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CoMFA, HQSAR, Pharmacophore and Docking studies on Pyridine analogs of nimesulide as Anti-Inflammatory Agents

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Abstract

The anti-inflammatory and antipyretic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are brought about by blockade of production of prostaglandins from arachidonic acid through inhibition of cyclooxygenase (COX) enzyme. Molecular modeling studies were performed on a dataset of 22 pyridinic analogs of nimesulide. The dataset was divided into a training set consisting of 16 and a test set comprising of 6 compounds on random basis. The COX-1 activity expressed in IC_{50} was converted into pIC_{50} and used as a dependent variable in the QSAR study. The molecular modeling studies were performed using SYBYL X 2.0 software running on a core-2 duo Intel processor workstation. The CoMFA model displayed good statistical significance in terms of internal cross validation (q^2) 0.458 and non-cross validation (r^2) 0.982 respectively. Also, the predicted r^2 values (r^2_{pred}) of 0.77 for the test set for the developed model suggested significant predicting ability of the models. In the HQSAR analysis, better statistical results were obtained in fragment size 5–7 and A/B/C/H distinct (q^2 0.987). Partial least square regression studies were performed by using COX inhibitory activity as dependent variable and structural properties of CoMFA and HQSAR as independent variables. Also, PLS of CoMFA was carried out with additional descriptors like ClogP, CMR and total dipole. Pharmacophore was developed using Galahad module of Sybyl and seven pharmacophoric features were depicted in molecules. The docking studies were carried out on pdb 1CQE (COX-1 with Flurbiprofen) and the interaction was obtained with Arg120. The studies revealed the importance of nitrogen as linker and triflouromethanesulfonamido group attached to pyridine ring.

Keywords: NSAIDs, Nimesulide, CoMFA, HQSAR, Docking, Pharmacophore.

Introduction

The anti-inflammatory and antipyretic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are brought about by blockade of production of prostaglandins from arachidonic acid through inhibition of cyclooxygenase (COX) enzyme[1,2]. However, gastroduodenal toxicity is a major adverse effect of NSAIDs, which is due to inhibition of prostaglandin (PG) synthesis in tissues where PGs are responsible for physiological homeostasis[3]. To provide an effective treatment for inflammatory disorders, the design of novel non-steroidal anti-inflammatory drugs is aimed at obtaining new drugs, devoid of the side-effects commonly associated with conventional NSAIDs[4]. The chemical modification of NSAIDs is aimed at improving their potency and decreasing their ulcerogenicity.

Such modification was achieved by synthesizing pyridinic analaogs of nimesulide, one of COX-2 preferential inhibitor by Renard et al and exploring their potential as anti-inflammatory drugs. Various computational techniques were applied on these derivatives, viz. CoMFA, HQSAR, docking and pharmacophore studies. The optimized and validated QSAR models can be used in many ways to aid in the designing of new compounds viz the activities of designed compounds can be predicted. The present study is aimed at carrying out CoMFA, HQSAR, docking and pharmacophore studies on reported pyridinic analogs of nimesulide. This study will prove useful in identifying the structural requirements for designing novel anti-inflammatory drugs.

Material and Methods

Twenty two pyridinic analogs of nimesulide were subjected to molecular modeling[5]. The QSAR methodology requires the data set to divide into test and training set. Thus the dataset was divided into the training set and test set randomly. Six molecules of dataset were used for external validation while the training set with remaining 16 molecules was used to generate the QSAR model. The biological activity [IC_{50} (nM)] was transformed into pIC_{50} (table-1). The transformed pIC_{50} was considered as dependent variable in the QSAR analysis for generation of the significant model.

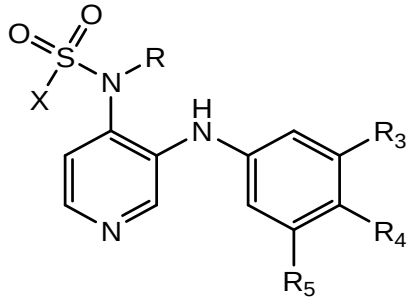
Molecular modeling studies were performed using SYBYL X 2.0 software[6] and core 2 duo Intel processor workstation as hardware. The common substructure, N-(3-Arylaminopyridin-4-yl) sulfonamide [figure-1(a)] was selected for alignment and the molecules were aligned [figure-1(b)] on it.

