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3D QSAR STUDIES IN CONJUNCTION WITH k-NEAREST NEIGHBOR MOLECULAR FIELD ANALYSIS (k-NN-MFA) ON A SERIES OF SUBSTITUTED 2-PHENYL-BENZIMIDAZOLE DERIVATIVES AS AN ANTI ALLERGIC AGENTS

NAVIN RAJ*, SANMATI K. JAIN
SLT Institute of Pharmaceutical Sciences, GGU, Bilaspur- 495009,Chhatisgarh, India

A set of forty one substituted 2-phenyl-benzimidazole with anti allergic activity against IgE was subjected to three dimensional quantitative structure activity relationship studies through recently introduced k- nearest neighbor molecular field analysis with step wise forward-backward as variable selection method to study the correlation between the molecular properties and the *In-vitro* IgE activities. In the present study k-NN-MFA calculations for both electrostatic and steric field were carried out. The master grid maps derived from the best model has been used to display the contribution of electrostatic potential and steric field. The k-NN-MFA models obtained by using 90 % of training set selection showed that electrostatic and steric interactions play major role in determining biological activity. The statistical results showed significant correlation coefficient r^2 (q^2) of 0.5757, r^2 for external test set (pred_r^2) 0.7238, coefficient of correlation of predicted data set (pred_r^2se) of 0.5799, degree of freedom 33 and k nearest neighbor of 2.The k-NN MFA contour plots provided further understanding of the relationship between the structural features of substituted-2-phenyl-benzimidazole derivatives and their activities, which should be applicable to design new, potential anti allergic agents.

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Keywords: IgE, Anti allergic, substituted-2-phenyl-benzimidazole derivative, k-Nearest Neighbor Molecular Field Analysis (k-NN-MFA).

1. Introduction

Allergic disorders such as rhinitis, sinusitis, atopic dermatitis, asthma, pollenosis and food allergy are most common causes of human diseases [1]. Hypersensitivity of the immune system to a specific antigen (allergens) plays a central role in the initiation of asthma and allergic rhinitis [2]. Key components of this process include Th2 lymphocytes, which are a major cellular infiltrate in asthmatic lung [3] and the antibody, immunoglobulin E (IgE), which is over produced in majority of people who suffer from allergic condition[4].However, numerous other components implicated for controlling IgE response are not always translated to prevent asthma[5-7].

Low affinity receptor for IgE (CD23) has been reported to have direct effects on IgE regulation, antigen presentation and airway hyper responsiveness [8-13]. Interleukin 4 (IL-4) and IL-13 also are required for IgE responses *in-vitro* and *in-vivo*[14-16], and have other putative roles in the development of allergy beside from their direct activation of IgE [17-19]. Degranulation of mast cells caused by antigen-antibody reactions triggers type-I allergic diseases and hypersensitivity of the immune system to a specific antigens like Th2 lymphocytes, IgE , IL-4 and 13 , required for IgE responses plays a central role in the initiation [20-22].Other numerous mediators too have been reported to contribute to the development of allergy and asthma[23-24]. This complexity of allergic pathology impedes our efforts to establish disease control.

*Corresponding author: pharmanavin@rediffmail.com

While there are a number of pharmacological agents available for the treatment of asthma and allergic rhinitis, a major shortcoming of many of these therapeutic alternatives is that they impact the disease state by targeting a single mediator that modifies a response at the target organ. By acting on effecter molecules, these drugs provide some symptomatic relief but do not modulate the course of the disease. Anti-histamines, for example, continue to be the drugs of choice for allergic rhinitis because they are somewhat effective and are linked to few side effects. However, anti-histamines provide little benefit for most cases of asthma, and require chronic dosing to achieve optimal effectiveness in allergic rhinitis.

Leukotriene receptor antagonists have more recently been developed for the treatment of asthma but their focus on a single effecter molecule limits efficacy to a minority of patients [25], hence the search of a therapeutic carrier is needed which affects the multiple mediators of allergic diseases.

Benzimidazole compounds constitute an important class of heterocyclic aromatic organic compounds for their versatile pharmacological activities such as antibacterial, antifungal, antihelmintic,antineoplastic, local analgesic, antihistaminic, vasodilative, hypotensive, spasmolytic [26-27]and antiallergic activities[28]. The main objective of the present study was the search for novel benzimidazole compounds that would show a promise to become useful antiallegic agents.

