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Receptor Surface Analysis of Some Antiinflammatory Benzimidazole Derivatives

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The information generated from three dimensional hypothetical receptor surface models built around template molecules has been used to produce highly predictive quantitative structure-activity relationship (QSAR) models for two series of benzimidazole derivatives with antiinflammatory activity. Molecule/receptor model interaction energies on thousands of points on the receptor surface served as input for the calculation of a QSAR relationship. Both electrostatic and van der Waals interaction energies have been used. A genetic algorithm has been used to search significant models. The QSAR models are significant comparable to those produced from using conventional physicochemical descriptors. The receptor surface models generated using high resolution computer graphics are visually intuitive and can be used for activity prediction of new candidate structures.

In the absence of information about three-dimensional structure of the protein active site to which a particular set of compounds can bind, one can attempt to build a hypothetical model of the receptor site that can provide insight about receptor site characteristics. This is known as a receptor site model[1,2]. This model must be deduced primarily from a set of known active molecules. The creation of receptor site models relies on the assumption of an underlying complementarity between the shape and properties of the receptor and the compounds than can bind. Ideally, the model should be predictive when evaluating new compounds and provide the medicinal chemist direction in the design of novel compounds.

In the present paper, a special type of receptor site model, called a receptor surface model[3,4] has been used to generate QSAR models for some antiinflammatory benzimidazole derivatives. A receptor surface model represents essential information about the hypothetical receptor site as a three-dimensional surface with associated properties mapped onto the surface model. The location and shape of the surface represent information about the steric nature of the receptor site; the associated properties represent other information of interest such as hydrophobicity, partial charge, electrostatic potential and hydrogen bonding propensity. Receptor surface models are simple to understand, easy to visualize and display, convey important information in an intuitive manner and can provide predictive capability for evaluating new compounds. The surface is represented internally as a set of points organized in a triangle mesh. The associated property data is stored with the points which comprise the mesh.

More recently, QSAR has been extended by including the analysis of three-dimensional information about the series, either through rectangular grid based data such as the comparative molecular field analysis (CoMFA) approach[5], or by three-dimensional shape descriptors, as illustrated by molecular shape analysis (MSA) approach[6]. In the present work, molecule/receptor model interaction energies mapped at thousands of points on the receptor surface model has been used as input for the calculation of a QSAR relationship. This methodol-

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ogy is called receptor surface analysis. The receptor surface is better able to sample the environment of the molecule than a rectangular grid, leading to better results[4].

In QSAR, generally, multiple linear regression (MLR) is used to combine different descriptors in the data set, MLR has proven difficult or impossible to use for data set that contained large number of descriptors. In the present work, an advanced methodology called genetic function approximation (GFA) analysis[7] that uses a genetic algorithm[8], has been used for analysis of the data set containing thousands of molecule/receptor model interaction energies to find an appropriate subset of descriptors, which are fitted in turn with MLR.

Many regression techniques develop a single model or a relatively small number of models. In contrast GFA develops a population of many models. The population is evolved by repeatedly performing the genetic crossover operation to recombine the terms of the better performing models. Upon completion, one typically selects the model from the population with the best score.

In search of novel non-acidic antiinflammatory compounds, we have been studying the QSARs of antiinflammatory benzimidazole derivative and in one of our previous papers we have reported QSAR of two series of benzimidazole derivatives (2-(substituted pyridinyl)benzimidazoles and 1H-benzimidazoles) using various physicochemical descriptors and stepwise multiple regression method[9]. The models obtained were not promising enough to be used for activity prediction of new designed compounds. Therefore, it was thought worthwhile to subject these two series of benzimidazole derivatives to receptor surface analysis to obtain QSAR models which can be used for activity prediction of new molecules.

EXPERIMENTAL

The antiinflammatory data of 2-(substituted pyridinyl) benzimidazoles (Series A, Table 1, Fig. 1) and 1H-benzimidazoles (Series B, Table 2, Fig. 1) were taken from Tsukamoto *et al.*[10] and Evans *et al.*[11] respectively. All the biological activity data (BioAct) has been converted to logarithmic equieffective molar doses (LogBA) for QSAR analysis. The software Cerius2 (Biosym/MSI)[12] installed on a Silicon Graphics Workstation has been used to perform all molecular modeling functions including receptor surface analysis and GFA.