CoMFA analysis: CoMFA analysis was performed by calculating steric (S) and electrostatic (E) fields at each lattice using a sp^3 hybridized carbon atom serving as the probe atom

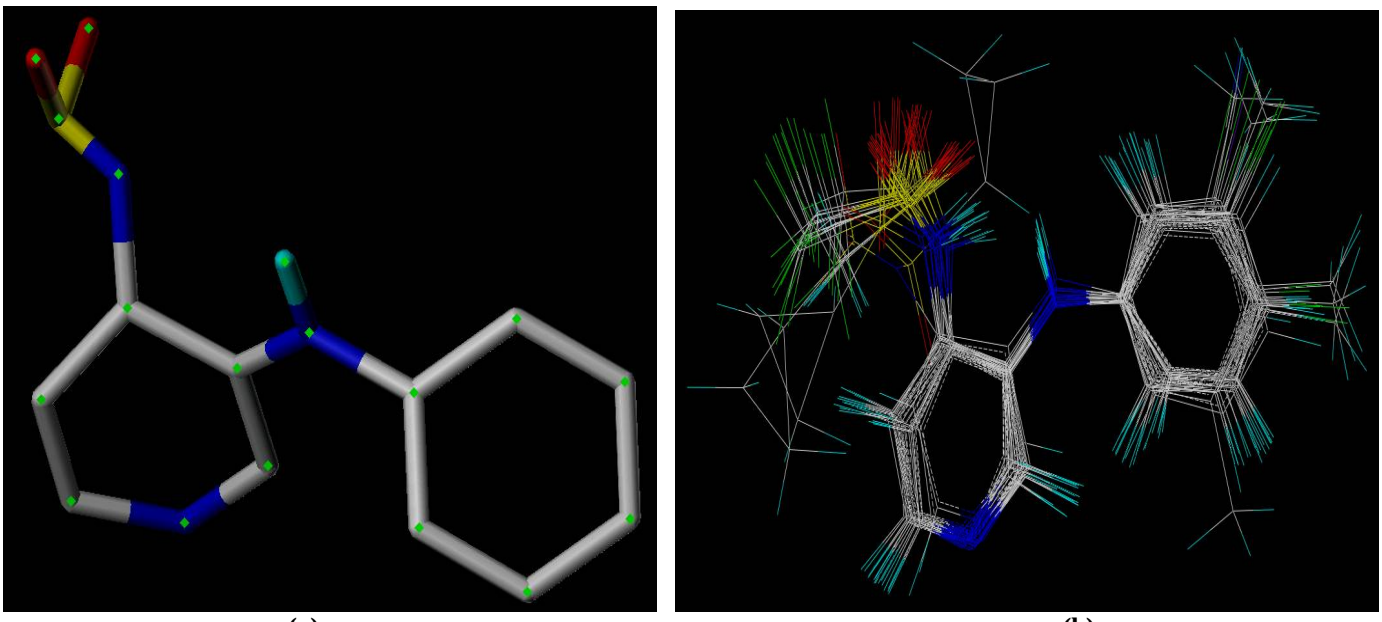
with a grid size of 2 Å and energy cut-off of 30 kcal/mol. Gasteiger, Gasteiger-Huckel, MMFF94, Del-Re and Pullman charges were used as the partial charges to develop the models[7].

Although all of these charges demonstrated good statistical significance, but the best CoMFA model was obtained using MMFF94 partial charges which was then chosen for further analysis. This model was then examined and contour maps were studied. The model was then used to predict the activities of test set molecules. The observed and predicted activities of test set are depicted in table-2.

Table-1
COX-1 inhibitory activities of pyridinic analogs of Nimesulide as anti-inflammatory agents