During the last 20 years quantitative structure activity relationship (QSAR) models have gained an extensive gratitude in physical, organic, analytical, pharmaceutical and medicinal chemistry. The success of the QSAR approach can be explained by the insight offered based on the structural determination of chemical properties, and the possibility to estimate the properties of new chemical compounds without the need to synthesize and test them among the homologous series[29].

Many different approaches to QSAR have been developed over the years. The rapid increase in three-dimensional structural information (3D) of bioorganic molecules, coupled with the development of fast methods for 3D structure alignment (e.g. active analogue approach), has led to the development of 3D structural descriptors and associated 3D QSAR methods. The most popular 3D QSAR methods are comparative molecular field analysis (CoMFA) and comparative molecular similarity analysis (CoMSIA)[30-31].

The CoMFA method involves generation of a common three dimensional lattice around a set of molecules and calculation of the steric and electrostatic interaction energies at the lattice points. The interaction energies are numerically very high when a lattice point is very close to an atom and special care needs to be taken in order to avoid problems arising because of this. The CoMSIA method avoids these problems by using similarity function represented as Gaussian. This information around the molecule is converted into numerical data using the partial least squares (PLS) method that reduces the dimensionality of data by generating components. However, a major disadvantage is that PLS attempts to fit a linear curve among all the points in the data set. Further, the PLS method does not offer scope for improvement in results. It has been observed from several reports that the predictive ability of PLS method is rather poor due to fitting of a linear curve between the available points.

In the case of the CoMSIA method, molecular similarity is evaluated and used instead of molecular field, followed by PLS analysis [32]. Variable selection methods have also been adopted for optimal region selection in 3D QSAR methods and shown to provide improved QSAR models as compared to the original CoMFA technique. These considerations provide an impulsion for the development of fast, generally nonlinear, variable selection methods for performing molecular field analysis.

With the above facts and in continuation of our research for newer antiallergic agents in the present study, we report here the development of a new method (kNN-MFA) that adopts a k-nearest neighbor principle for generating relationships of molecular fields with the experimentally reported activity to provide further insight into the key structural features required to design potential drug candidates of this class. This method utilizes the active analogue principle that lies at the foundation of medicinal chemistry.

2. Computational methods

2.1. Methodology

We hereby report the models, as generated by k-NN-MFA in conjunction with stepwise (SW) forward-backward variable selection methods. In the k-NN-MFA method, several models were generated for the selected members of training and test sets, and the corresponding best models are reported herein. VLife Molecular Design Suite (VLifeMDS), allows user to choose probe, grid size, and grid interval for the generation of descriptors. The variable selection methods along with the corresponding parameters are allowed to be chosen, and optimum models are generated by maximizing q2. k-nearest neighbor molecular field analysis (kNN-MFA) requires suitable alignment of given set of molecules. This is followed by generation of a common rectangular grid around the molecules. The steric,hydrophobic and electrostatic interaction energies are 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 in the following discussion to indicate field values at the lattice points. The optimal training and test sets were generated using the random selection method. This algorithm allows the construction of training sets covering descriptor space occupied by representative points. Once the training and test sets were generated, kNN methodology was applied to the descriptors generated over the grid[32].

2.2 Chemical Data

Forty seven substituted 2-phenyl-benzimidazole derivatives as antiallergic agents were taken from the literature and used for k-NN-MFA analysis[28]. The above reported substituted 2-phenyl-benzimidazole derivatives showed wide variation in their structure and potency profiles. k-NN-MFA (3D QSAR) models were generated for these derivatives using a training set of 36 molecules. Predictive power of the resulting models was evaluated by a test set of 5 molecules with uniformly distributed biological activities. Selection of test set molecules was made by considering the fact that test set molecules represent structural features similar to compounds in the training set. The various substituents of all compounds along with their actual and predicted biological activities are shown in **Table 1**.

2. 3. Biological Activities

The negative logarithm of the measured IgE *In-vitro* IC_{50} (nM) [$pIC_{50} = -\log (IC_{50} \times 10^{-9})$] were used as dependent variable, thus correlating the data linear to the free energy change. Since some compounds exhibited insignificant activity, hence such compounds were excluded from the present study. Hence the study concerned here with forty-one compounds only.

