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Research Article

3D QSAR studies of Substituted Benzamides as Nonacidic Antiinflammatory Agents by kNN MFA Approach

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ABSTRACT

A series of N- (4, 6 dimethyl-2-pyridinyl) benzamides as non acidic anti-inflammatory drugs was subjected to a 3D-quantitative structure activity relationship using kNN MFA approach. In the mentioned model it can be seen that the kNN MFA model obtained by using the stepwise variable selection method shows that electrostatic interactions (2 of 3 descriptors in SW are electrostatic field descriptors) plays major role in determining biological activity. It can also be noted that descriptor E_ 300 is implying the significant role of this electrostatic field interaction for structure activity relationship.

Keywords: Substituted Benzamide, Anti-inflammatory agent, 3D QSAR study.

INTRODUCTION

Gastric irritation, the most prominent side effect to the widely used non-steroidal antiinflammatory drugs, compels the medicinal chemists to discover novel category of non acidic antiinflammatory drugs which are partially or fully devoid of gastrointestinal toxic effects such as ulceration, hemorrhage and perforation. Novel categories of drugs are being developed based on new mechanism of action and pathogenesis of inflammation. The non-acidic anti-inflammatory compounds of the previously reported benzamides prompted us to establish QSAR in the given series having N- (4, 6 dimethyl 2-pyridinyl) moiety as a basic nucleus. The observed antiinflammatory activity elicited by the inhibition of carragenan induced rat paw edema in shown in Table 1. The compounds were reported in the publications [1- 3].

MATERIALS AND METHODS

The molecular modeling calculations were performed using Molecular Design Suite (MDS) by Vlifesiences[4]. The X-ray Crystallographic data for these ligands-AT1 complexes are not deposited in the protein data bank; hence all the molecules were constructed using the standard geometry with 2D molecular module of Molecular Design Suite. The calculation of 2D descriptors and by regression and partial least square analysis was performed on Pentium IV workstation using Molecular Design Suite. In 3D QSAR kNN-MFA common rectangular grid around the molecules was build. The steric and electrostatic interaction energies were computed at the lattice points of the grid using a methyl probe of charge 1. These interaction energy values are considered for relationship generation and utilized as descriptors to decide nearness between molecules. The term descriptor is utilized to indicate field values at the lattice points. 2080, 3D descriptors (1040 for each electrostatic and electronic field, which theoretically form a continuum) were calculated[5-7] setting charge types as Gasteiger–Marsili (GM) and dielectric constant value at 1.0. The probe atomic number 6 with probe charge 1 with electrostatic cutoff of 10 and steric cutoff 30 was employed to perform descriptor calculation. The descriptors with no variation in values were rejected; descriptor with constant value will not contribute to QSAR.

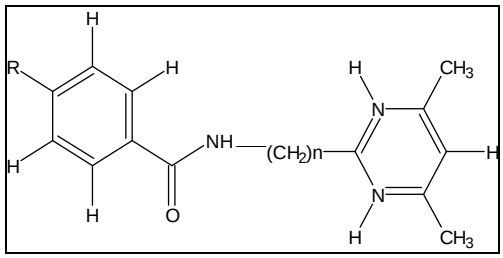
k- Nearest Neighbor (kNN) Method.
The kNN methodology relies on a simple distance learning approach whereby an unknown member is classified according to the majority of its k-nearest neighbors in the training set. The nearness is measured by an appropriate distance metric (e.g., a molecular similarity measure calculated using field interactions of molecular structures). This method employs the kNN classification principle combined with the stepwise variable selection procedure for optimization of (i) the number of nearest neighbors (k) used to estimate the activity of each compound and optimization of (ii) selection of variable from the original pool of all molecular descriptors (steric and electrostatic fields at the lattice points) that are used to calculate similarities between compounds (i. e. distances in n-var – dimensional descriptor space). Further, step-by-step search procedure that begins by developing a hypothetical pharmacophore (HP) model with a single independent and adds independent variables one step at a time, examining the fit of the model at each step (using weighted k-nearest neighbor cross validation procedure as described below) until there are no more significant variables remaining outside the model.
The descriptors that get selected in a given model are the field points either of steric of electrostatic nature at particular locations in a common grid around reported set of molecules. For utilizing these descriptors for new ligand design, we consider the field values at different grid points of compounds cluster having most active compound. The extrema of field values of compounds in the cluster of most active compounds decide range of field values which is preferred and recommended for new compound design.

kNN - MFA with Stepwise (SW) Variable Selection. This method employs a stepwise variable selection procedure combined with kNN to optimize
(i) The number of nearest neighbors (k)
(ii) The selection of variables from the original pool.
The step by- step search procedure begins by developing a trial model with a single independent variable and adds independent variables, one step at a time, examining the fit of the model at each step.