Comp ID	X	R	R_3	R_4	R_5	IC_{50} in µM (COX-1)
NM1	CF_3	H	H	H	H	0.18
NM2	CF_3	H	Br	H	H	0.91
NM3	CF_3	H	H	Cl	H	1.15
NM4	CF_3	H	Cl	H	H	1.09
NM5	CH_3	H	Cl	H	H	7.15
NM6	CF_3	H	F	H	H	0.44
NM7	CF_3	H	F	F	H	0.27
NM8	CH_3	H	F	H	H	1.37
NM9	CH_3	H	F	F	H	2.09
NM10	CF_3	H	I	H	H	1.93
NM11	CF_3	H	CN	H	H	6.65
NM12	Cyclopropyl	SO_2-Cyclopropyl	H	H	H	19.62
NM13	Cyclopropyl	H	H	H	H	3.61
NM14	CF_3	H	CH_3	H	H	0.99
NM15	CF_3	H	CH_3	H	CH_3	4.11
NM16	CF_3	H	CH_3	CH_3	H	0.71
NM17	CH_3	H	CH_3	CH_3	H	3.28
NM18	CF_3	H	Cl	CH_3	H	1.47
NM19	CF_3	H	Br	CH_3	H	1.4
NM20	CF_3	H	CH_3	Br	H	2.32
NM21	CF_3	H	Cl	F	H	1.74
NM22	CF_3	H	Br	F	H	1.35



(a) **(b)**

Figure-1
Template (a) used for alignment of dataset (b)

HQSAR analysis: The basis of HQSAR is that since the structure of a molecule is encoded within its 2D fingerprint and that structure is responsible for all molecular properties (including biological activity), the activity of a molecule can be predicted from its fingerprint[8]. The 2D structure of the molecules of training set was loaded in the HQSAR window. HQSAR analysis was performed by specifying hologram length, fragment size and fragment distinct.

The available fragment distinction are atoms (A), bonds (B), connections (C), hydrogen atoms (H), chirality (Ch), and donor and acceptor (DA) and hologram length options are 97, 151, 199, 257, 307 and 353 while default fragment size ranges from a minimum value of 4 and maximum 7 number of connected atoms in a fragment. The number of components was selected as 6.

HQSAR analysis was performed by using default hologram length values ranging from 53 to 401 bins, initially using the fragment size default (4–7)[9]. The fragment distinction based model generated the statistical parameter on the basis of which further analysis was performed. The model with significant statistical value was selected from different combinations used. This model (A/B/C/H) was further optimized using different fragment sizes.

PLS and predictive r^2 analysis: PLS analysis was performed by using COX-1 inhibitory activity in the form of dependent variable and dependent variable as properties of CoMFA and HQSAR. Also, PLS of CoMFA was carried out with additional descriptors like ClogP, CMR and total dipole. In addition to this, predictive r^2 was also calculated.

Pharmacophore analysis: GALAHAD module of SYBYL was used to generate pharmacophore using population size of 90 and maximum generations as 50. 10 models were generated and the best model was selected with low energy and high values of steric and hydrogen bonding.

Molecular Docking: Surflex-Dock employs an empirical scoring function and a patented search engine to dock ligands into a protein's binding site[10]. The COX-1 protein structure was retrieved from the RCSB Protein Data Bank (PDB entry code: 1CQE). The docking analysis requires the preparation of protein molecule. Surflex-Dock uses the residues identifying the active site for the sole purpose of generating the protomol. The protomol was generated on ligand extracted site (flurbiprofen). All the water molecules were removed from protein molecule. Also polar hydrogen atoms and AMBER7FF99 charges were added. The bloat value and the threshold value were taken as 1 and 0.5 respectively for generation of protomol.

Results and Discussion

CoMFA analysis: CoMFA model with MMFF94 charges was developed using pIC_{50} as depending variable and CoMFA fields as independent variables. The PLS statistics of CoMFA model is depicted in table-2 and the correlation between experimental and predicted activities (pIC_{50}) of training and test set by CoMFA and HQSAR analysis is shown in table-3 and figure-2. Also, the prominent predicting ability of the developed model is reflected by its predicted r^2 values (r^2_{pred}) of 0.77 for the test set.

