

Design of new chemicals entities as anti-inflammatory using structure optimization by molecular modeling studies

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Abstract

The research on design of non toxic anti-inflammatory analgesic agent is continuously going on since more than last 40 years. Keeping the same objective in mind as an attempt to develop potent and nontoxic, nonsteroidal anti-inflammatory & analgesic agents, we have optimized the tetrahydropyrimidine pharmacophore by using molecular modeling studies. In this paper we present results of 2D and 3D QSAR studies of series 18 molecules containing tetrahydropyrimidine pharmacophore as cyclooxygenase (COX) inhibitors. The 3D QSAR studies were performed using simulated annealing k-nearest neighbor molecular field analysis (SA kNN-MFA) methods. The 2D QSAR studies were performed using multiple regressions. The output of present research work is the 2D QSAR studies indicated contribution of different physicochemical descriptors and the result of 3D QSAR studies indicated the exact steric and electronic requirement in the ranges at various positions around tetrahydropyrimidine pharmacophore. Thus the pharmacophore requirement for COX inhibition was optimized and requirement at various positions around tetrahydropyrimidine pharmacophore were defined.

Keywords: Nonsteroidal anti-inflammatory, Tetrahydropyrimidine, Cyclooxygenase (COX) inhibitors. QSAR.

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*Article History:

Received: 21/02/2019

Revised: 24/03/2019

Accepted: 26/03/2019

DOI: <https://doi.org/10.7439/ijpc.v9i3.5220>

QR Code



How to cite: Modhave N. S. Design of new chemicals entities as anti-inflammatory using structure optimization by molecular modeling studies. *International Journal of Pharmaceutical Chemistry* 2019; 9(3): e5220. Doi: 10.7439/ijpc.v9i3.5220
Available from: <https://ssjournals.com/index.php/ijpc/article/view/5220>

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently used medications worldwide to treat pain, fever, and inflammation. The multifunctional pyrimidine represents a heterocyclic system of remarkable pharmacological efficiency [1-5]. In the recent years, several novel approaches for reducing the GI toxicity of NSAIDs with promising results have been reported [6]. These mainly involve structural modifications of existing NSAIDs such that inhibition of COX is maintained, but other attributes are added that diminish GI (and other) toxicity and in some cases boost efficacy and/or potency [7]. Recent strategies adopted to minimize the side effects of NSAIDs include the use of the dual LOX/COX inhibitors, the use of selective COX-2 inhibitors, and the use of hybrid molecules made up of non-selective or selective COX inhibitors together with a nitric oxide-releasing functional group [8]. The strategy of designing

hybrid molecules made up of non-selective COX inhibitors together with a nitric oxide-releasing moiety constitutes one of the most promising approaches, because nitric oxide supports several endogenous GI defence mechanisms, including increase in mucus, bicarbonate secretions, increase in mucosal blood flow and inhibition of the activation of proinflammatory cells [9-11]. Moreover, because of the beneficial cardiovascular effects (vasodilation) of Nitric Oxide, such drugs are expected to be devoid of the cardiovascular adverse effects associated with the use of selective COX-2 inhibitors [10,12]. The major goal of this work is to optimized substituent's on to the tetrahydropyrimidine pharmacophore, in order to increase inhibition of COX and in turn enhance anti-inflammatory activity by using 3-D QSAR studies and with the aim of designing compounds with a wider margin of safety, especially with reference to the Gastrointestinal ulcerogenicity.

3. Materials and Methods

3.1 Biological Data

A data set containing eighteen molecules, reported for their analgesic and anti-inflammatory activity was selected for the present study [5]. The structures of the compounds and their biological data are presented in **Table 1**. The activity of all compounds used in the QSAR study was measured by the same assay and is reported as % inhibition at 10mg/ml. For the QSAR study, the activity values were transformed in **Eq.1** as follows:

$$\text{Activity (pIC}_{50}) = -\log c + \text{logit}$$

----- **Eq.1**

Where c is the molar concentration

$$= \frac{\text{concentration } (\mu\text{g/ml}) \times 0.001}{\text{Molecular weight}}$$

$$\text{logit} = \log [\% \text{ inhibition} / (100 - \% \text{ inhibition})]$$

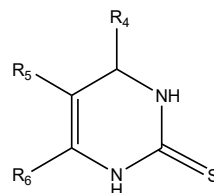


Table 1: The selected series of 4, 6-(4-substitutedaryl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidin-5-yl]-acetic acid derivatives along with their Anti-inflammatory activity data