kNN - MFA with Genetic Algorithm
The genetic function approximation (GFA) algorithm offers a new approach to the problem of building quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) models. Replacing regression analysis with the GFA algorithm allows the construction of models competitive with or superior to those produced by standard techniques and makes available additional information not provided by other techniques[8]. Unlike most other analysis algorithms, GFA as a result provides multiple models, and the population of the model is created by evolving random initial model using a genetic algorithm. Genetic algorithms are derived from an analogy with the spread of mutations in a population. In this analogy, "individuals" are represented as a one-dimensional string of bits. An initial population of individuals is created, usually with random initial bits. A fitness function is used to estimate the "quality" of an individual, so that the "best" individuals receive the best fitness scores. Individuals with the best scores are more likely to propagate their genetic material to offspring through crossover, in which pieces of genetic material are taken from each parent and recombined to create the child. After many such mating steps, the average fitness of the individuals in the population increases, as good combinations of genes are discovered and spread through the population. Genetic algorithms are especially good at searching problem spaces having a large number of dimensions, since they conduct a very efficient, directed sampling of the large space of possibilities. The genetic parameter setting includes the parameters like cross correlation limit, population, Convergence criteria, mutation probability, chromosome length, and print after iterations.

RESULTS AND DISCUSSION:
3D MODEL BUILIDING
The descriptors that get selected in a given model are the field points either of steric of electrostatic nature at particular locations in a common grid around reported set of molecules. For utilizing these descriptors for new ligand design, we consider the field values at different grid points of compounds cluster having most active compound (Figure 1). The extrema of field values of compounds in the cluster of most active compounds decide range of field values which is preferred and recommended for new compound design. The plot of the kNN-MFA shows the relative position and ranges of the corresponding important electrostatic and steric fields in the model provide guidelines for new molecule design.
As discussed in paper Parate et. al., the electrostatic contributions were found to be the highest of all parameters, and combined electrostatic and steric fields as well as calculated molar refractivity and Z component when studied together showed (r^2_{cv} = q^2=0.465 and a r^2=0.976) the best predictions. Only model of 0.46 and above and a fraction of variance r^2 over 0.85 for the optimum number of components were further considered.

TABLE 1: SERIES OF COMPOUNDS N-(4, 6- DIMETHYL – 2-PYRIDINYL) BENZAMIDES



S. No.	Comp.No.	R	% Inhibition of Carragenan induced rat paw edema (P)[a] Dose = 200 mg/ kg body weight
1.	1	H	84.7±5.4
2.	2	4-NO_2	54.4±12.5
3.	3	3-NH_2	53.8±8.9
4.	4	4-NH_2	63.6±6.4
5.	5	3-$NHCOCH_3$	44.5±7.5
6.	6	4-$NHCOCH_3$	53.3±7.7
7.	8	4-F	60.4±8.4
8.	10	3-Cl	61.1±2.5
9.	11	4-Cl	54.5±5.5
10.	12	3-Br	78.3±.3.4
11.	13	4-Br	38.3±8.6
12.	14	3-CH_3	47.5±8.4
13.	15	3-CF_3	65.8±6.2
14.	16	2-OCH_3	33.0±5.7
15.	17	3-OCH_3	61.3±7.0
16.	18	4-OCH_3	48.3±8.2
17.	19	4-OC_2H_5	45.0±6.1
18.	20	4-SCH_3	45.0±8.4
19.	24	4-CN	57.3±5.5
20.	25	3-NO_2	78.8±8.9
21.	27	4-CN	57.3±5.5
22.	28	3-F	91.4±2.9
23.	29	$(CH_2)_2$-C_6H_6	29.2±11.0

Table 2: Statistical Analysis of kNN Stepwise forward backward variable selection and CoMFA

3D kNN- MFA Model 1	**3D kNN- MFA (Not Significant, Genetic Algorithm)**	**CoMFA model**	
Training Set Size = 15	Training Set Size = 15	No. of compounds=	23
Test Set Size = 8	Test Set Size = 8		
Selected Descriptors:	**Selected Descriptors:**	Principal components	5
S_165	S_246	r^2cv	0.465
E_300	E_412	F test	54.38
S_219		r^2d	0.976
Statistics:	**Statistics:**	P Value	0.000
k Nearest Neighbour= 4	k Nearest Neighbour= 5	SEE	0.072
n = 15	n = 15	**Descriptor Range:**	
Degree of freedom = 11	Degree of freedom = 11	Steric Contribution	(1.331, .316)
q^2 = 0.7962	q2 = 0.5378	Electrostatic contribution	(2.044, 0.486)
q2_se = 0.1379	q2_se = 0.2078	CMR	(0.723, 0.172)
Predr2 = 0.4015	Predr2 = 0.1677	Z component	(0.109, 0.0260)
pred_r2se = 0.3747	pred_r2se = 0.4419		
Descriptor Range:	**Descriptor Range:**		
S_165 -0.1748 1.9815	S_246 -0.0612 -0.0346		
E_300 -4.0622 1.2855	E_412 -9.2546 -5.9132		
S_219 -0.4530 1.1280			