Table-2
PLS Statistics of CoMFA Studies

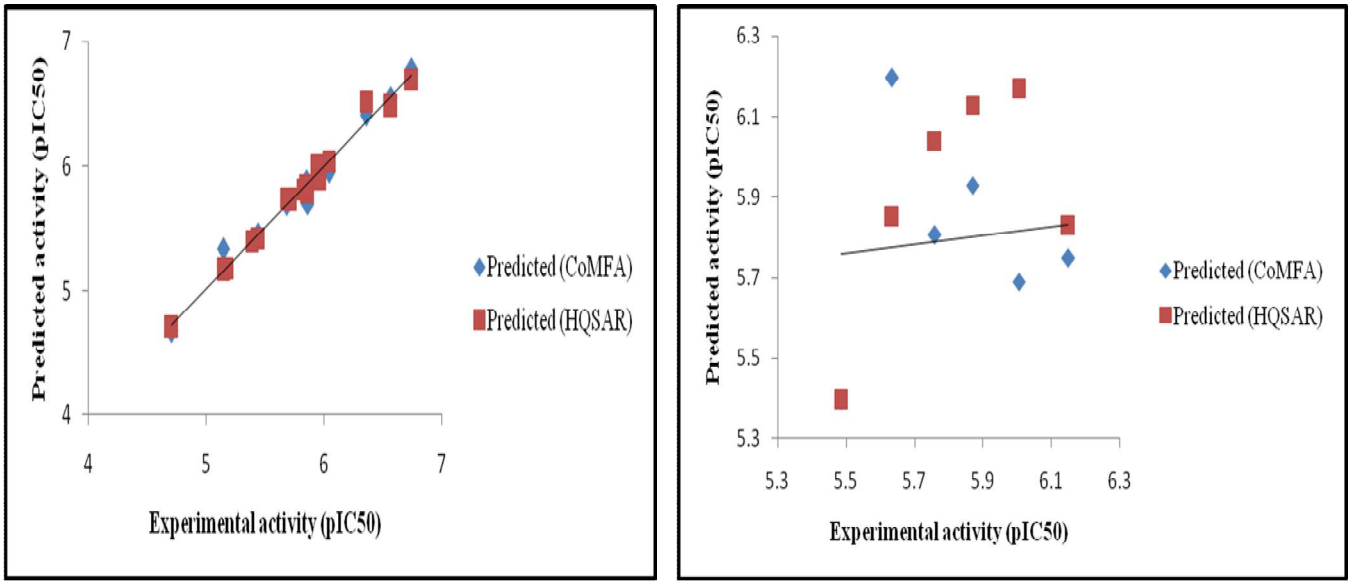
Fields	CoMFA	CoMFA with additional descriptors		
		ClogP	Total Dipole	CMR
r^2	0.982	0.958	0.746	0.965
N	4	4	2	4
q^2	0.458	0.35	0.294	0.242
SEE	0.084	0.126	0.287	0.115
F-value	147.653	63.324	19.086	76.587
Field Contribution (%)	0.428 (S) 0.572 (E)	0.381 (S) 0.523 (E) 0.096 (ClogP)	0.3 (S) 0.377 (E) 0.323 (Total Dipole)	0.432 (S) 0.568 (E) 0.001 (CMR)

r^2 = coefficient of determination, N = optimal number of component, q^2 = cross validated correlation coefficient, SEE = standard error of estimate, F = Fischer test,

Table-3
Experimental and predicted activities of training and test set by CoMFA and HQSAR