Receptor surface analysis:

Generation of the receptor : According to the technique described by Hahn[3], two separate hypothetical receptor surface models were generated for Series A and Series B. Following general steps were followed:

Selection of the templates: Some of the most active compounds of series A and Series B were selected as templates to build the receptor model. For receptor model of Series A, Compound no. 5, 6, 7, 14, 17, 19, 22, 23, 24, 26, 27, 34, 36 and 39 and for receptor model of Series B, Compound no. 4, 5 and 9 were selected for this purpose.

Conformational analysis: All the compounds including the templates of both the series were subjected to conformational analysis using Boltzmann jump method and the most stable conformation was stored for further operations.

Alignment of the templates: The selected templates were aligned on the biologically most active molecule (Compound no. 27 for Series A and Compound no. 5 for Series B) using the flexible root mean square (RMS) alignment method. During the flexible alignment, the target molecule is fixed and the moving molecule is rotated and translated, additionally, the conformation of the molecule is varied to minimize the RMS over the distances between the matched atoms.

Building the receptor model: After the alignment of the templates the receptor surface was built around the templates using van der Waals field function and Marching Cubes algorithm[13]. The grid spacing during the generation of the receptor model was adjusted to be 0.50 Å and receptor's tightness of fit (surface fit) was adjusted at 0.10 Å. The transparency of the receptor surface model was adjusted at 50%. The receptor models for Series A and Series B are shown in Fig. 2 and Fig. 3 respectively.

Property mapping: Electrostatic potential, charge, hydrogen bonding propensity and hydrophobicity characteristics were mapped on the receptor surface one at a time. Property maps were displayed as color regions of the receptor surface. These properties reflect the anticipated characteristics of the receptor that is being modeled. The intensity of the color reflects the magnitude of the mapped property at a particular location.

Calculation of receptor/ligand interaction energies: All the molecules of Series A and Series B were energy minimized inside their respective receptor models. Due to this minimization step, molecules adopt a conforma-

TABLE 1 : SUBSTITUENTS AND ANTIINFLAMMATORY ACTIVITY FOR 2-(SUBSTITUTED PYRIDINYL) BENZIMIDAZOLES

No.	R1	R2	BioAct+	BA++	LogBA
1	H	H	22	0.0429	-1.3671
2	H	3-Me	17	0.0356	-1.4489
3	H	4-Me	-4	-0.0084	—
4	H	5-Me	19	0.0398	-1.4006
5	H	6-Me	44	0.0921	-1.0359
6	H	5-Et	44	0.0982	-1.0077
7	H	6-Et	41	0.0915	-1.0383
8	H	5-n-Bu	-16	-0.0402	—
9	H	5-CH_2OH	-10	-0.0225	—
10	H	6-CH_2OH	-13	-0.0293	—
11	H	5-COOEt	-16	-0.0428	—
12	H	6-COOEt	31	0.0829	-1.0817
13	H	6-Cl	30	0.0689	-1.1618
14	H	6-OMe	47	0.1059	-0.9752
15	H	6-$CONH_2$	13	0.0309	-1.5090
16	H	6-OH	-7	-0.0148	—
17	Me	H	35	0.0732	-1.1353
18	OMe	H	34	0.0766	-1.1159
19	Cl	H	36	0.0827	-1.0826
20	Me	5-Me	7	0.0156	-1.8061
21	Cl	5-Me	-5	-0.0122	—
22	Me	6-Me	42	0.0938	-1.0279
23	OMe	6-Me	40	0.0957	-1.0190
24	Cl	6-Me	39	0.0950	-1.0221
25	OH	6-Me	6	0.0135	-1.8692
26	Me	5-Et	35	0.0831	-1.0806
27	OMe	5-Et	58	0.1469	-0.8329
28	Cl	5-Et	-2	-0.0052	—
29	OH	5-Et	25	0.0598	-1.2232
30	NO_2	5-Et	-5	-0.0134	—
31	NH_2	5-Et	25	0.0596	-1.2249
32	NHAc	5-Et	5	0.0140	-1.8534
33	Me	6-Et	10	0.0237	-1.6247
34	OMe	6-Et	52	0.1317	-0.8804
35	Cl	6-Et	3	0.0077	-2.1117
36	H	5,6$(Me)_2$	37	0.0826	-1.0829
37	Me	5,6$(Me)_2$	-19	-0.0451	—
38	Me	6-OMe	32	0.0766	-1.1159
39	Me	6-Cl	36	0.0877	-1.0569
40	Me	6-OH	-6	-0.0135	—

+ Percent inhibition of paw edema by 100.0 mg/kg of drug

++ Percent paw edema inhibition per micromolecule of drug per kg of body weight

TABLE 2 : SUBSTITUENTS AND ANTIINFLAMMATORY ACTIVITY FOR 1H-BENZIMIDAZOLE (SERIES B)

