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Development of CoMFA, advance CoMFA and CoMSIA models in pyrroloquinazolines as thrombin receptor antagonist☆

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Abstract—3D QSAR studies namely CoMFA, advance CoMFA and CoMSIA have been carried out on a series of pyrroloquinazolines for their thrombin receptor antagonistic activity. The predicted activities by highly significant CoMFA ($q^2 = 0.66$) and CoMSIA ($q^2 = 0.67$) models were in good accordance with observed activities and the models may be useful for optimization of thrombin receptor antagonistic activity.

1. Introduction

Thrombin is a plasma protease that plays a critical role in hemostasis as well as in thrombotic disorders. It is an important mediator of platelet aggregation under physiological and pathological conditions. These and other cellular effects of thrombin are due to its proteolytic activity. Inhibition of proteolytic activity not only inhibits aggregation but also presents potential liabilities such as hemorrhage, etc. Many of the cellular actions of thrombin are mediated by a G-Protein coupled receptor PAR-1 (protease activated receptor-1).[1] Thrombin cleaves the extracellular domain of the PAR-1 receptor at Arg 41-Ser 42 site to expose a new N-terminus, which binds intramolecularly to an undefined extracellular site and acts as a tethered ligand for the receptor.[2] This PAR-1 receptor holds the key for desired selectivity of function.[3] Thus selective blockade of intramolecular activation step of thrombin receptor should result in moderation of platelet activation and aggregation without interfering with the enzymatic activity of the thrombin in coagulation cascade. Hence thrombin receptor antagonists may be a safer antiplatelet agent for the treatment of thrombotic disorders.[4]

In order to understand the influence of different physicochemical and structural parameters on thrombin receptor antagonistic activity, 3D QSAR studies have been carried out on a series of thrombin receptor antagonists using CoMFA and CoMSIA techniques and the results are presented in this paper.

2. Materials and methods

The in vitro inhibitory activity data of pyrroloquinazolines against thrombin receptor ($TRIC_{50}$) was taken from Ahn et al.[5] (Table 1).

2.1. Computational approach

Molecular modeling techniques viz CoMFA, advance CoMFA and CoMSIA were done on a Silicon Graphics Octane R12000 workstation using SYBYL6.9 molecular modeling software.[6] Catalyst[7] running on a SGI O2 was used for common feature hypothesis generation.

2.2. Molecular structures and alignment

The most active compound was built from fragments in the SYBYL fragment library and the geometry was fully optimized using the standard SYBYL settings. Partial charges were computed using Gasteiger–Huckel method and it was submitted for conformational search protocol using multisearch method with the following settings: maximum cycles 400, maximum conformers 400, energy cutoff 70 kcal/mol, number of hits 12, maximum rms gradient 3.0 and tolerance 0.40. The conformer of most active compound with the lowest energy was then used

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Keywords: Thrombin receptor antagonist; QSAR; CoMFA; CoMSIA.

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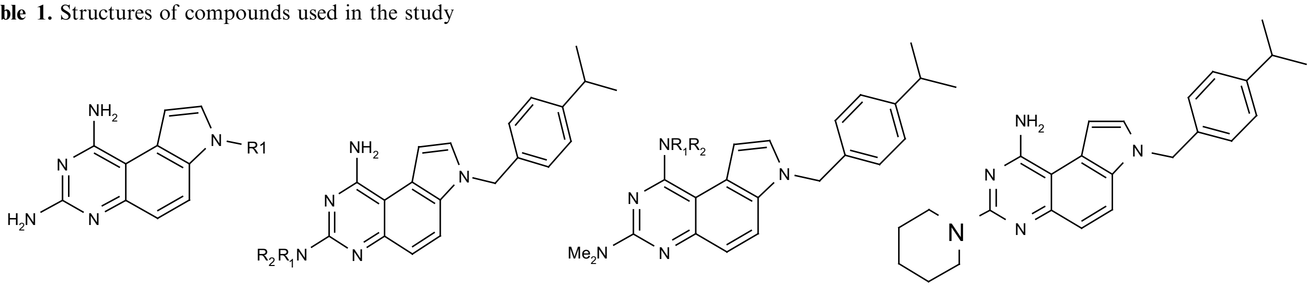
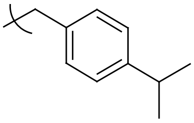
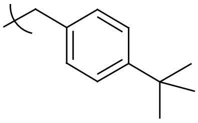
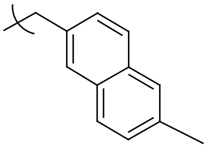
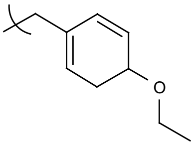
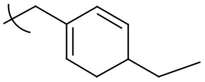
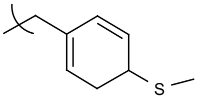
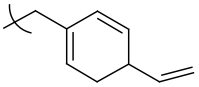
Table 1. Structures of compounds used in the study