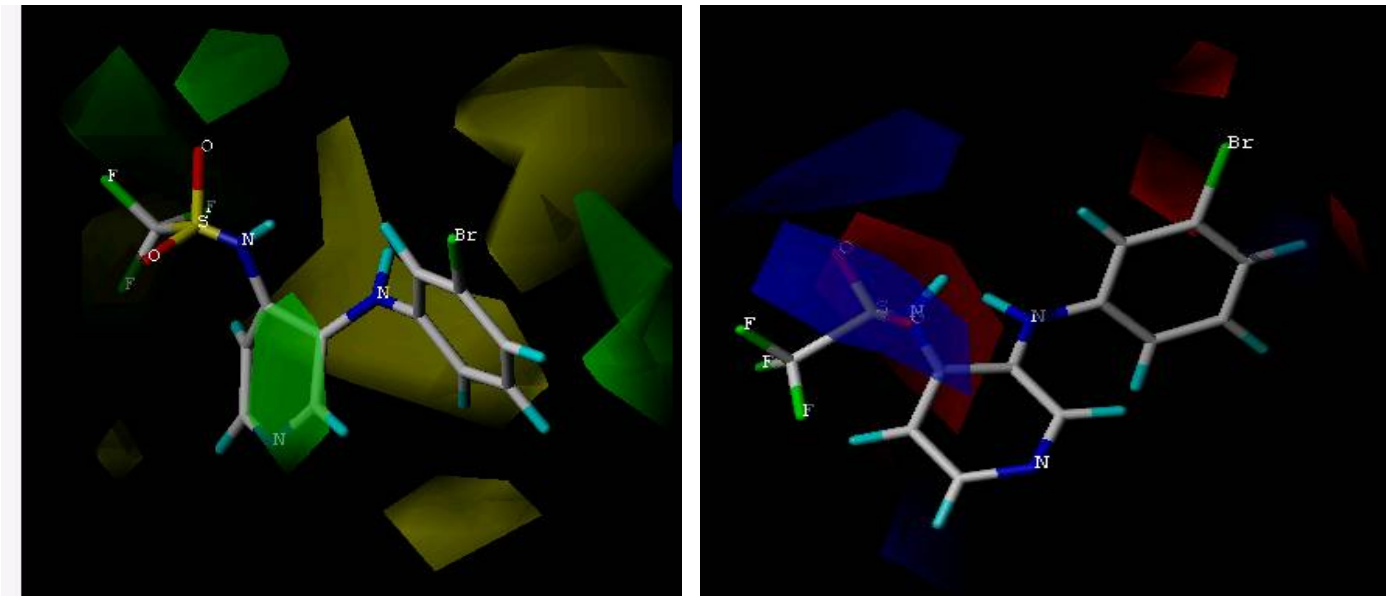
S. No.	Experimental pIC_{50}	CoMFA	HQSAR
		Predicted	Predicted
1	6.7447	6.7754	6.7021
2	6.041	5.9507	6.0431
3	5.9393	5.9572	6
4	5.9626	5.9361	5.8971
5	5.1457	5.3283	5.1631
6	6.3565	6.4084	6.5196
7	6.5686	6.5587	6.4771
8	5.8633	5.698	5.7856
9	5.6799	5.6867	5.7431
10	5.7144	5.7232	5.7248
11	5.1772	5.164	5.1795
12	4.7073	4.6765	4.7168
13	5.4425	5.4483	5.4216
14*	6.0044	5.6898	6.168
15	5.3862	5.4174	5.3964
16*	6.1487	5.7498	5.828
17*	5.4841	5.3953	5.3953
18	5.8327	5.806	5.8155
19	5.8539	5.8809	5.8305
20*	5.6345	6.1975	5.849
21*	5.7595	5.8059	6.041
22*	5.8697	5.93	6.129

* Test set



(a) **(b)**

Figure-2
Experimental versus predicted activity (CoMFA and HQSAR) of (a) training set and (b) test set



(a) **(b)**

Figure-3
CoMFA contour maps of NM1 (a) Steric (b) Electrostatic

CoMFA contour map analysis and HQSAR contribution analysis: The contours and their contributions can be viewed and analyzed using color coding where the color ranges from green/yellow (steric) for sterically favorable and unfavorable regions respectively while blue/red (Electrostatic) contours indicate regions that favour electropositive substituents and electronegative substituents, respectively.

Steric contour map shows sterically favorable region on pyridine ring and unfavorable region on phenyl. The electrostatic contour maps show positive charge desirable on nitrogen and negative charge desirable on oxygen of trifluoromethanesulfonamido group. In addition to this, requirement of electron withdrawing groups is clearly indicated by red contours on ortho and para positions to nitrogen linker on phenyl ring.

The contribution of various fragments can be viewed and analyzed using specific HQSAR color coding were the color ranges from green to white and finally red indicating positive contributing, moderately contributing and finally unfavourable contributing fragments respectively. HQSAR contribution maps clearly indicated the important role played by nitrogen linker towards anti-inflammatory activity and ortho and para positions to this linker are important for placing various substituents.

