

Experimental and QSAR Studies on Antimicrobial Activity of Benzimidazole Derivatives

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Twenty eight analogues of benzimidazoles had been synthesized and tested for their antimicrobial activity against four bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus pumilus* and *Proteus vulgaris*) and two fungi (*Aspergillus flavus* and *Aspergillus niger*). Compounds with R as C₆H₄NO₂ and R' as SO₂C₆H₄–CH₃(p), with R as C₆H₄OCH₃ and R' as SO₂C₆H₄–CH₃(p), and with R as CH₂C₆H₅ and R' as CH₂(CH₂)₉Cl exhibited comparable or higher antibacterial activity than Ciprofloxacin against *S. aureus* and *E. coli* and, higher activity than Nystatin against *A. flavus*. Several other compounds showed better activity than the standard antibiotic for *E. coli*. Compounds with R as CCl₃ and R' as SO₂C₆H₄–CH₃(p) or COC₆H₅ exhibited the lowest activity against all the organisms. Addition of methylene groups in the R' position increased activity. Many of the compounds showed better activity than Ciprofloxacin for one or more organisms. Compound with R as CH₂OC₆H₅ and R' as CH₂(CH₂)₉Cl exhibited higher activity against both the fungi than the control Nystatin. Quantitative structure activity relationships (QSARs) developed were good for all the organisms ($R^2=0.65$ to 0.88 ; $R^2_{\text{adj}}=0.63$ to 0.86) and the predictive capability of the developed models was also reasonable ($q^2=0.52$ to 0.83). The models had two to three independent variables. The data for the models which had three independent variables were divided into training and test/validation sets. The former set was used to develop the QSAR and these developed models were used to predict the activity of the test set data. In all the three cases the predictive capability of the models was good. The molecular descriptors identified were predominantly log P, electronic parameters, molecular size, shape and area. A positive correlation existed between the antibacterial activity and the first principal component.

Key words benzimidazole; quantitative structure activity relationship; anti-infective activity; electronic descriptor; Log P; Genetic function algorithm

With increase in the incidence of multidrug-resistant gram-positive and gram-negative bacteria it becomes imperative to continuously search for small molecules as anti-infective agents. Benzimidazoles fit this requirement well since they have demonstrated a diverse set of biological activities that include antibacterial, antiamebic, antiviral, antifungal,^{1–4} anthelmintic,⁵ anti-HIV,⁶ antihistaminic,^{7–9} anti-ulcer,^{10,11} cardiotoxic,¹² antihypertensive^{13,14} and neuroleptic.¹⁵ They are also widely in clinical use. Their observed activity depends upon the functional group attached to the moiety. In order to obtain more effective chemotherapeutic agents, a variety of reports have been presented on the synthesis and biological evaluation of new benzimidazoles.¹⁶

A systematic structure based design may help in identifying new benzimidazole analogues with higher activity. Developing quantitative structure activity relationship (QSAR) and using the relationship for designing newer structures have shown reasonable success. QSAR studies on benzimidazole chloroaryloxyalkyl derivatives indicated that their *in vitro* activity against *Salmonella typhi* O-901 and *Staphylococcus aureus* A 15091 depended on three descriptors namely, HOMO energy, hydration energy and number of primary carbon atoms of the molecule.⁴ 2-Aminobenzimidazole moiety connected to the 4-(5) position of an imidazole ring through C₂- or C₃-methylene chains when tested on rat brain membranes exhibited activity and it is found to have a parabolic dependence on Log P.¹⁷ Activities of benzimidazoles against *Bacillus subtilis* also indicated a parabolic relation with Log P. Sener *et al.* have studied the QSAR of antibacterial activity of benzimidazoles against *Klebsiella pneu-*

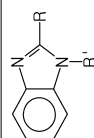
*moniae*¹⁸) and concluded that the more potent compound would possess an oxazolopyridine ring system substituted with an electron withdrawing group at position 5 and benzyl moiety at position 2.¹⁹ They found that the antifungal activity of these compounds against *Candida albicans* highly correlated with LUMO, molecular weight, *R* (resonance effect) and HOMO.²⁰ Oxazolopyridine ring system with the substitution of a benzyl moiety at position 2 exhibited activity against *K. pneumoniae*. Also a group which possessed hydrogen accepting capability improved the activity.²¹ In this paper we report the synthesis of analogues of benzimidazole with a variety of electron withdrawing groups at the *N*–1 position, their antimicrobial activity against four bacterial and two fungal species and the corresponding QSAR studies.