Compound 1-7 Compound 8-13 Compound 14-15 Compound 16

Compound	R_1	R_2	BA*	−logBA	Pred. Act.[a]	Pred. Act.[b]	RA[a]	RA[b]
1		—	0.300	0.523	0.61	−0.017	−0.087	0.54
2		—	1.230	−0.09	−0.56	−0.375	0.47	0.29
3		—	1.750	−0.24	−0.44	0.053	0.2	−0.29
4		—	2.625	−0.42	−0.54	−0.405	0.12	−0.02
5		—	4.500	−0.65	−0.64	−0.431	−0.01	−0.22
6		—	6.875	−0.84	−0.61	−0.839	−0.23	−0
7		—	9.600	−0.98	−0.56	−0.703	−0.42	−0.28
8	H	$-CH_3$	0.870	0.06	0.11	0.09	−0.05	−0.03
9	CH_3	$-CH_3$	0.100	1	1	1.216	0.00	−0.22
10	H	$-C_2H_5$	0.524	0.281	0.28	0.21	0.001	0.07
11	H	$-C_2H_4OH$	4.900	−0.69	−0.57	−0.823	−0.12	0.13
12	H	Cyclopropyl	0.056	1.252	1.15	1.079	0.102	0.17
13	CH_3	Cyclopropyl	0.052	1.284	1.20	1.18	0.084	0.1
14	H	CH_3	3.200	−0.51	−0.323	−0.454	−0.187	−0.06
15	CH_3	CH_3	1.250	−0.096	−0.354	−0.083	0.258	−0.02
16	—		0.100	1	1.15	1.09	1.148	−0.09

* = Inhibitory activity in μM against thrombin receptor.

[a] Predicted using best CoMFA model.

[b] Predicted using best CoMSIA model.

as a template to build structures of all other compounds. The partial charges for all compounds were calculated using Gasteiger–Huckel method and the molecules were optimized for their geometry using Tripos force field with a distance dependent dielectric function and energy convergence criterion of 0.001 kcal/mol Å using 1000 iterations and standard SYBYL settings.

Alignment is a crucial problem in 3D QSAR studies, there are various approaches suggested in the literature. Some of them are:

Maximum Common Structure (MCS) based alignment: Maximum common structure is used for alignment of molecules using the most active molecule or the molecule having highest number of functional groups as a criterion.

Rigid body field fit: The molecules are aligned according to their electrostatic and steric field, etc. on the template molecule. Multifit: A number of attributes like electrostatic and steric fields, particular atom or groups, etc. can be used for alignment of the molecules to the template molecule by flexible fitting.

The approaches, which were used in the present study, are:

1. Alignment 1: Common structure based fitting using the following core and compound number **13** as criterion for alignment (Fig. 1).
2. Alignment 2: Rigid body field fit alignment using the steric and electrostatic fields.
3. Alignment 3: This alignment was done by catalyst using common feature hypothesis and the aligned molecules were than exported to SYBYL, the Gasteiger–Huckel charges were then calculated before using them in CoMFA and CoMSIA field calculation (Fig. 2).

2.3. CoMFA[8] studies

It is a 3D QSAR method in which the superimposed molecules are kept in a 3D grid and steric and electrostatic fields are then calculated at various grid points using Lennard–Jonnes and Coulombic potentials, respectively. The CoMFA calculations were done using Tripos Advance CoMFA module in SYBYL. For each alignment an sp^3 carbon atom having a charge of +1 and a radius of 1.52 Å was used as a probe to calculate various steric and electrostatic fields, the grid spacing was set at 2 Å and the region was calculated automatically. To investigate the influence of different parameter settings on CoMFA various steric and electrostatic cutoffs and grid spacing were also tried as suggested by Crammer et al.