2. 4. Molecular Modeling and Alignment

The molecular modeling was carried out on HCL PC having intel core 2 duo processor and windows XP operating system, using the software namely: Molecular Design Suite supplied by the VLife Sciences, Pune (VLife MDS)[33]. The structures were constructed using the 2D draw application and converted to 3D structures. Energy minimization and geometry optimization was conducted using Merck Molecular Force Field (MMFF) method with Root Mean Square (RMS) gradient set to 0.01 Kcal/mol Å and iteration limit to 10000. Alignment of all the forty-one compounds was done using template based alignment in MDS; the aligned structures were used for the study. In the template based alignment method, a template structure was defined and used as a basis for alignment of a set of molecules. In the present case, alignment was done using benzimidazole nucleus as template (**Fig.1)** for template-based alignment, as it was common to all the structures and most active compound as reference (**Fig.2)**. The alignment of molecules is shown in **Fig.3**.

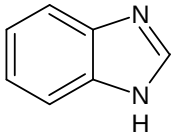

Fig.1. Benzimidazole moiety as a template for alignment.

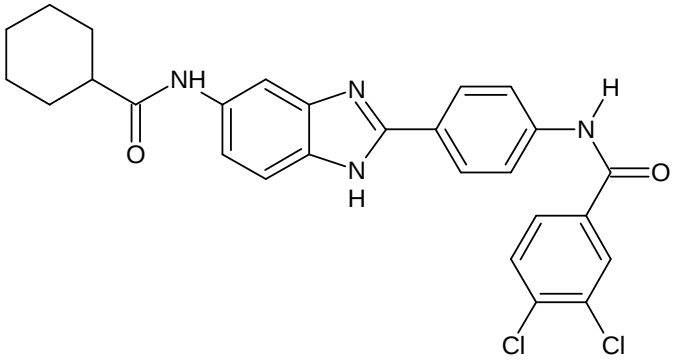

Fig.2. Reference molecule (1gg) used for alignment by template based alignment

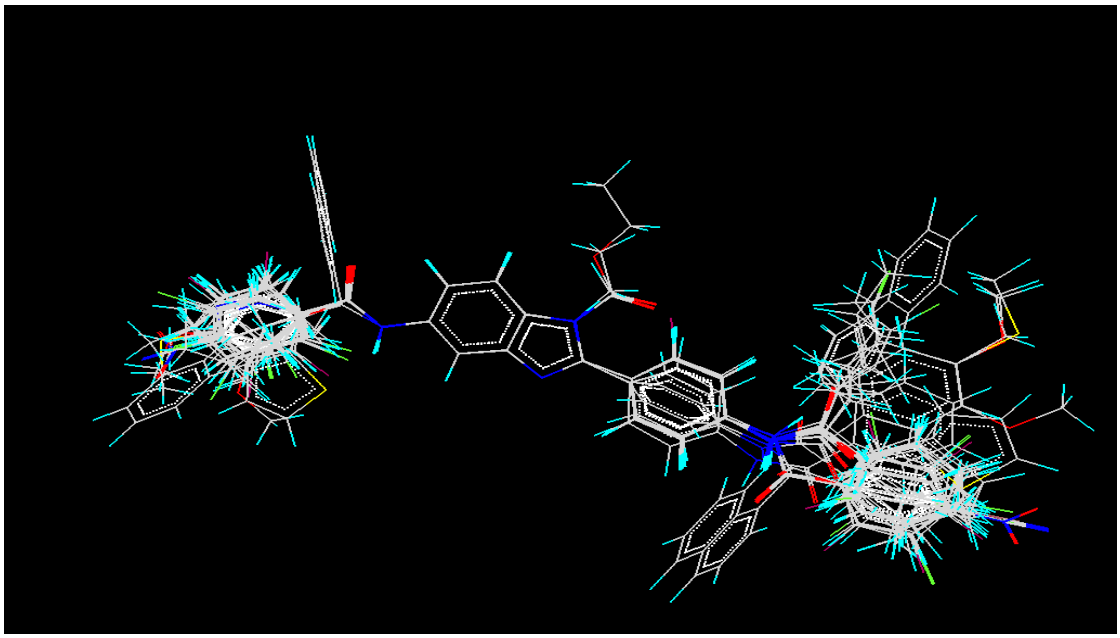

Fig.3. Template based alignment of Molecules.

For calculation of field descriptor values, using Tripos force field, all three electrostatic, hydrophobic and steric field type with cut offs 10.0 and 30.0 Kcal/mol respectively were selected and charge type was selected as Gasteiger – Marsili. Dielectric constant was set to 1.0 considering the distance dependent dielectric function. Probe setting was carbon atom with charge 1.0 and grid setting as follows:

From	To	Interval
X: -16.009	18.5522	2.0000
Y: -11.1832	8.317	2.0000
Z: -9.3227	15.3238	2.0000

This resulted in calculation of 7020 field descriptors (2340 for each electrostatic, hydrophobic and steric) for all the compounds in separate columns. For performing QSAR analysis, all the invariable columns were removed from the work sheet, as they do not contribute to QSAR[34].