Sr.no.	Molecule no.	-R ₄	-R ₅	-R ₆	IC ₅₀	pIC ₅₀
1.	4a		-COOH		17	0.8224
2.	4b		-COOH		30	1.1869
3.	4c		-COOH		32	1.2221
4.	4d		-COOH		21	0.9436
5.	4e		-COOH		19	0.8677
6.	4f		-COOH		15	0.7591
7.	4g		-COOH		20	1.1869
8.	4h		-COOH		25	1.1176
9.	4i		-COOH		35	1.321
10.	4j		-COOH		16	0.841
11.	4k		-COOH		09	0.5388
12.	4l		-COOH		11	0.6477
13.	4m		-COOH		28	1.1193
14.	4n		-COOH		22	1.0219
15.	4o		-COOH		33	1.2588
16.	4p		-COOH		10	0.5829
17.	4q		-COOH		11	0.6079
18.	4r		-COOH		07	0.4074

All computational studies were performed using V-Life Molecular Design Software Version 3.0 [13]. Both 2D and 3D QSAR models were generated using a set of 18 molecules. Test set of 5 molecules with distributed biological were used to access the predictive power of generated QSAR models using training set of 13 molecules with varied chemical and biological activities. In addition, the molecules in the test set should be selected in such a way that there lie few similarities with the compounds in training set. For example the variation in structural composition, biological activity ranges etc. Additional care was taken while selecting compounds in training and test set, that all these compounds retain similarity in chemical composition with respect to mode and locus of binding at active binding pocket of COX enzyme. The goal was to ensure the accuracy of predictive abilities of models developed.

3.2 2D QSAR studies:

The most widely used Multiple Linear regression (MLR) analysis was used to correlate biological activities with physicochemical properties and in turn Chemical composition of the selected series of compounds. All Physicochemical properties were computed using V-Life Molecular Design Suite Software and were auto loaded in worksheet. Different QSAR equation were generated using MLR method and the best model was selected based on value of r^2 (Square of correlation coefficient for training set of compounds), q^2 (Cross- validated r^2) and pred_r^2 (Predictive r^2 for test set of compounds) shown in **Table 2**.

3.3 3D QSAR Studies using kNN MFA

The compounds were constructed from the fragments in the V-Life molecular builder database with standard bond lengths and bond angles and geometry optimization was carried out using the standard Merck molecular force field (MMFF) [14] with distance dependant –dielectric function and energy gradient of 0.001 kcal/mol Å. The template used for alignment is shown in **figure 2**. Alignment of compounds is a very important feature for developing kNN MFA analysis. For each alignment, the steric and electrostatic potential fields for KNN MFA were calculated at each lattice intersection of a regularly spaced grid box. The lattice spacing was set to value of 2.0 Å in all X, Y and Z directions. A distance–dependant dielectric constant of 1.0 was used an sp³ carbon atom with van der Waals radius of 1.52 Å and + 1.0 charge was served as the probe atom to calculate steric and electrostatic fields (Figure 3). All 18 molecules in the training set were considered as observation to generate QSAR equations using Simulated Annealing (SA) kNN MFA methods.

Figure 2: Common template used for alignment of tetrahydropyrimidine series of compounds.

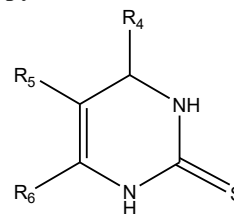
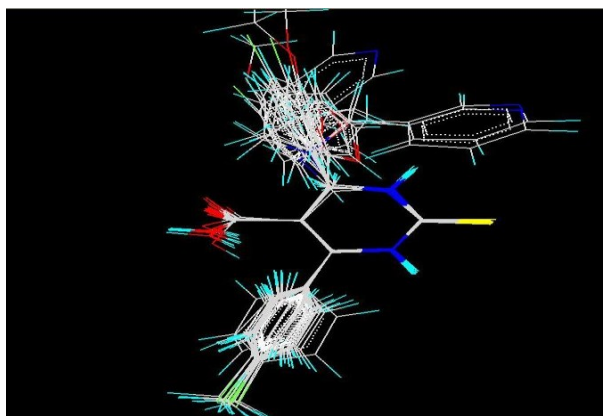


Figure 3: Overlain of all [4, 6-(4-substituted aryl)-2-thioxo-1,2,3,4 tetrahydropyrimidin 5-yl] acetic acid derivatives aligned using template based alignment method tool of V-Life MDS.