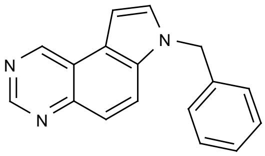


Figure 1. The core used for common structure based alignment.

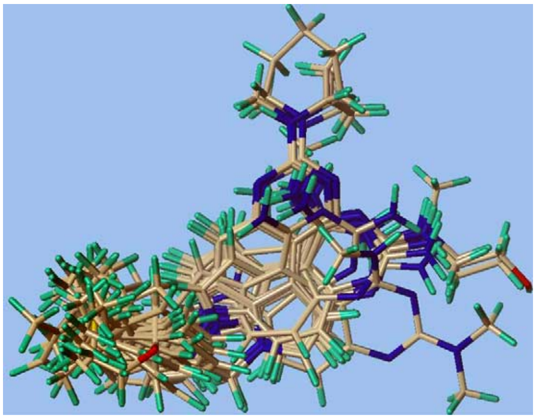


Figure 2. Alignment of molecules using catalyst.

2.4. Advance CoMFA

H-bonding, indicator and parabolic fields are used in advanced CoMFA routine to extend the scope of CoMFA H-bond fields are special indicator fields based on the description by Bohacek and McMartin.[9] Lattice points with steric energies below the steric cutoff energy are assigned nominal energies equal to the steric cutoff energy if they are close to H-bond accepting or donating atoms. Indicator fields[10] replace all lattice energies with magnitudes below a designated threshold with zero values. All energies at or above that threshold are replaced with a nominal energy value equal in magnitude to the relevant field cutoff value. When both fields are included in a single CoMFA column, the greater of the steric and electrostatic cutoffs is used. The sign of the original lattice energy is retained. Parabolic fields are those in which the magnitude of a standard steric and/or electrostatic field at each lattice point has been squared; the original sign of the energy is retained.

2.5. CoMFA region focusing

CoMFA region focusing is a method of application of weights to the lattice points in a CoMFA region to enhance or attenuate the contribution of these points to subsequent analysis, here '*StDev*Coefficients*' values as weights and different weighing factors were applied in addition to grid spacing for getting the better models.

2.6. CoMSIA studies

The CoMSIA[11] technique was introduced by Klebe in 1994 in which similarity indices are calculated at different points in a regularly spaced grid for prealigned molecules. It has several advantages over CoMFA technique like greater robustness regarding both region shifts and small shifts within the alignments; no application of arbitrary cutoffs and more intuitively interpretable contour maps. The standard settings (Probe with charge +1, radius 1 Å and hydrophobicity +1, hydrogen-bond donating +1, hydrogen-bond accepting +1, attenuation factor α of 0.3 and grid spacing 2 Å) were used in CoMSIA to calculate five different fields viz steric, electrostatic, hydrophobic, acceptor and donor.

2.7. Partial least square analysis

PLS is used to correlate thrombin receptor antagonistic activity with the CoMFA and CoMSIA values containing magnitude of steric and electrostatic potentials. The models were assessed by their cross-validated r^2 (q^2)

using leave one out (LOO) procedure by SAMPLS method[12] as implied in SYBYL. CoMFA standard scaling was applied to all the CoMFA analysis. The full PLS analysis was run with a column filtering of 2.0 kcal/mol to reduce the noise and to speed up the calculation. For CoMSIA too SAMPLS method was used, thereafter a full PLS was run using column filtering of 2 kcal/mol. Autoscaling was applied to all CoMSIA analysis.

3. Results and discussion

A 3D QSAR study is considered statistically significant if the q^2 is greater than 0.3, although a q^2 greater than 0.4 is normally preferred. The CoMFA model having a q^2 of 0.66 and CoMSIA model having a q^2 of 0.67 are clearly significant. The optimal number of components was determined by selecting highest q^2 value, which most often corresponds to lowest s_{press} value. In addition to LOO cross validation the cross validation in groups was also carried out using four equally sized groups, repeating the procedure 10 times. This resulted in q^2 comparable to the corresponding results (q^2 ranges between 0.485 and 0.642). Progressive scrambling of the dataset was also carried out as implied in SYBYL6.9 to check possible chance correlations. The results of progressive scrambling further confirm consistency of the model as defined by the critical slope, which was found to be between 0.60 and 0.80 for 50 different runs of progressive scrambling. The external validation of the model was not possible as the dataset had only 16 compounds, which is too small to spare any compound for test set, but the results of LOO, group cross validation and progressive scrambling indicate that the model is stable enough.