2. 6. Selection of Training and Test Set

The dataset of 41 molecules was divided into training and test set by Random selection method from a diverse range of 60% to 90% ,out of which 90% of training set for model 1 and model 2 with pIC_{50} activity field as dependent variable and various 3D descriptors calculated for the compounds as independent variables.

3. Experimental

All the forty one compounds were built on workstation of molecular modelling software VlifeMDS, which is a product Vlife Sciences Pvt Ltd., India[33]. We hereby report the models, as generated by k-NN-MFA in conjunction with stepwise (SW) forward-backward variable selection methods shown in **Table 3**. In the present k-NN-MFA study, (-16.009 18.5522) x (-11.1832 to 8.317) y (-9.3227to 15.3238) z, A^0grid at the interval of 2.00 was generated around the aligned compounds. The hydrophobic, steric and electrostatic interaction energies were computed at the lattice points of the grid using a methyl probe of charge +1 of Gasteiger-Marsili type. These interactions energy values were considered for relationship generation and utilized as descriptors to decide nearness between molecules.

3.1. Building k-NN-MFA Models

Since there was a large pool of descriptors available to build model, stepwise variable selection method was used along with k-nearest neighborhood (k-NN) to find optimal sub-set of descriptors for k-NN-MFA model. The k-NN-MFA models were developed using step wise forward-backward method with cross correlation limit set to 0.5 and term selection to 4.0 and F-test ‘out’ to 3.99. As some additional parameters, variance cut-off was set as 2 Kcal/mol Å and scaling as auto scaling, additionally the k-nearest neighbour parameter setting was done within the range of 2-5 and prediction method was selected as distance based weighted average. The method described above has been implemented in software, Vlife Molecular Design Suite (VlifeMDS)[32], which allows user to choose probe, grid size, and grid interval for the generation of descriptors. The variable selection methods along with the corresponding parameters are allowed to be chosen, and optimum models are generated by maximizing q2.

Steps involved in k-NN-MFA method [32]

Molecules are optimized before alignment optimization is done by MOPAC energy minimization and optimization is necessary process for proper alignment of molecules around template.

1. K-NN-MFA method requires suitable alignment of given set of molecules, alignment are template based.
2. This is followed by generation of common rectangular grid around the molecules; the hydrophobic, steric and electrostatic interaction energies were computed at the lattice points of the grid using a methyl probe of charge +1.
3. The optimal training and test set were generated using random selection method.
4. Model was generated by k-NN method, and models validated internally and externally by leave one out, external validation.
5. Predict the activity of test set of compounds.

Since the final equation is not very useful to represent efficiently the k-NN-MFA models, 3D master grid maps of the best models are displayed. They represent area in space where steric and electrostatic field interactions are responsible for the observed variation of the biological activity.

4. Results and discussion

Training set of 36 and test set of 5 substituted-2-phenyl-benzimidazole derivatives having different substitution were employed. Following statistical parameters were used to correlate biological activity and molecular descriptors: n = number of molecules, Vn = number of descriptors, k = number of nearest neighbor, df = degree of freedom, r^2= coefficient of determination, q^2 = cross validated r^2 (by the leave-one out method), pred_ r^2 = r^2 for external test set, pred_ r^2se = coefficient of correlation of predicted data set. Selecting training and test set by random selection method, the Unicolumn statics was performed which is shown in **Table 2**. The unicolumn statistical analysis can be interpreted that the mean and standard deviation for the training and test sets provide insight into the relative difference in the mean and point density distribution of the two sets. The minimum and maximum values in both the training and test sets are compared such that the maximum of the test set should be less than that of the training set. The minimum of the test set should be greater than that of the training set, suggesting the interpolative behaviour of the test set (i.e., derived within the minimum–maximum range of the training set) which is prerequisite analysis for further QSAR study.

The observed and predicted pIC_{50} values for model 1 are shown in **Table 1.** The plot of observed vs. predicted activity is shown in **Fig.5.** From the plot it can be seen that k-NN-MFA model is able to predict the activity of training set quite well (all points are close to regression line) as well as external.