Modelling Methodology The structures of the twenty eight benzimidazoles as listed in Table 1 was drawn and its energy was minimized by using Cerius² software[®] (Accelrys Inc, U.S.A.) with Consistent Valence Force Field (CVFF) force field. This force field, also known as universal force field, is well suited for small organic molecules (not with compounds having metals) and it is parameterized using peptide and protein structures.²² Two hundred and forty nine descriptors or structural features that include topological, charge, geometrical, aromaticity, constitutive, quantum mechanics and thermodynamics were evaluated for all these structures. These descriptors describe the structural features of the molecule. Several literature reports give a very detailed description of these descriptors.^{23–25} Selecting a short set of descriptors to be used in the QSAR from this large pool is always a challenge. Several techniques have been re-

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Table 1. Physical Property and Antimicrobial Activity of the Twenty Eight Compounds at Two Loadings ($\mu\text{g}^{(a)}$)

Compound	R ^(b)	R ^(b)	mp (°C)	Yield (%)	Antibacterial activity						Antifungal activity											
					S. aureus			E. coli			B. pumilus			P. vulgaris			A. flavus			A. niger		
					100	200	100	100	200	100	200	100	200	100	200	100	200	100	200	100	200	
1a	CCl ₃	COC ₆ H ₅	112—114	94	14±0.2	15±0.17	12±0.16	14±0.11	11±0.11	13±0.11	14±0.07	16±0.05	15±0.04	16±0.04	14±0.03	16±0.07						
1b	CCl ₃	SO ₂ C ₆ H ₄ —CH ₃ (p)	226—228	92	14±0.18	16±0.089	13±0.14	14±0.044	10±0.12	12±0.13	16±0.04	17±0.04	10±0.11	12±0.044	12±0.089	14±0.083						
1c	CH ₂ C ₆ H ₅	COC ₆ H ₅	308—310	91	16±0.03	18±0.13	18±0.11	19±0.055	14±0.14	14±0.15	17±0.089	18±0.054	16±0.15	18±0.055	15±0.094	17±0.055						
1d	CH ₂ C ₆ H ₅	SO ₂ C ₆ H ₄ —CH ₃ (p)	118—120	90	17±0.054	17±0.13	19±0.14	21±0.071	11±0.032	12±0.15	14±0.084	16±0.071	19±0.15	20±0.063	12±0.077	14±0.054						
1e	CH ₂ OC ₆ H ₅	COC ₆ H ₅	284—286	88	16±0.21	18±0.094	18±0.18	20±0.055	14±0.071	15±0.14	16±0.089	17±0.063	20±0.11	21±0.031	10±0.089	12±0.071						
1f	CH ₂ OC ₆ H ₅	SO ₂ C ₆ H ₄ —CH ₃ (p)	193—195	95	14±0.19	15±0.16	18±0.11	21±0.031	11±0.077	13±0.15	14±0.11	16±0.055	16±0.071	18±0.077	12±0.083	14±0.063						
2a	C ₆ H ₅	COC ₆ H ₅	206—208	88	14±0.184	15±0.187	17±0.10	18±0.114	14±0.084	14±0.032	16±0.095	17±0.045	16±0.11	17±0.032	14±0.055	15±0.071						
2b	C ₆ H ₄ NO ₂	SO ₂ C ₆ H ₄ —CH ₃ (p)	252—256	86	21±0.032	22±0.00	20±0.095	21±0.095	18±0.032	19±0.141	18±0.084	19±0.145	19±0.145	20±0.055	12±0.055	13±0.055						
2c	C ₆ H ₄ NH ₂	COC ₆ H ₅	236—237	78	17±0.141	18±0.110	19±0.077	19±0.084	16±0.145	17±0.148	16±0.118	18±0.179	14±0.148	17±0.063	10±0.055	12±0.071						
2d	C ₆ H ₄ CH ₃	SO ₂ C ₆ H ₄ —CH ₃ (p)	221—223	72	18±0.110	19±0.100	18±0.032	20±0.032	15±0.152	16±0.145	15±0.045	16±0.176	18±0.141	19±0.145	14±0.084	16±0.077						
2e	C ₆ H ₄ OH	COC ₆ H ₅	245—247	89	16±0.148	16±0.138	18±0.071	19±0.032	14±0.032	16±0.141	17±0.141	18±0.145	15±0.130	16±0.089	13±0.089	13±0.063						
2f	C ₆ H ₄ OCH ₃	SO ₂ C ₆ H ₄ —CH ₃ (p)	215—217	91	20±0.158	20±0.084	19±0.045	22±0.100	15±0.155	17±0.045	17±0.110	19±0.148	17±0.105	19±0.145	12±0.095	13±0.063						
3a	CH ₂ OC ₆ H ₅	CH ₂ C ₆ H ₅	220—222	86	15±0.230	16±0.045	12±0.089	12±0.095	14±0.179	20±0.105	14±0.176	22±0.152	13±0.095	13±0.084	14±0.071	14±0.114						
3b	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₃ Cl	235—237	81	16±0.205	16±0.063	13±0.158	14±0.095	15±0.110	21±0.176	16±0.084	22±0.148	13±0.145	14±0.045	14±0.089	14±0.077						
3c	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₄ Cl	239—241	78	16±0.145	17±0.071	14±0.084	16±0.089	16±0.032	21±0.148	17±0.176	22±0.045	14±0.084	15±0.110	15±0.077	16±0.114						
3d	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₅ Cl	248—249	90	16±0.145	16±0.118	15±0.089	17±0.071	17±0.134	22±0.118	18±0.063	22±0.095	14±0.089	16±0.095	16±0.105	17±0.138						