3.1. CoMFA advance CoMFA and CoMFA region focusing

The results of CoMFA studies are summarized in Tables 2–4. The best results were obtained at a cutoff of 30 kcal/mol for both steric and electrostatic fields.

Alignment 1: PLS analysis showed a high q^2 value of 0.65 with hydrogen bonding field using three principal components. The other fields like indicator field also showed significant q^2 of 0.50 while Tripos standard and indicator fields showed a q^2 of 0.48 and 0.46 with high conventional r^2. When these fields were focused, the q^2 improved and the HB field produced highest q^2 of 0.71 while the parabolic field produced q^2 of 0.64 with high conventional r^2. All the models are significant statistically as evident by q^2, s_{press}, SE, F-value and r^2_{bs}. The optimal number of components was selected on the basis of lowest s_{press} value (Table 2).

Alignment 2: The molecules were aligned using field fit procedure. HB field alone produced q^2 above 0.50. When the fields were focused using *StDev*Coefficient* at a weight of 0.5 the fields gave best q^2 values for this alignment. HB field showed highest q^2 of 0.73 while indicator and parabolic showed q^2 of 0.64 the Tripos standard field showed q^2 of 0.624. These results are also significant statistically (Table 3).

Alignment 3: The conformers of the molecules were generated in catalyst using BEST conformer approach and then they were submitted for hypothesis generation using common features. The alignment produced by the best hypothesis was then transferred to SYBYL. Gasteiger–Huckel charges were calculated and the molecules were submitted for CoMFA and CoMSIA studies. This alignment produced the most consistent results among all alignments. Tripos standard field produced the best model having a q^2 of 0.662 after region focusing. In most of the results only one component was used as it corresponds to lowest s_{press} value, adding other components resulted in negligible increase in q^2 (<5%) but increased s_{press} value considerably so the less complex model was chosen. Most of the fields showed q^2 above 0.6 before and after region focusing indicate steric factors play major role in determination of biological activity as evident from the results below. The region focusing not only increased q^2, but resulted in the refinement of contour maps as well (Table 4).

To test the predictability of the analysis the activities were calculated from the CoMFA model using CoMFA (RF) field for alignment 3, the observed and the predicted values are found to be in good agreement with each other (Table 1).

Table 2. CoMFA, advance CoMFA and CoMFA region focusing (Alignment 1)

Parameters*	CoMFA	CoMFA (RF) Gs = 1 Å	Indicator	Indicator (RF) Gs = 1 Å	HB	HB (RF) Gs = 1 Å	Parabolic	Parabolic (RF) Gs = 1 Å
q^2	0.48	0.61	0.50	0.58	0.65	0.71	0.46	0.64
s_{press}	0.65	0.55	0.633	0.55	0.50	0.46	0.65	0.53
R^2	0.95	0.96	0.92	0.93	0.89	0.92	0.94	0.93
SE	0.20	0.184	0.256	0.23	0.283	0.24	0.222	0.234
F	50.77	61.06	30.11	50.43	31.68	33.29	40.95	36.66
Component	4	4	4	3	3	3	4	4
Fraction								
Steric	0.84	0.65	0.99	1.0	0.37	0.47	0.89	0.78
Electrostatic	0.16	0.35	0.01	0.0	0.63	0.53	0.11	0.22
$r^{2\,(100\text{ runs})}_{\text{bs}}$	0.97	0.981	0.962	0.96	0.901	0.925	0.97	0.97

* = The parameters have their usual meanings. $Pr^2 = 0$ is 0.000 for all, RF = region focusing, weight for focusing = 0.5 for all analyses, Gs = grid spacing.