Table 1. Substituents, Experimental and Predicted Activity of substituted 2-phenyl benzimidazole used in training and test set by model-1. IC_{50} = Compound concentration in nano mole that led to 50% reduction in IgE, $pIC_{50} = -\log (IC_{50} \times 10^{-9})$[values converted from nM to M]: Training and Test data set developed using model 1, T = Test set molecule.

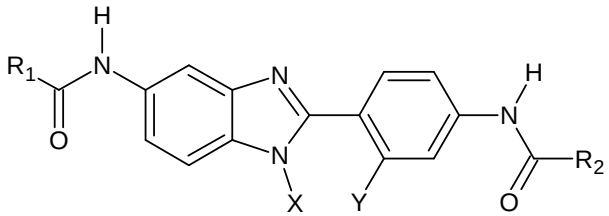

Fig.4. Parent structure of substituted-2-phenyl-benzimidazole derivatives

Compound Name	R1	R2	X	Y	IgE *In-vitro* IC50 (nM)	IgE *In- vitro* IC50 (M)	
						Experimental activity	Predicted activity

Compound Name	R1	R2	X	Y	IgE *In-vitro* IC50 (nM)	IgE *In- vitro* IC50 (M)	
						Experimental activity	Predicted activity
1a	Phenyl	Phenyl	H	H	20	7.698970	7.38638
1b	4-bromophenyl	4-bromophenyl	H	H	200	6.698970	6.71295
1e	3-cholorophenyl	3-cholorophenyl	H	H	25	7.602060	7.42236
1f	2-cholorophenyl	2-cholorophenyl	H	H	45	7.346780	7.84218
1g	3,4-di-cholorophenyl	3,4-di-cholorophenyl	H	H	40	7.397940	7.55046
1h	2,3-di-cholorophenyl	2,3-di-cholorophenyl	H	H	10	8.00000	8.309
1i	3,5-di-cholorophenyl	3,5-di-cholorophenyl	H	H	70	7.154900	7.64799
1j(T)	2,4-di-cholorophenyl	2,4-di-cholorophenyl	H	H	30	7.522870	6.8733
1k	2,6-di-cholorophenyl	2,6-di-cholorophenyl	H	H	400	6.397940	7.68033
1m	Penta -fluoro-phenyl	Penta -fluoro-phenyl	H	H	4	8.397940	7.77429
1n	Phenyl	4-choloro phenyl	H	H	90	7.045750	7.02779
1o	4-nitro phenyl	4-nitro phenyl	H	H	150	6.823900	7.00843
1q	4-Cyanophenyl	4-Cyano phenyl	H	H	100	7.000000	6.83339
1r	4-Methoxyphenyl	4-Methoxyphenyl	H	H	30	7.522870	7.82023
1s	3,5-Methoxyphenyl	3,5-Methoxyphenyl	H	H	700	6.154900	6.37188
1v	4-S-Methyl-Phenyl	4-S-Methyl-Phenyl	H	H	150	6.823900	7.61186
1w	4-Methyl Phenyl	4-Methyl Phenyl	H	H	20	7.698970	7.14843
1y	1-Naphthalene	1-Naphthalene	H	H	80	7.096910	7.0000
1z	CH2-2-thiophene	CH2-2-thiophene	H	H	500	6.301030	6.89056
1aa	Cyclo-hex-3-ene	Cyclo-hex-3-ene	H	H	40	7.397940	6.45345
1cc	Phenyl	Cyclohexyl	H	H	10	8.000000	8.45201
1dd	CH3	Cyclohexyl	H	H	100	7.000000	7.09691
1ee(T)	3,4-dichlorophenyl	Cyclohexyl	H	H	0.8	9.096910	8.30988
1ff	4-Chlorophenyl	Cyclohexyl	H	H	06	8.221840	8.3385
1gg(T)	Cyclohexyl	3,4-dichlorophenyl	H	H	0.4	9.397940	8.61109
1hh(T)	Cyclohexyl	4-Chlorophenyl	H	H	8	8.096910	8.61109
1ii	1-Adamantyl	2-fluorophenyl	H	H	10	8.000000	7.49638
1jj	1-Adamantyl	4-fluorophenyl	H	H	10	8.000000	7.92383
1kk	2-Pyridyl	1-Adamantyl	H	H	06	8.221840	8.19813
1ll	3-Pyridyl	1-Adamantyl	H	H	20	7.698970	8.2023