No.	R1	R2	R3	BioAct+	BA++	LogBA
1	H	4-ClC_6H_4	5(6)-MeO	2	0.0157	-1.8037
2	H	4-ClC_6H_4	5(6)-OH	4	0.0296	-1.5293
3	Me	4-ClC_6H_4-N⟨ ⟩	5-O-$(CH_2)_2$-N	6	0.0671	-1.1731
4	Me	4-ClC_6H_4	5-CH(OMe)Me	33	0.3006	-0.5220
5	C_6H_5	4-ClC_6H_4	6-O-$(CH_2)_2NEt_2$	43	0.5474	-0.2617
6	C_6H_5	4- ClC_6H_4	6-NH-$(CH_2)_2OH$	10	0.1098	-0.9593
7	C_6H_5	4- ClC_6H_4	6-NH-$(CH_2)_3Me$	2	0.0229	-1.6408
8	C_6H_5	4- ClC_6H_4	6-N⟨ ⟩N	0	0.0001*	-4.0000
9	C_6H_5	4-ClC_6H_4	5-CH(NHEt)Me	21	0.2209	-0.6558

+ Percentage improvement in the joint of the paw compared to controls by 33.0 mg/kg of drug
++ Percent improvement in the joint of the paw per micromolecule of drug per kg of body weight
* Converted to a very low value for log conversion

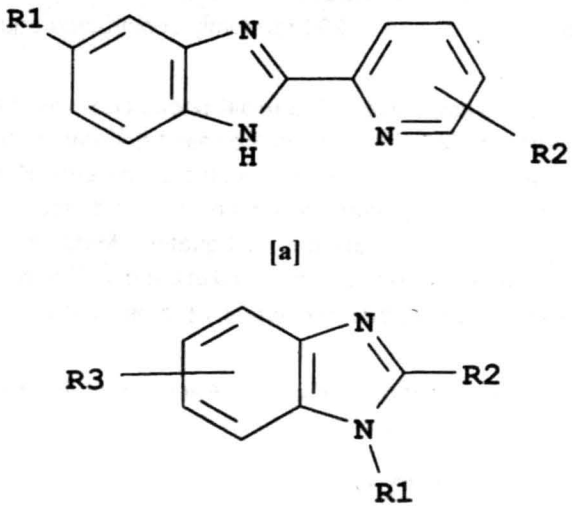


Fig. 1 : 2-(Substituted pyridinyl) benzimidazoles [a] 1H-benzimidazoles [b] used in the study

tion which is in accordance with the receptor surface. The receptor models of Series A and Series B contain 13 332 and 18 564 surface points respectively. On each surface point, van der Waals and electrostatic interaction energies between the surface and the each ligand

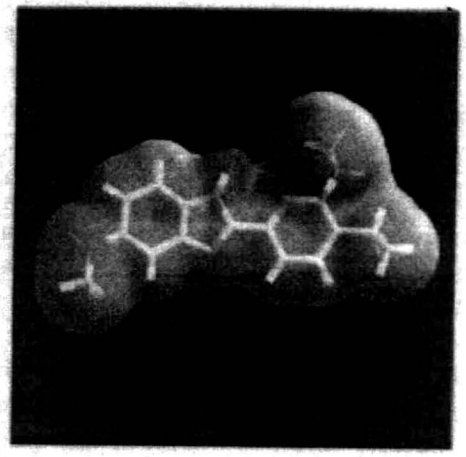


Fig. 2 : Receptor surface model for Series A (shown as a translucent surface around the template molecules)

molecule were calculated. The van der Waals interaction energy was calculated using the following formula[3]:

$$E_{(vdw)} = K((RA/r)^{12}-2D(RA/r)^6) \quad [1]$$

$$RA=VDWrC_h \quad [2]$$

RA is the hybridization corrected van der Waals radius for the atom, r is the distance between the atom and the surface point (the radius of the surface point is

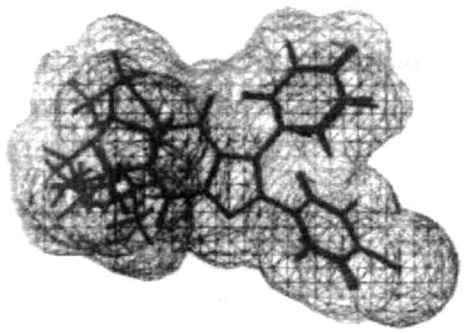


Fig. 3 : Receptor surface model for Series B (shown as a wire frame model around the template molecules)

implicitly zero), K is the well depth constant and is set to 0.1 for all van der Waals atom/point interactions and D is an empirically derived point density scaling factor, which scales the van der Waals energy and forces so that ideal atom/surface interactions yield a van der Waals value of 0.0125 kcal/Å^2 of surface contact. D is set to 0.01 for the grid resolution of 0.5 Å and surface point density of 6 points/Å^2.