Table 3. CoMFA, advance CoMFA and CoMFA region focusing (Alignment 2)

Parameters*	CoMFA	CoMFA (RF) Gs = 1 Å	Indicator	Indicator (RF) Gs = 1 Å	HB	HB(RF) Gs = 1.5 Å	Parabolic	Parabolic (RF) Gs = 1 Å
q^2	0.484	0.624	0.44	0.64	0.52	0.732	0.46	0.64
s_{press}	0.635	0.542	0.66	0.53	0.62	0.44	0.65	0.53
R^2	0.95	0.96	0.96	0.97	0.93	0.93	0.94	0.934
SE	0.19	0.175	0.18	0.164	0.234	0.23	0.218	0.23
F	57.18	67.71	60.82	77.00	36.63	50.434	42.414	38.88
Component	4	4	4	4	4	3	4	4
Fraction								
Steric	0.84	0.66	0.91	0.86	0.40	0.31	0.89	0.78
Electrostatic	0.16	0.34	0.09	0.14	0.60	0.69	0.11	0.22
$r^{2\,(100\text{ runs})}_{\text{bs}}$	0.97	0.98	0.98	0.98	0.96	0.94	0.97	0.97

* = The parameters have their usual meanings. $Pr^2 = 0$ is 0.000, RF = region focusing, weight for focusing = 0.5 for all analyses, Gs = grid spacing.

Table 4. CoMFA, advance CoMFA and CoMFA region focusing (Alignment 3)

Parameters*	CoMFA	CoMFA (RF) Gs = 1 Å	Indicator	Indicator (RF) Gs = 1 Å	HB	HB (RF) Gs = 1 Å	Parabolic	Parabolic (RF) Gs = 1 Å
q^2	0.621	0.662	0.504	0.635	0.51	0.653	0.613	0.65
S_{press}	0.484	0.457	0.554	0.501	0.57	0.48	0.49	0.47
R^2	0.737	0.737	0.77	0.77	0.903	0.937	0.751	0.743
SE	0.404	0.404	0.427	0.375	0.254	0.205	0.392	0.399
F	39.72	39.19	33.59	47.54	60.57	96.86	42.27	40.49
Component	1	1	1	1	2	2	1	1
Fraction								
Steric	0.47	0.46	0.39	0.60	0.46	0.39	0.63	0.60
Electrostatic	0.53	0.54	0.61	0.40	0.54	0.61	0.37	0.40
$r^{2\,(100\text{ runs})}_{\text{bs}}$	0.77	0.76	0.78	0.80	0.940	0.97	0.792	0.777

* = The parameters have their usual meanings. $Pr^2 = 0$ is 0.000, RF = region focusing, weight for focusing = 0.5 for all analyses, Gs = grid spacing.

3.2. CoMSIA

The CoMSIA analysis was done at a grid spacing of 2 Å and the column filtering was set at 2.0 kcal/mol the other parameters were set as default.

Alignment 1: The CoMSIA results also show a high significance of the models. A q^2 of 0.625 was obtained when all fields were taken but when individual fields steric, electrostatic, etc. were taken it gave the best q^2 of 0.63 with steric field. The hydrophobic field also showed q^2 of 0.45 while donor showed q^2 of 0.41. No significant result with acceptor fields was obtained. The model containing steric and donor is best based on q^2 (0.67) and low press value (0.49) (Table 5).

Alignment 2: Steric factors are playing major role as the steric field gave highest q^2 of 0.653. The donor and hydrophobic fields also showed significant q^2 values while the electrostatic and acceptor fields did not produce significant results (Table 6).

Alignment 3: This alignment again produced best results as in CoMFA. Hydrophobic field showed highest q^2 of 0.67, similarly steric and donor fields also showed good q^2 values. It is important to note that electrostatic field is also showing a q^2 of 0.62 that was absent in previous alignments. The acceptor field remained insignificant as in previous alignments. When steric, electrostatic and hydrophobic fields were taken together they gave a q^2 of 0.63 with two principal components. Similar to CoMFA only one component was used again as it corresponds to lowest s_{press} value, adding other components resulted in negligible increase in q^2 (<5%) but increased s_{press} value considerably (Table 7).