Compound Name	R1	R2	X	Y	IgE *In-vitro* IC50 (nM)	IgE *In- vitro* IC50 (M)	
						Experimental activity	Predicted activity
1mm	Cyclohexyl	Cyclohexyl	H	H	4	8.397940	8.11158
1nn	1-Adamantyl	1-Adamantyl	H	H	4	8.397940	7.84826
1oo	Cycloheptyl	Cycloheptyl	H	H	1.5	8.823900	7.78798
1qq	Cyclobutyl	Cyclobutyl	H	H	400	6.397940	6.58576
1rr	Cyclopropyl	Cyclopropyl	H	H	1000	6.000000	6.77284
1ss	4-methyl-cylcohexyl	4-methyl-cylcohexyl	H	H	4	8.397940	7.99696
1vv	Cinnamyl	Cinnamyl	H	H	70	7.154900	7.3888
1xx	Phenyl	Phenyl	CH3	H	800	6.096910	6.87683
1yy	Cyclohexyl	Cyclohexyl	COOCH2CH3	H	7	8.154900	8.55255
1zz	Cyclohexyl	Cyclohexyl	COCH3	H	1.5	8.823900	8.11095
1aaa(T)	Cyclohexyl	Cyclohexyl	H	2-F	2	8.698970	8.27301

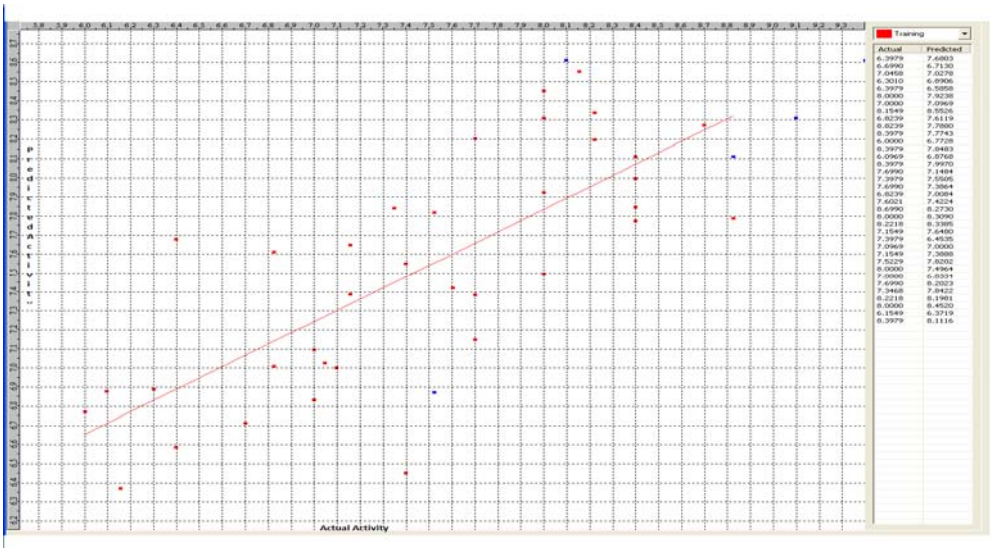

Fig 5. Graph of Actual vs. Predicted activities for training and test set molecules from the k-NN-MFA model 1, A) Training set (Red dots) B) Test set (Blue dots)

Table 2. Unicolumn Statics of Training and Test Sets.

Unicolumn statics	Average	Max	Min	Std. Deviation
For Training Set	7.6011	9.3979	6.0000	0.8109
For Test Set	7.5046	8.8239	6.0969	1.2744

During the k-NN-MFA investigation, selection of training and test set from 60 % to 90 % were investigated. The 90 % selection produced a significant result as compare to others. The k-

NN MFA for substituted-2-phenyl-benzimidazole derivatives using SW variable selection methods resulted in several statistically significant models, of which corresponding best models are reported herein.

The model selection criteria being the value of q^2, the internal predictive ability of the model, and that of pred_ r^2, the ability of model to predict the activity of external test set. For activity against IgE, model 1 generated with SW variable selection method, found to be statistically most significant especially with respect to external predictive ability. The model showed internal Predictive ability of about 58 % (q^2 =0.5757) and external predictive ability of about 70 %(pred_ r^2 =0.7238). Another statistically significant model 2 was obtained for antiallergic activity against IgE, through SW k-NN MFA justified by internal and external predictive ability of the model as 59% (q^2 =0.5923) and 51% (pred_ r^2=0.5127) respectively. The statistical results are depicted in **Table 3.**

Table 3. Stastical results of k-NN-MFA method.

