contour map for the same are shown in Fig. 3 and Fig. 4 respectively.

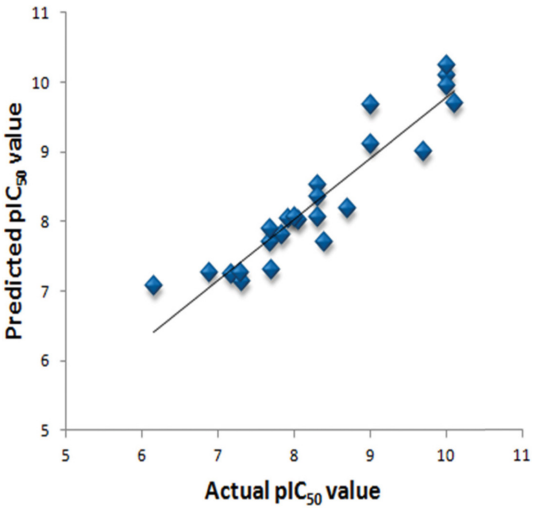


Fig. 3. Scatter plot diagram for final ligand-based CoMFA model.

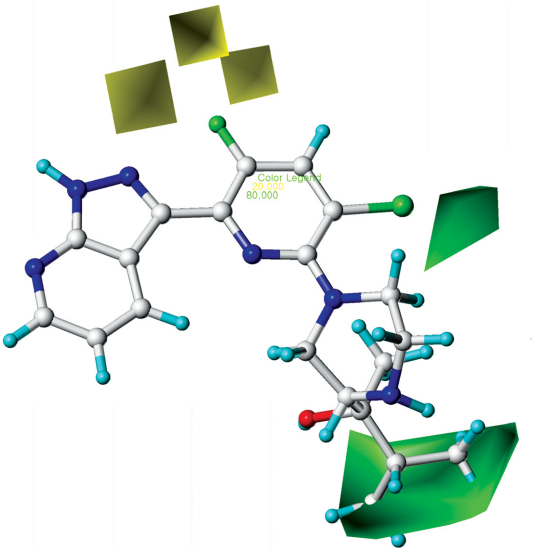


Fig. 4a. CoMFA Steric contour map. The green contours indicate sterically favored regions and the yellow contours denote the sterically unfavorable regions.

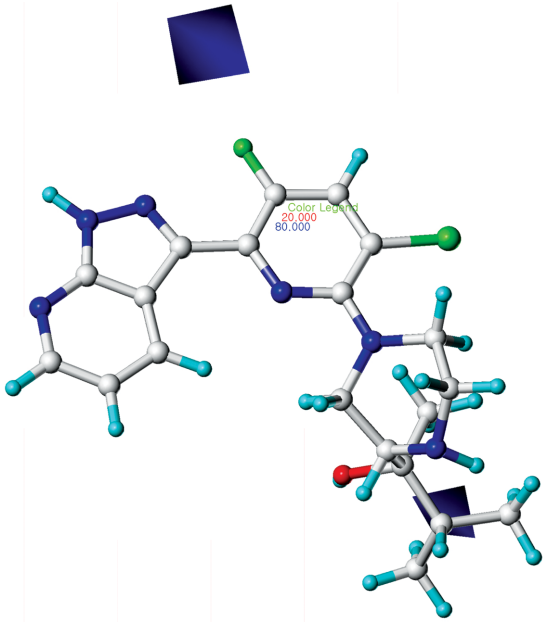


Fig. 4b. CoMFA Electrostatic contour map. The blue colored areas favor electropositive substituents.

3.2. CoMFA Contour Maps

The contour maps were developed by using the STDEV*COEFF field type with the default 80% and 20% level contributions for favorable and unfavorable regions, respectively. The most active compound 24 was shown superimposed inside the contour map. The steric contour map of the ligand-based CoMFA is shown in Fig. 4a. The green color signifies regions that favor sterically bulky groups and yellow color signifies the regions that are not favored for bulky substitution. A big green colour contour near the R^4 position of the piperzine ring suggest that the bulky substitution that region could increase the activity of the comnpound. This could the reason for the better activity of compounds **13**, **15**, **20**, **21**, **22** and **23** including the most active compound 24 which possess bulky substitution in that position. Similarly, a green contour near the R^3 position indicates that the bulky substitution in this region could favor to enhance the activity. The yellow contours near the fluoride atom of the R1 position suggest that bulky substitution at this position could decrease the activity.

The electrostatic contour map of the ligand-based CoMFA model is shown in Fig. 4b. The blue color signifies positive charge is favored and red color signifies that negative charge is favored to increase the activity of the compound. The small blue contour seen near the R^1 substitution implies that positive substitution in that position could increase the activity. Another blue color contour near the R^2 position suggests that positive substitution in that particular position could enhance the activity. This could validate the fact that compounds **09**, **10**, **13**, **15, 20**, and **23** including the most active compound **24** that contain positive substitution at this position possess better activity.

4. Conclusion

The critical role of PKC-θ in proliferation, differentiation and survival of T-cells makes it an interesting therapeutic target for autoimmune diseases. In this study, we have taken a series of pyridylpyrazolopyridine as potent antagonist for PKC-θ kinase. The ligand-based CoMFA model was developed with acceptable statistical values. The developed model was validated using bootstrapping and progressive sampling. The validation results showed that the model is predicable and robust. Futhermore, the analysis of the contour maps generated for the ligand-based CoMFA model highlighted the regions to increase the activity of the compounds. The overall contour map results suggest that

bulky positive substitution in R2 position could enhance the activity. Likewise, position substitution in R3 position can help to enhance the activity of the compounds. Bulky substitution must be strongly avoided in the R1 position to increase the activity of these compounds. The useful information provided by the contour maps could be used to develop a more potent compound of pyridylpyrazolopyridine series as PKC-θ inhibitor.

Acknowledgements

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