Table 5. CoMSIA studies (Alignment 1)

Parameter*	S	E	D	H	S and H
q^2	0.63	0.236	0.41	0.45	0.54
S_{press}	0.513	0.74	0.68	0.63	0.57
R^2	0.82	0.17	0.55	0.77	0.80
SE	0.36	0.77	0.59	0.42	0.37
F	17.65	0.83	3.42	9.36	16.36
Component	3	3	4	3	3
Relative contribution					
S	1.0	—	—	—	0.56
E	—	1.0	—	—	—
D	—	—	1.0	—	—
H	—	—	—	1.0	0.44
$r^{2\,(100\text{ runs})}_{\text{bs}}$	0.843	0.44	0.724	0.77	0.84

* = The parameters have their usual meanings. S = steric, E = electrostatic, D = donor, H = hydrophobic, $Pr^2 = 0$ is 0.000.

3.3. CoMFA and CoMSIA contour maps

The alignment 3 produced most consistent and highly significant results; therefore this alignment was used for

Table 6. CoMSIA studies (Alignment 2)

Parameter*	*S*	*E*	*D*	*H*	*S* and *D*
q^2	0.653	0.154	0.52	0.54	0.648
S_{press}	0.614	0.78	0.613	0.58	0.502
R^2	0.905	0.77	0.82	0.915	0.911
SE	0.272	0.41	0.38	0.26	0.25
F	26.23	13.03	12.50	29.76	41.10
Component	4	3	4	3	3
Relative contribution					
S	1.0	—	—	—	0.38
E	—	1.0	—	—	—
D	—	—	1.0	—	0.62
H	—	—	—	1.0	—
$r_{\text{bs}}^{2\ (100\ \text{runs})}$	0.95	0.841	0.87	0.95	0.947

* = The parameters have their usual meanings. *S* = steric, *E* = electrostatic, *D* = donor, *H* = hydrophobic, $Pr^2 = 0$ is 0.000.

Table 7. CoMSIA studies (Alignment 3)

Parameter*	*S*	*E*	*D*	*H*	SEH
q^2	0.602	0.62	0.58	0.67	0.63
S_{press}	0.49	0.49	0.53	0.47	0.48
R^2	0.72	0.704	0.71	0.76	0.734
SE	0.42	0.43	0.425	0.386	0.406
F	35.43	33.27	34.05	44.21	38.59
Component	1	1	1	1	1
Relative contribution					
S	1.0	—	—	—	0.232
E	—	1.0	—	—	0.446
D	—	—	1.0	—	—
H	—	—	—	1.0	0.322
$r_{\text{bs}}^{2\ (100\ \text{runs})}$	0.74	0.73	0.744	0.81	0.75

* = The parameters have their usual meanings. *S* = steric, *E* = electrostatic, *D* = donor, *H* = hydrophobic, $Pr^2 = 0$ is 0.000.

generation of contour maps for CoMFA and CoMSIA using the field type *StDev*Coefficient*. The contour maps using CoMFA region focusing are of better quality because the region focusing resulted in a sort of image enhancement. Mostly the CoMFA contour maps are from region focusing but Tripos standard field without focusing is shown in Figure 3 for the sake of comparison. The contour maps of CoMFA denote the region in the space where the aligned molecules would favourably or unfavourably interact with the receptor while the CoMSIA contribution maps denote those areas within the specified region where the presence of a group with a particular physico-chemical property will be favoured or disfavoured for good biological activity. The advantage of CoMSIA contour maps over CoMFA is that they are easier to interpret. The contours for normal and focused Tripos standard field are shown in Figures 3 and 4 while that of CoMSIA steric and electrostatic field are shown in Figures 5 and 7, respectively. The contour maps (Fig. 3–10) show contribution for favourable and unfavourable interactions with the receptor in terms of steric (80% green, 20% yellow) and electrostatic (80% blue and 20% red).

Greater values of bio-activity are correlated with more bulk near green, less bulk near yellow more positive charge near blue and more negative charge near red. The greater size of green-yellow region than red-blue region indicates a greater contribution of steric fields than electrostatic fields in the determination of biological activity. The CoMFA and CoMSIA contour plots mainly consist of three regions in this series. The first one is where the green polyhydra is largest (at

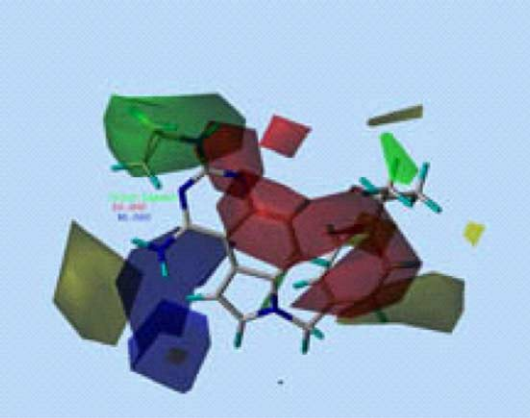


Figure 3. Contour plot of tripos standard field (unfocused).