4. Result & Discussion:

4.1 2D-QSAR studies

Multiple regression is widely used method for building QSAR model. It is simple to interpret a regression model, in which contribution of each descriptor could be seen by the magnitude and sign of its regression coefficient. A descriptor coefficient magnitude shows its relative contribution with respect to other descriptors and sign indicates whether it is directly (+) or inversely (-) proportional to the activity. The QSAR model is presented as Eq.2

$$\begin{aligned} \text{pIC}_{50} = & 0.7660(\pm 0.0263) \text{SaaCHE-index} \\ & -0.3874(\pm 0.0295) T_2_N_2 \\ & 0.5886(\pm 0.0121) \text{chiV0} \\ & 0.6421(\pm 0.0241) \text{SaaCH count} \\ & -6.1256 \end{aligned} \quad \text{-----Eq.2}$$

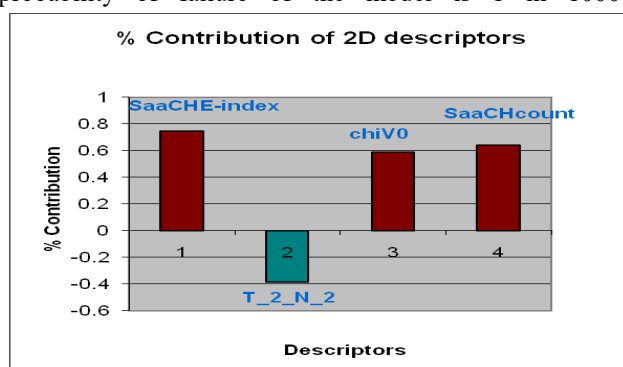
The relative contribution of each descriptor for the biological activity as selective COX-2 inhibitors is presented in (**Table 2**).

Table 2: Statistical results of 2D QSAR generated by MLR Analysis for Tetrahydropyrimidine derivatives

Sr.No.	Statistical parameters	Result	Contributing Descriptors (contribution)
1	r^2	0.9043	
2	q^2	0.8330	1. SaaCHE-index 0.7660(± 0.0263)
3	Pred r^2	0.6561	
4	Pred r^2 SE	0.1138	2. T_2_N_2 (-0.3874(± 0.0295))
5	F-Test	35.2488	
6	Alpha Rand q^2	0.05	3. chiV0 (0.5886(± 0.0121))
7	Best Rand q^2	0.16163	
8	Z-score q^2	2.21369	4. SaaCHcount (0.6421(± 0.0241))

The equation explains 90 % ($r^2 = 0.90$) of the total variance in the training set as well as it has internal (q^2) and external (pred r^2) predictive ability of ~83 % and ~65% respectively.

The F test = 35.24 shows probably the statistical significance 99.99% of the model which means that probability of failure of the model is 1 in 10000.

**Figure 4: % Contribution of 2D descriptors**

The regression equation indicated that the positively contributing descriptors were directly proportions to the biological activity, increasing of these properties in the molecules will increases COX activity. Whereas negative correlated descriptors were inversely proportion to COX activity and it was required to decrease these properties in the compound for better COX activity.

- **SaaCHE-index:** - It is a topological descriptor which defines electrotopological state indices for number of –CH group connected with two aromatic bonds. It is positively contributing to activity. Means the -CH which is attached to the aromatic ring is important.
- **T_2_N_3:-** It is alignment independent descriptors which is the count of number of double bounded atoms (i.e. any double bonded Atom, T_2) separated from nitrogen atom by 3 bonds in a molecule. Contribution is negative (-0.3874) for the activity which indicates that any doubly bonded atom separated from nitrogen atom, should not be more than 3 bonds.
- **chiV0:-** This descriptor signifies atomic valence connectivity index (order 0), which indicates degree of branching in molecules. Different arrangements of atoms affect the activity. chiV0 indicates there is no branching. It might be that N1 and N3 of

Tetrahydropyrimidine should be unsubstituted to contribute directly for the activity.