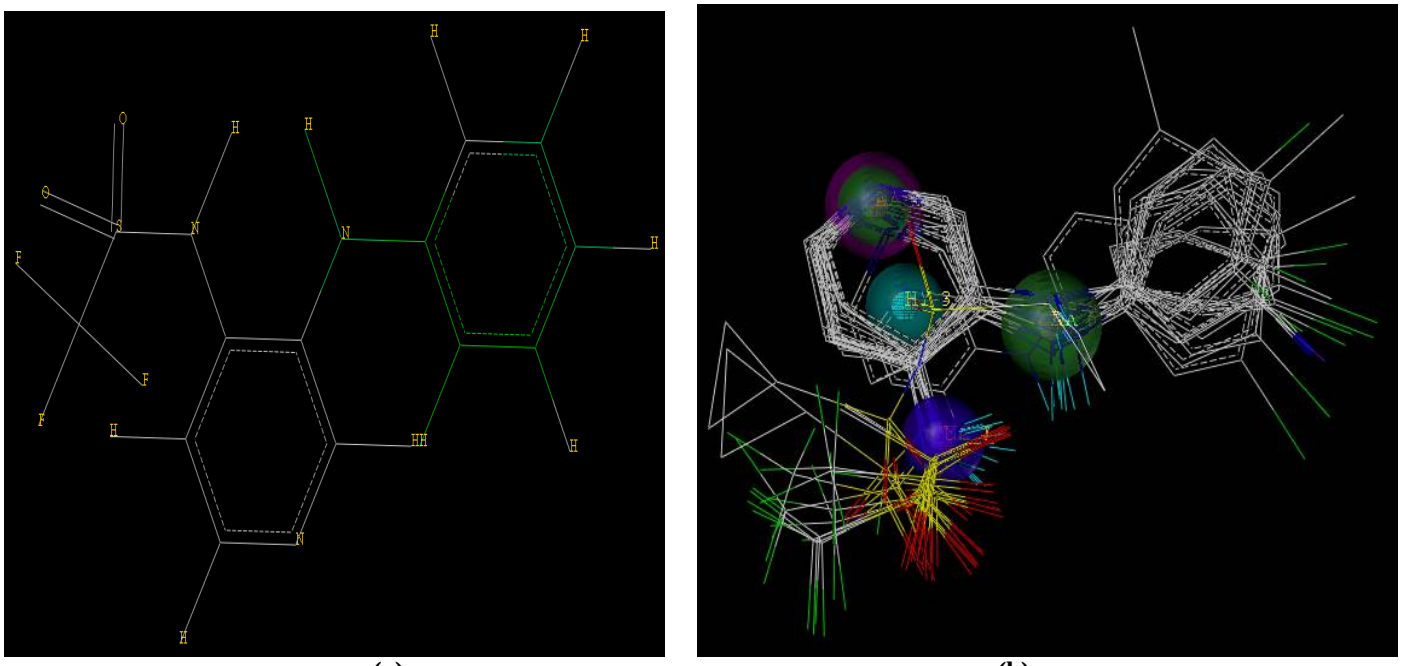
HQSAR model with A/B/C/H distinct and default fragment size (4-7) and optimized model with same fragment distinct and 5-7 fragment size are shown in table-4.

Pharmacophore studies: The best pharmacophore model was chosen with low energy and high value of steric and hydrogen bonding**.** Seven pharmacophoric features namely three donor atoms (DA-1,4,5), two acceptor atoms (AA-2,6), one hydrophobic centre (HY-3) and one negative center (NC-7) were identified. The donor atoms are three nitrogens; viz. nitrogen of pyridine ring, linker and trifluoro-/methanesulfonamido group attached to pyridine ring. The two acceptor atoms are the two oxygens of trifluoro-/methanesulfonamido group attached to pyridine ring and the hydrophobic centre is the pyridine ring itself while the negative centre is in the form of nitrogen of trifluoromethanesulfonamido group.

Docking studies: The oxygen of trifluoromethanesulfonamido group attached para to pyridine ring of NM1 was found to hydrogen bond with nitrogen of Arg120 of COX-1 enzyme [figure-5 (a)]. These two oxygens also form part of the pharmacophore required for anti-inflammatory activity. The MOLCAD (molecular computer aided design) program was employed to analyze the binding mode between the inhibitor and protomol pocket. The color coded display in the workspace, the electrostatic potential (EP) and lipophilicity (LP), hydrogen bonding (HB), cavity depth (CD) suggest better insights into the understanding of the binding affinity [figure-5 (b-e)].