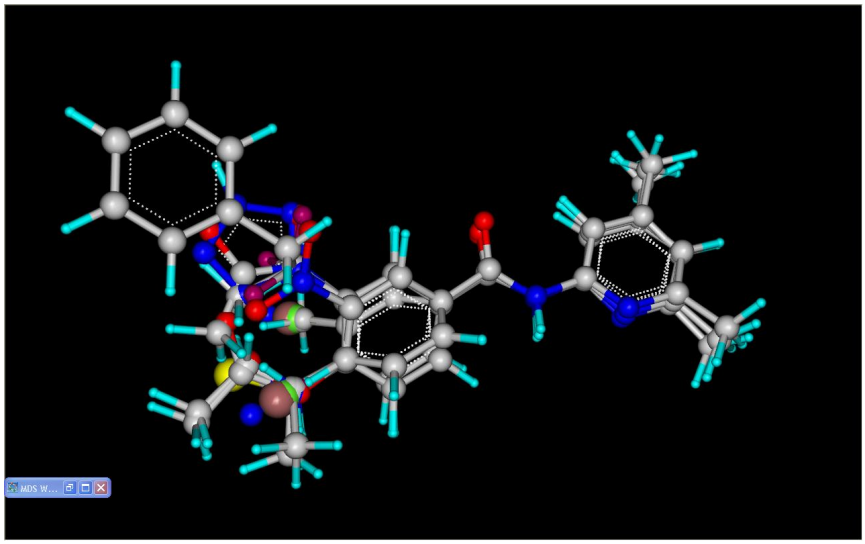


Figure 1: Template based alignment of molecules.

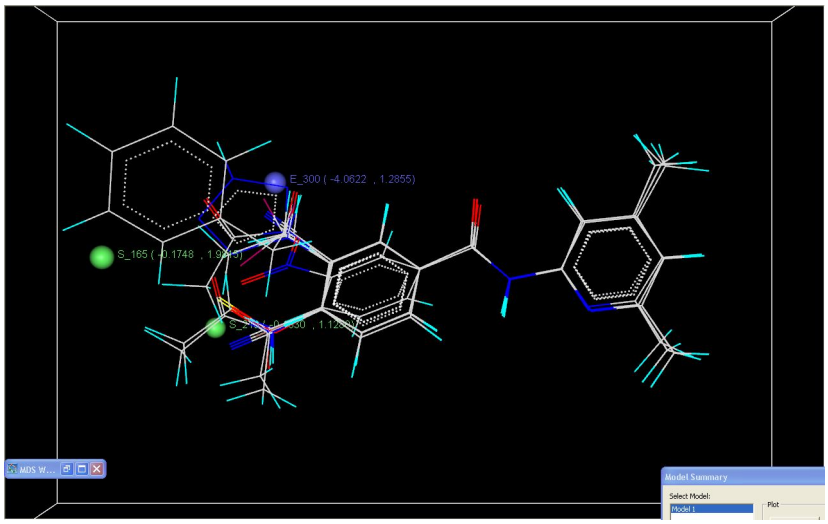


Figure 2: Distribution of chosen points for stepwise forward backward kNN MFA analysis (Model 1)

RESULT AND DISCUSSION

Amongst all the models obtained Model 1, (Table 2) is is depicted that the kNN MFA model obtained by using the two stepwise forward backward variable selection method shows that electrostatic interactions (2 of 3 descriptors in SW are electrostatic field descriptors) plays major role in determining biological activity. It can also be noted that descriptor E_ 300 is implying the significant role of this electrostatic field interaction for structure activity relationship. Statistically SW-kNN MFA triparametric model is comparatively better than the other two with respect to the internal (q^2 = 0. 7962) as well as the external (pred_r^2 = 0. 4015) model validation and correctly predicts activity ~ 80% and ~ 40% for the training and test set respectively. It uses 2 electrostatic field descriptors along with its 3 k nearest neighbor (k = 3) to evaluate the activity of new molecule. The plot of the kNN MFA which shows the relative position and ranges of the corresponding important electrostatic/ steric fields in the model provides the following guidelines for design of new molecule The negative range of electrostatic field indicates that negative electrostatic potential is favorable for increase in the activity and hence a more electronegative substituent group is preferred in that region. The negative range of Steric field indicates that negative steric potential is favorable for increase in the activity and hence less bulky substituent group is preferred in that region. Taking clues from these guidelines and from the developed SW-kNN MFA model field plot and corresponding important electrostatic fields range shows the ranges as shown in Figure 2 are more towards negative side, meaning increasing electro negativity of the substituent group is favorable at the respective substitution site. This is in line with the fact that most of the active molecules have electrostatic field values in negative range only. The above study suggests that the, electronegative character of the substituents preferably at meta position of benzamide moiety result in significant enhancement of biological activity. It suggests that an inclusion of electronegative groups (at meta position) like - SCH_3, $-CHF_2$, $-CF_2CH_3$, $-CHFCH_2$, affect the electron distribution and will improve pharmacological activity of the molecule.

CONCLUSION

The results obtained in both the kNN MFA and CoMFA methods are confirming that the presence of electronegative groups at the required position are playing a significant role in governing the biological activity in the molecules. The compounds thus designed and predicted by the aforementioned models confirm the inclusion of electronegative and less bulky functional moieties which will result in optimally active molecules with appreciable anti-inflammatory activity.

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