- **SaaCHcount:** - This descriptor defines the total number of carbon atoms connected with hydrogen along with two aromatic bonds. This descriptor is positively contributing means that aromatic ring is important for activity.

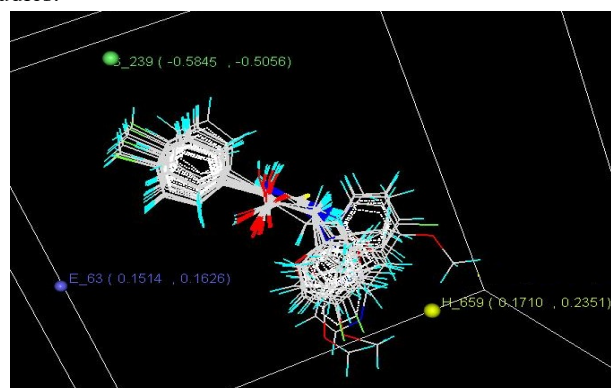
4.2 3D QSAR Studies using kNN MFA [15]

Several 3D QSAR Model were generated for selected compounds of training and test sets of tetrahydropyrimidine derivatives by KNN method using Simulated Annealing methods and best model generated by (SA), based on q^2 and pred r^2 is reported here as shown in **Table 3**.

Table 3: Statistical results of 3D QSAR generated by kNN-MFA Analysis for Dihydropyrimidine derivatives

Parameter	SA –kNN method
q^2	0.7469
q^2_{se}	0.1445
Pred r^2	0.8199
Pred r^2_{se}	0.1254
Hydrophobic	H 659 (0.1710 0.2351)
Electrostatic	E 63 (0.1514 0.1626)
Steric	S 239(-0.5845 -0.5056)

All the statistical results, cross-validation were analyzed by considering the fact that a value of cross-validated r^2 (r^2_{cv}) is above 0.5 indicating the probability of getting correlation value by chance is less than 5%. The statistical results obtained by both SA and SW kNN-MFA studies.

**Figure 5: Stereo view of the molecular rectangular field grid around the superposed molecular units of tetrahydropyrimidine series of compounds using SA- kNN method**

The generated model shows best internal as well as external predictivity ($q^2=0.7469$, $\text{pred}_r^2=0.8199$) and also error occurred in predictivity was very low so model was considered satisfactory for optimization. Thus this model was used to optimize the electronic as well as hydrophobic

requirement around pharmacophore. The actual, predicted activities and the residuals thereof (actual - predicted activities) for the training set and test set molecules are presented in **Table 4** and **Table 5**.

Table 4: Training set of tetrahydropyrimidine derivatives along with biological activity, predicted activity data and residuals obtained thereof for SA-kNN-MFA model.

Sr. No.	Molecules	IC ₅₀	pIC ₅₀	SA -kNN method	
				Predicted Activity	Residual Activity
1)	4c	32	1.2221	1.2588	-0.0367
2)	4e	19	0.8677	0.7591	0.1086
3)	4f	15	0.7591	0.8677	-0.1086
4)	4g	30	1.1869	1.321	-0.1341
5)	4h	25	1.1176	1.0219	0.0957
6)	4i	35	1.321	1.1193	0.2017
7)	4j	16	0.841	0.9436	-0.1026
8)	4l	11	0.6477	0.5388	0.1089
9)	4m	28	1.1193	1.321	-0.2017
10)	4n	32	1.0219	1.1176	-0.0957
11)	4p	10	0.5829	0.5388	0.0441
12)	4q	11	0.6079	0.5388	0.0691
13)	4r	07	0.4074	0.6079	-0.2005

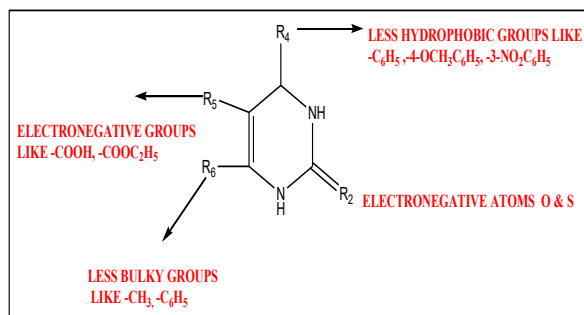
Table 5:-Test set of dihydropyrimidine derivatives along with biological activity, predicted activity data and residuals obtained thereof for SA-kNN-MFA model.