Parameters	Model 1	Model 2
N	36	36
K	2	2
q^2	0.5757	0.5923
pred_ r^2	0.7238	0.5127
pred_ r^2 se	0.5799	0.4102
Descriptors	S_592 30.0000 30.0000	S_1473 -0.4357 -0.2719
	E_2018 -0.5941 -0.5569	E_2018 0.7396 0.7974
Vn	2	2

It is known that the CoMFA method provides significant value in terms of a new molecule design, when contours of the PLS coefficients are visualized for the set of molecules. Similarly, the k-NN-MFA models provide direction for the design of new molecules in a rather convenient way. The points which contribute to the k-NN-MFA model 1 are shown in **Fig. 6**. The range of property values for the chosen points may help in the design of new potent molecules (**Fig. 6**).

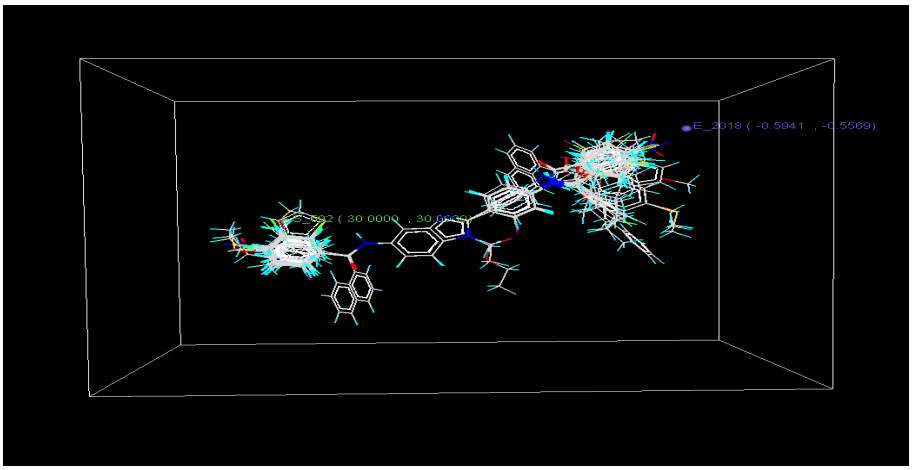

Fig.6. 3D-alignement of molecule with the important steric and electrostatic point Contributing to the model with range of values shown in parenthesis

The k-NN-MFA models obtained by using 90 % of training set selection showed that electrostatic and steric interactions play major role in determining biological activity S_592 in model 1 and S_1473 in model 2 are steric field descriptors similarly E_2018 in model 1 and E_2018 in model 2 are electrostatic field descriptors. Negative value in electrostatic field descriptors indicates that negative electronic potential is required to increase activity and more electronegative substituent group is preferred in that position, positive range indicates that group that imparting positive electrostatic potential is favourable for activity so less electronegative group is preferred in that region. Similarly negative range in steric descriptors indicates that negative steric potential is favourable for activity and less bulky substituent group is preferred in that region, positive value of steric descriptors reveals that positive steric potential is favourable for increase in activity and more bulky group is preferred in that region.

6. Conclusion

The model developed to predict the structural features of substituted-2-phenyl-benzimidazole derivatives against IgE, reveals useful information about the structural features requirement for the molecule. The master grid obtained for the various k-NN-MFA models show that negative value in electrostatic field descriptors indicates the negative electronic potential is required to increase activity and more electronegative substituent group is preferred in that position, positive range indicates that the group which imparts positive electrostatic potential is favourable for activity so less electronegative group is preferred in that region. Negative range in steric descriptors indicates that negative steric potential is favourable for activity and less bulky substituent group is preferred in that region. Positive value of steric descriptors reveals that positive steric potential is favourable for increase in activity and more bulky group is preferred in that region . In both the optimized models, Model 1 is giving very significant results. The developed models also possess promising predictive ability as discerned by testing on the external test set and should be useful to elucidate the relationship between compound structure and biological activities and to facilitate design of more potent and selective anti allergic agents.