The electrostatic term is a monopole-monopole Coulombic function and is calculated using the following equation[3]:

$$E_{(ele)} = (322.1\ Q_A Q_P/r)DS(r) \quad [3]$$

r is the separation between the atom and the surface, Q_A is the partial atomic charge of the atom, and Q_P is the charge at the surface point. The point charge can be obtained from either the partial charge associated with point or the electrostatic potential value associated with point. D is same point density correction factor used in the van der Waals calculation and S(r) is an atom based switching function :

$$S(r) = (r^2_{off}-r^2)^2(r^2_{off}+2r^2-3r^2_{on})/(r^2_{off}-r^2_{on})^3 \quad [4]$$

for $r_{on} < r < r_{off}$

$S(r) = 1$ for $r < r_{on}$

$r_{on} = 7$ Å and $r_{off} = 8$ Å

This cubic function yields a continuous potential energy and force.

Calculation of interaction energies on each surface point has resulted in 26 664 column entries for Series A and 37 128 column entries for Series B which was large enough to overload the memory of the computer and therefore every 4th surface point was considered for analysis.

QSAR analysis: In this step all the van der Waals and electrostatic interaction columns and biological activity (LogBA) of Series A and Series B were subjected to QSAR analysis separately to obtain significant QSAR equations. Since in the present case the number of independent variables is huge therefore genetic function approximation (GFA) method was adopted to obtain QSAR models. GFA consists of following steps :

Building the initial population: The analysis begins by building a population of 200 randomly constructed equations.

Evolving the population: The initial population was then evolved for 50000 generations. For each generation, two better scoring equation were selected as parents. Parts of each parent equation were then used and crossover was performed to create a child equation. Mutation operations were performed on the child at creation. The worst rated equation was then replaced by the new child equation.

Evaluating the QSAR equations: Each equation of the

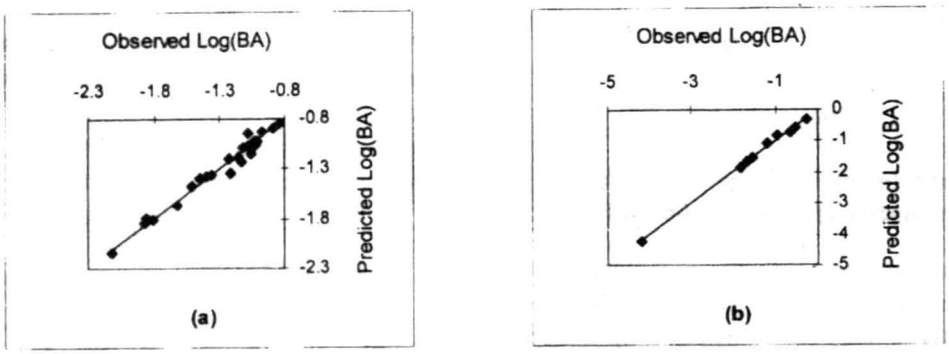
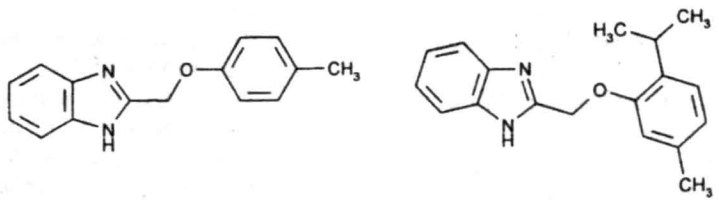


Fig. 4 : Plot of predicted vs. observed LogBA for (a) Equation [5] and (b) Equation [7]



I II

Fig. 5 : Structures of two new benzimidazoles designed using receptor surface analysis and shown comparable antiinflammatory activity as compound no. 27 after synthesis

evolved population was then evaluated using various statistical measures such as number of data points (n), correlation coefficient (r), squared correlation coefficient (r^2), cross-validated r^2 (cvr^2), bootstrap r^2 (bsr^2), F-test (F), standard deviation (std), squared correlation coefficient (r^2) and predicted sum of squares (PRESS).