Sr.No.	Molecules	IC ₅₀	pIC ₅₀	SA-kNN method	
				Predicted Activity	Residual Activity
1.	4a	17	1.1869	0.8677	-0.0453
2.	4b	30	1.2221	0.7591	0.4278
3.	4d	21	0.9436	0.841	0.1026
4.	4k	9	0.5388	0.6079	-0.0691
5.	4o	33	1.2588	1.1176	0.1412

Tables 4 and 5 showed residual obtained by subtraction of predicted activities from biological activities in both model was low, thus error occurred in prediction of biological activity by both model is near to zero which indicated power of predicating the biological activity is good. Thus both SA and SW kNN MFA model will be used for designed of new potent compounds containing tetrahydropyrimidine nucleus for inhibition of COX enzyme.

4.3 Optimisation of pharmacophore:-

The information obtained from 3D and 2D QSAR studies was used to optimize the electrostatic, hydrophobic and steric requirement around the tetrahydropyrimidine pharmacophore for their anti-inflammatory activity.



4.4 Design of new chemical entities:-

Designing of NCE's was purely based on information obtained from literature survey and by 3D and 2D QSAR studies by using CombiLib[®] tool in VLife MDS software.

About thousands of molecules were generated which followed the Lipinski's rule, but we have selected only 12 molecule on the basis of activity predicted by MLR equation generated by 2D QSAR .

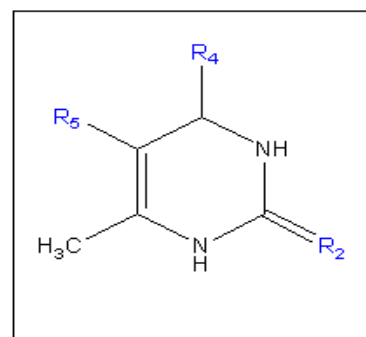


Table 6: Newly Designed compound along with their predicted activity.

Mol. No.	-R ₂	-R ₄	-R ₅	-R ₆	Screen result	Screen score	Predicted activity
N ₁	-O	-C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	0.8435
N ₂	-S	-C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	0.8120
N ₃	-O	-3- NO ₂ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	0.9825
N ₄	-S	-3- NO ₂ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	1.2011
N ₅	-O	-2-ClC ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	0.9912
N ₆	-O	-4-OCH ₃ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	2.314
N ₇	-O	-4- F C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	1.9874
N ₈	-S	-4-OCH ₃ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	1.9843
N ₉	-O	-4- NO ₂ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	1.9877
N ₁₀	-S	-2-Cl C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-CH ₃	ADRXWS	6	0.9476
N ₁₁	-O	-4-OCH ₃ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-C ₆ H ₅	ADRXWS	6	0.3245
N ₁₂	-S	-4-OCH ₃ C ₆ H ₅	-NHNHCOCH ₂ ONO ₂	-C ₆ H ₅	ADRXWS	6	0.2467

The relevant findings of present work are summarized below.

Designing of NCE's was purely based on information obtained from literature survey and by 3D and 2D QSAR studies by using Combilib tool in VLife MDS software.

- 1) At R₄, hydrophobic points was generated so different hydrophobic functional groups were placed at that position, and those which showed good predicted activity are reported here.
- 2) At R₆ less steric groups were placed like -CH₃, and -C₆H₅ and it was observed that -CH₃ at R₆ shows good predicted activity.
- 3) At R₅ different electronegative groups were substituted. The NO group reported to possess ulcer healing properties was also incorporated in the side chain and also these NO group containing compounds have shown good predicted activity.
- 4) At R₂, either O or S was placed and it was found that compounds containing O at R₂ show good predicted activity as compared to S at R₂.

5. Conclusion

The thorough investigations of result of 2D and 3D QSAR studies have helped us to decide about the electronic and steric nature of substitution pattern around the selected Tetrahydropyrimidine nucleus. At various positions on the common template the substitution pattern was carried out and the same data was used for the design of NCEs. The regression equation obtained was used for prediction of activity of designed compounds in silico. In all the overall outcomes of these studies have provided great help to optimize the pharmacophore and to design the potent, hybrid molecule with nitric acid releasing group compounds.

Acknowledgement

The authors are thankful to The Principal, A.I.S.S.M College of Pharmacy, Pune, for providing laboratory facilities.

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