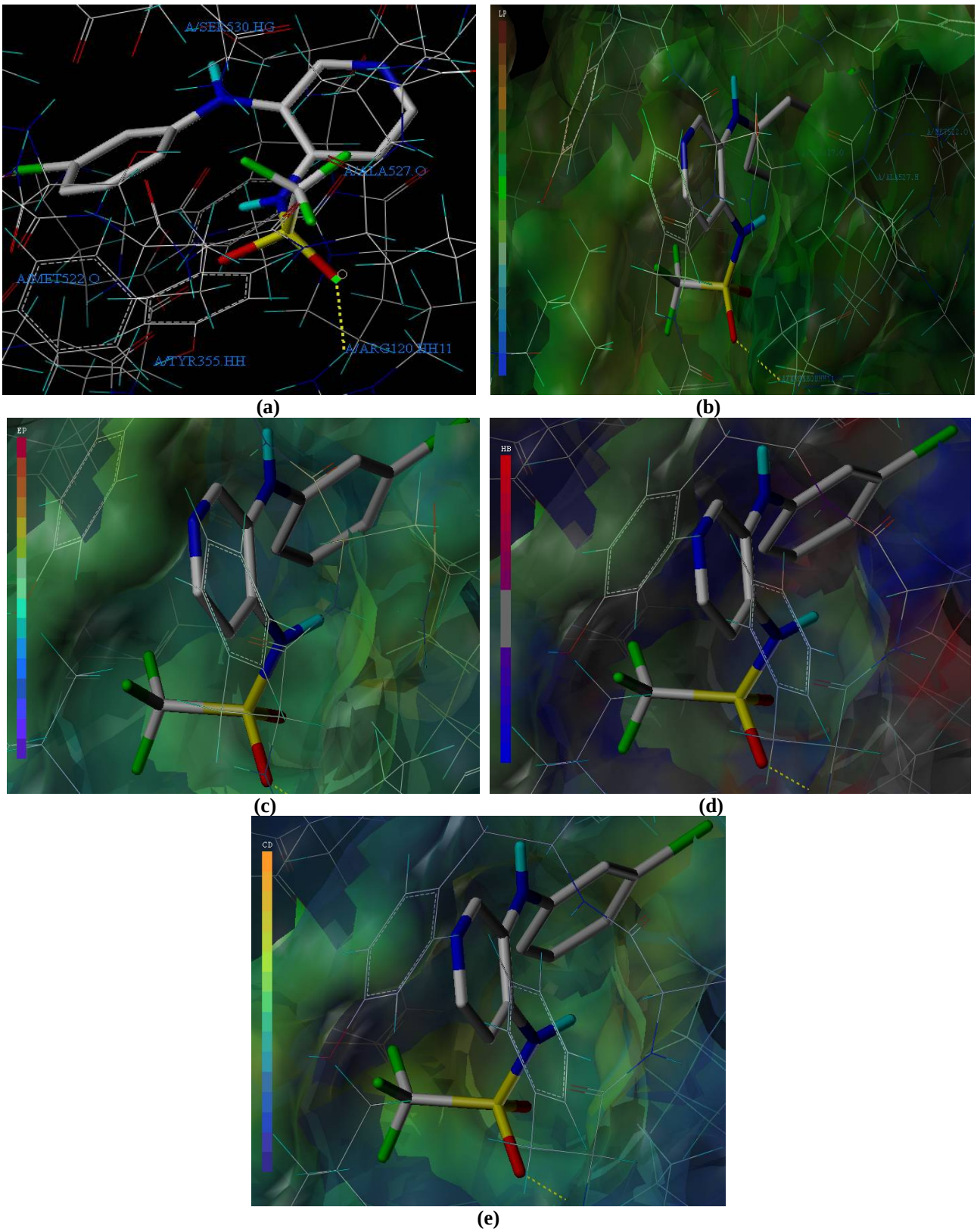
Table-4
Summary of HQSAR models

Type	N	r^2_{cv}	r^2	S.E.	S.E. (cv)	Best length	r^2 (ensemble)	S.E. (ensemble)	Fragment Size
A/B/C/H	6	0.986	0.986	0.082	0.082	151	0.982	0.091	4-7
A/B/C/H	6	0.987	0.987	0.079	0.079	151	0.987	0.079	5-7



(a) **(b)**

Figure-4
(a) HQSAR contribution map (b) Pharmacophore requirements



(a) **(b)**

(c) **(d)**

(e)

Figure-5: Docking poses of NM1
(a) Hydrogen bond interaction with Arg120 of COX-1 (b) MOLCAD surfaces Lipophilic potential (LP)
(c) Electrostatic potential (EP) (d) Hydrogen bonding (HB) (e) Cavity depth (CD)

Conclusion

The docking and pharmacophore analysis results are correlating with CoMFA revealing the importance of oxygen of trifluoromethanesulfonamido group involved in hydrogen bonding with Arg120 of COX-1. The HQSAR studies revealed the importance of nitrogen as a linker and also the importance of ortho and para position to the nitrogen linker being available for substitutions. These structural insights can aid in the designing of novel anti-inflammatory agents.

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