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Reference

[1] G.J. Rodrigo, C. Rodrigo, J.B. Hall, Chest **125** (2004).
[2] N.R. Raj, S.K. Jain, C.N. Raj, A.B. Panda, International Journal of Pharmaceutical Sciences and Nanotechnology,**3**,2(2010)
[3] C. Walker, E. Bode, L. Boer, T.T. Hansel, K. Blaser, J.C. Virchow Jr.,Am. Rev. Respir. Dis. **146** (1992).
[4] S. Matsushita, M.L. Richards, D.H. Katz, in: E.B. Weiss, M. Stein (Eds.), Bronchial Asthma, Mechanisms and Therapeutics, third ed, Little, Brown and Company, Boston (1993).
[5] H. Milgrom, R.B. Fick Jr., J.Q. Su, J.D. Reimann, R.K. Bush, M.L. Watrous, et al., N. Engl, J. Med. **341** (1999).
[6] M. Haak-Frendscho, K. Robbins, R. Lyon, R. Shields, J. Hooley, M. Schoenhoff, et al., Immunology **82** (1994).
[7] H. Lin, K.M. Boesel, D.T. Griffith, C. Prussin, B. Foster, F.A. Romero, et al., J. Allergy Clin. Immunol. **113** (2004).
[8] P. Yu, M. Kosco-Vilbois, M.L. Richards, G. Kohler, M.C. Lamers, Nature 369 (1994).
[9] L. Flores-Romo, J. Shields, Y. Humbert, P. Graber, J.P. Aubry, J.F. Gauchat, et al., Science **261** (1993).
[10] A. Saxon, M. Kurbe-Leamer, K. Behle, E.E. Max, K. Zhang, J. Immunol. **147** (1991) .

[11] A. Stief, G. Texido, G. Sansig, H. Eibel, G. Le Gros, H. van der Putten, J. Immunol. **152** (1994).
[12] G. Texido, H. Eibel, G. Le Gros, H. van der Putten, J. Immunol. **153** (1994).
[13] A. Haczku, K. Takeda, E. Hamelmann, A. Oshiba, J. Loader, A. Joetham, et al., Am. J. Respir. Crit. Care Med. **156** (1997).
[14] R.M. Ten, M.J. McKinstry, G.D. Bren, C.V. Paya, J. Allergy Clin. Immunol.**104** (1999).
[15] M.R. Kehry, L.C. Yamashita, Proc. Natl. Acad. Sci. USA **86** (1989).
[16] U. Pirron, T. Schlunck, J.C. Prinz, E.P. Rieber, Eur. J. Immunol. **20**(1990).
[17] E.A. Clark, J.A. Ledbetter, Nature **367** (1994).
[18] L.B. Bacharier, R.S. Geha, J. Allergy Clin. Immunol. **105** (2000).
[19] G. Grunig, M. Warnock, A.E. Wakil, R. Venkayya, F. Brombacher, D.M. Rennick, et al., Science,**282** (1998).
[20] N. Miyahara, K. Takeda, T. Kodama, A. Joetham, C. Taube, J.W. Park, et al., J. Immunol. **172** (2004).
[21] G. Brusselle, J. Kips, G. Joos, H. Bluethmann, R. Pauwels, Am. J. Respir. Cell Mol. Biol. **12** (1995).
[22] D.C. Webb, A.N. McKenzie, A.M. Koskinen, M. Yang, J. Mattes, P.S. Foster, J. Immunol. **165** (2000).
[23] P.J. Barnes, K.F. Chung, C.P. Page, Pharmacol. Rev. **50** (1998).
[24] C. Taube, A. Dakhama, E.W. Gelfand, Int. Arch. Allergy Immunol. **135**(2004).
[25] J. Drazen, Am. J. Respir. Crit. Care Med. **157** (1998).
[26] Saleem K., Khan S.A., Singh N., Eur. J. Med Chem.(2007).
[27] Block H., Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Eleventh edition, Lippincott-Raven Publisher.(2004).
[28] Mark L. Richards, Shirley Cruz Lio, Anjana Sinha, Homayon Banie, Richard J. Thomas, Michael Major, et al., European Journal of Medicinal Chemistry, **41** (2006).
[29] O. Ivanciuc, T. Ivancicu, A.T. Balaban, Internet Electron. J. Mol. Des. **1**(2002).
[30] R. D. Cramer, D. E. Patterson, J. D. Bunce, J Am Chem Soc, **110**, 5959 (1988).
[31] G. Klebe, U. Abraham, T. Mietzner, J Med Chem, **37**, 24 (1994).
[32] Malleshappan. Noolvi,H.M.Patel,Navdeep Singh Sethi, Digest Journal of Nanomaterials and Biostructures ,**5**,2(2010).
[33] VLifeMDS 3.5; Molecular Design Suite, Vlife Sciences Technologies Pvt. Ltd., Pune, India (2004), www.vlifesciences.com
[34] Joohee Pradhan, Rajesh Sharma, Anju Goyal, International Journal of Pharma Research and Development – Online,**2**,6(2010).