RESULTS AND DISCUSSION

In 2-(substituted pyridinyl) benzimidazoles[10] (Series A) all the interaction energies and LogBA for this series was subjected to GFA analysis and the following equation was obtained as the best equation in the population of 200 equations:

LogBA = +(33.321017)1640_vdw + (131.581898) 1676_ele + (-46.498375) 4492_vdw + (76.640386) 4648_ele + (26.450969) 8044_vdw + (-50.363588) 13012_vdw + (-0.859082) [5]

n=29, r^2=0.975, r=0.987, press=0.134, cvr^2=0.955, bsr^2=0.975, F=140.502, std=0.059

Where, 1640_vdw, 4492_vdw, 8044_vdw and 13012_vdw are van der Waals interaction energy at 1640th, 4492nd, 8044th and 13012th receptor surface point respectively and 1676_ele, 4648_ele are electrostatic interaction energy at 1676th and 4648th receptor surface points respectively.

In 1H-benzimidazoles[11] (Series B) all the interaction energies and LogBA for this series was subjected to GFA analysis and the following two equations were obtained as best equations in the population of 200 equations:

LogBA = +(-186.299427)336_ele+(-1361.046178)1696_vdw+ (-1.831943) [6]

n=9, r^2=0.976, r=0.988, press=0.710, cvr^2=0.935, bsr^2=0.969, F=120.518, std=0.210

LogBA= +(-220.640345)324_ele +(-218.950601)336_ele + (-1469.472465)1696_vdw+(-2.182661) [7]

n=9, r^2=0.997, r=0.998, Press=0.099, cvr^2=0.991, bsr^2=0.996, F=493.367, std=0.086

Where, 1696_vdw is van der Waals interaction energy at 1696th surface point and 336_ele, 324_ele are electrostatic interaction energy at 336th and 324th surface points respectively.

It can be observed that the overall statistics of these equations are excellent and their prediction ability is also very good which is evident from their cross-validated r^2 values. The predicted vs. observed biological activities using equations [5] and [7] is presented in Fig 4.

These equation and the receptor models generated can be used for antiinflammatory activity predication of new molecules. In our laboratory, these models have been used for designing of new antiinflammatory benzimidazole derivatives. Two of such compounds are shown in Fig. 5. Compound I and compound II has shown an experimental 41 and 55 per cent inhibition of paw edema respectively at a dose of 100 mg/kg, whereas, ibuprofen and mefenamic acid has shown 45 and 56 per cent inhibition, respectively, at the same dose. The predicted paw edema inhibition of compound I and compound II using equation [5] was 75 and 81 respectively.

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REFERENCES

1. Kato, Y., Itai, A. and Iitaka, Y., **Tetrahedron,** 1987, 43, 5229.
2. Doweyko, A.M., **J. Med. Chem.,** 1988, 31, 1396.

3. Hahn, M., **J. Med. Chem.,** 1995, 38, 2080.
4. Hahn, M. and Rogers, D., **J. Med. Chem.,** 1995, 38, 2091.
5. Cramer, R.D., Patterson, D.E. and Bunce, J.D., **J. Am. Chem. Soc.,** 1988, 110, 5959.
6. Hopfinger, A.J. and Burke, B.J., In; Johnson, M.A. and Maggiora, G.M., Eds., Concepts and Applications of Molecular Similarity, John Wiley and Sons, New York, 1990, 11.
7. Rogers, D. and Hopfinger, A.J., **J. Chem. Inf. Comput. Sci.,** 1994, 34, 854.
8. Holland, J.H., In; Adaptation in Natural and Artificial Systems, The University of Michigan Press, Ann Arbor, 1975, 1.
9. Chakravarti, S.K. and Chaturvedi, S.C., **Indian J. Pharm. Sci.,** 1999, 61, 206.
10. Tsukamoto, G., Yoshino, K., Kohno, T., Ohtaka, H., Hajime, K. and Ito, K., **J. Med. Chem.,** 1980, 23, 734.
11. Evans, D., Hicks, T.A., Williamson, W.R.N., Dawson, W., Meacock, S.C.R. and Kitchen, E.A., **Eur. J. Med. Chem.,** 1996, 31, 635.
12. Cerius2, Biosym/Molecular Simulations Inc., 9685 Scranton Road, California, USA 92121, 3752.
13. Lorensen, W.E. and Cline, H.E., **Comput, Graphics,** 1987, 4, 163.