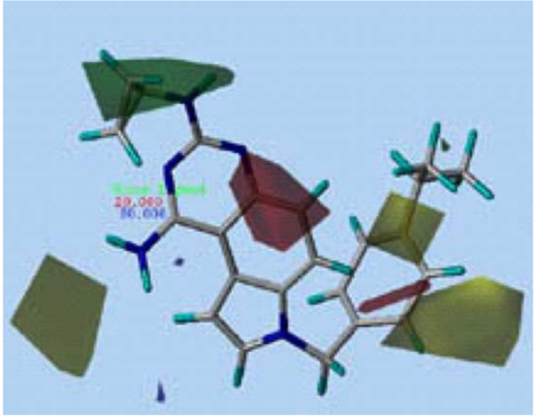


Figure 4. Contour plot of focused tripos standard field.

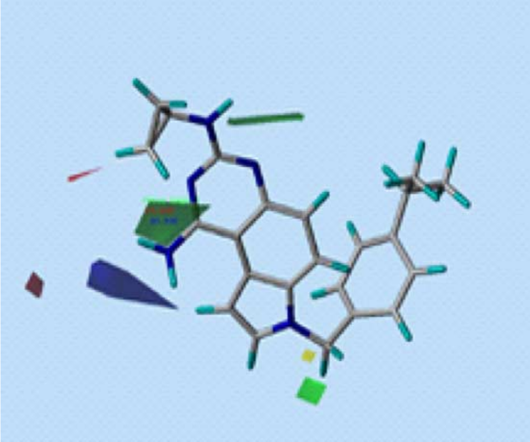


Figure 5. Contour plot using CoMFA hydrogen bonding field.

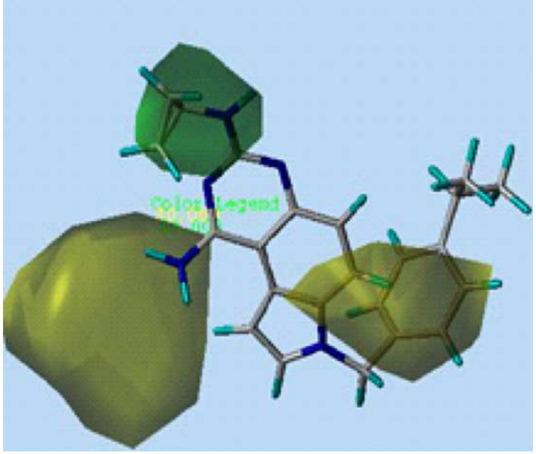


Figure 6. Contour plot using CoMSIA steric field.

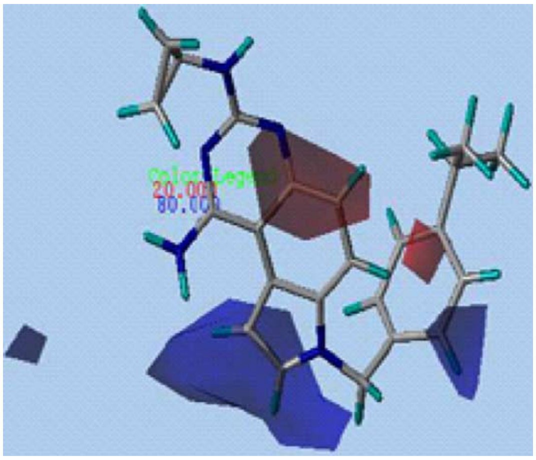


Figure 7. Contour plot using CoMSIA electrostatic field.

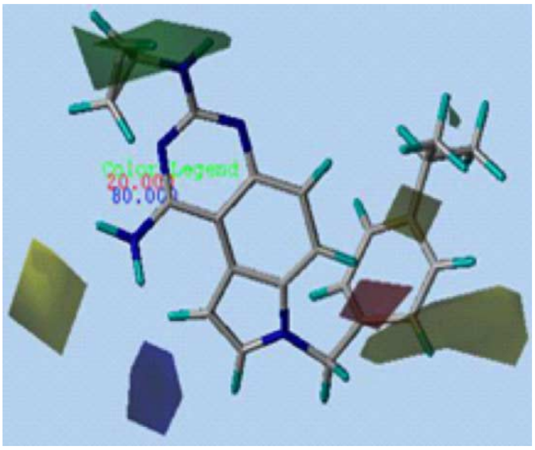


Figure 8. Contour plot using parabolic field.

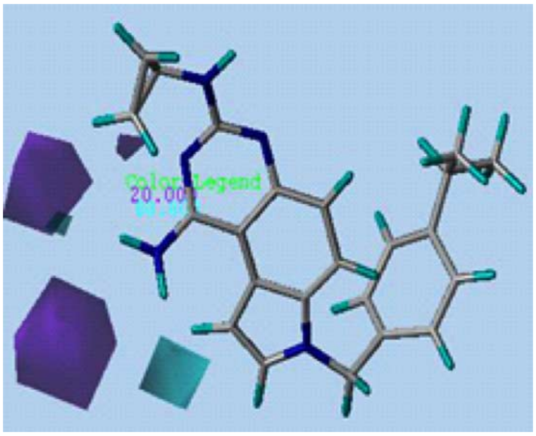


Figure 9. Contour plot using donor field.

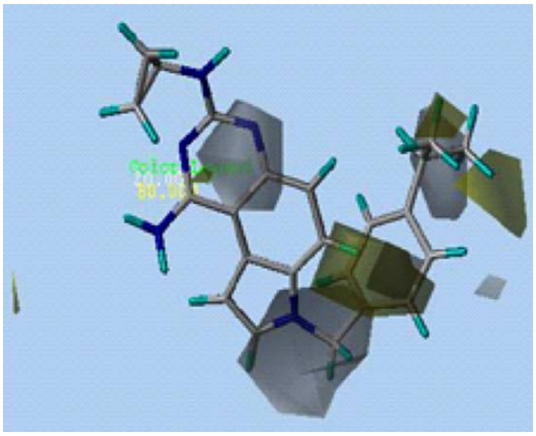


Figure 10. Contour plot using hydrophobic field.

N-cyclopropyl group in Figures 3–5). The second one is at the free NH_2 group (near blue region in Fig. 5) and the third one is around the propyl group present on the phenyl ring at pyrole nitrogen (yellow polyhydra in Fig. 4). The green polyhydra larger than others at the first region in all the contours signifies the importance of steric bulk here. Its image enhancement on application of region focusing resulted in locating the region where steric bulk is preferred for higher activity. The CoMSIA contour map for steric field also has a similar green polyhydra indicating the importance of steric bulk at the same region thus it is an important region as almost all contours show a similar sized green polyhydra and indicate the importance of bulk in this region. The second region enclosed by yellow polyhydra (Fig. 6) indicates that the substitution of hydrogens by bulkier group will decrease the activity. However this region is of importance due to the presence of a blue polyhydra in both the CoMFA and CoMSIA contour plots (Fig. 5 and 7) thus indicating that there could be some possibility of making H-bonds with the binding site by involving free NH_2 group, which is also a part of TRAP. The contour plot of donor field as shown by magenta polyhydra (Fig. 9) suggests favourable H-bond donor type interaction. Overall analysis of the contours at second region in terms of yellow polyhydra for less steric bulk, blue region in HB contours and magenta polyhydra for hydrogen bond donor type interactions explains the less activity of compounds where bulky groups substitute hydrogens at NH_2. The third region enclosed by green polyhydra again shows the importance of steric bulk in the region, which may be important for hydrophobic interactions also, as in the hydrophobic contour plot (Fig. 10) this region is enclosed in a white polyhydra indicating that hydrophobic interactions will be favourable for activity.

4. Conclusion

The 3D QSAR studies carried out using CoMFA and CoMSIA have led to the identification of the regions important for steric, hydrophobic and electronic interactions and the derived models well explain the observed variance in the activity and also provide important insight into structural variations that can lead to the design of NCEs with high activity.

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