3e	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₆ Cl	255—257	84	17±0.152	17±0.110	16±0.100	17±0.077	18±0.118	22±0.114	16±0.071	23±0.126	14±0.084	15±0.084	16±0.110	16±0.134						
3f	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₇ Cl	262—264	88	17±0.122	18±0.141	16±0.089	18±0.032	20±0.148	22±0.045	16±0.110	24±0.134	18±0.114	19±0.110	15±0.114	18±0.105						
3g	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₈ Cl	269—271	79	18±0.100	18±0.077	15±0.130	18±0.055	21±0.071	22±0.063	18±0.114	24±0.130	14±0.110	16±0.126	19±0.130	22±0.134						
3h	CH ₂ OC ₆ H ₅	CH ₂ (CH ₂) ₉ Cl	278—280	92	18±0.114	20±0.105	16±0.095	18±0.032	21±0.032	22±0.055	18±0.130	25±0.138	18±0.122	19±0.134	20±0.126	23±0.134						
4a	CH ₂ C ₆ H ₅	CH ₂ CH ₂ CH ₂ Cl	208—210	91	15±0.170	16±0.138	13±0.105	14±0.105	14±0.138	16±0.045	16±0.134	18±0.114	09±0.352	10±0.232	11±0.134	12±0.045						
4b	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₃ Cl	218—220	82	14±0.458	16±0.122	16±0.130	17±0.032	13±0.141	15±0.141	17±0.130	18±0.138	12±0.045	13±0.110	12±0.130	14±0.118						
4c	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₄ Cl	234—236	85	15±0.138	16±0.100	14±0.110	15±0.100	14±0.095	15±0.138	17±0.089	18±0.055	12±0.084	13±0.138	13±0.138	14±0.110						
4d	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₅ Cl	243—245	90	16±0.179	16±0.095	17±0.084	18±0.045	15±0.089	16±0.110	16±0.130	16±0.045	18±0.138	19±0.000	14±0.141	15±0.089						
4e	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₆ Cl	256—258	87	17±0.141	17±0.055	16±0.10	17±0.045	17±0.032	17±0.063	18±0.045	21±0.063	15±0.071	14±0.077	0.077	0.077						
4f	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₇ Cl	267—269	96	16±0.032	18±0.063	18±0.032	20±0.032	14±0.032	18±0.084	17±0.032	21±0.032	14±0.110	15±0.032	15±0.179	15±0.110						
4g	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₈ Cl	278—280	85	20±0.045	21±0.032	19±0.055	21±0.032	16±0.089	23±0.045	17±0.084	22±0.032	14±0.114	16±0.055	15±0.176	17±0.045						
4h	CH ₂ C ₆ H ₅	CH ₂ (CH ₂) ₉ Cl	296—298	86	18±0.032	20±0.055	16±0.084	20±0.032	20±0.045	23±0.045	19±0.045	26±0.032	17±0.10	18±0.045	18±0.161	20±0.055						
Ciprofloxacin					19±0.031	0±0.044	17±0.045	20±0.031	22±0.055	24±0.044	20±0.032	28±0.055	16±0.045	17±0.032	20±0.063	22±0.063						
Nystatin					—	—	—	—	—	—	—	—	—	—	—	—						
Control					—	—	—	—	—	—	—	—	—	—	—	—						



^{a)} Average value of diameter of the inhibition zone in mm—determined by disc diffusion method. ^{b)} mp, melting point.

ported in the literature for performing this task. A genetic algorithm technique was used to select the descriptors from this large pool that would give the best QSAR model. The genetic function algorithm performs a search over the space of possible QSAR models using the lack of fit (LOF=difference between the predicted and the actual activity values) score to estimate the fitness of each model. Lack of fit indicates a difference between the model predictions and actual values. Such evolution of a population of randomly constructed models leads to the discovery of highly predictive QSAR. The populations of the models are created by evolving random initial models using a genetic algorithm.²⁶⁾ This approach can build models using not only linear polynomials but also higher-order polynomials, and other nonlinear functions. The QSAR is nothing but a multiple regression containing these selected descriptors as the independent variables and the activity as the dependent variable. The goodness of the regression fits were estimated using parameters such as, R^2 ($=1-\text{SSE}/\text{TSS}$), R^2_{adj} ($=1-(n-1)(1-R^2)/(n-p-1)$), q^2 ($=1-\text{PRESS}/\text{TSS}$), and F ratio ($=(n-2)R^2/(1-R^2)$) where, TSS=total sum of squares, PRESS=predictive sum of squares based on leave-one-out method.²⁷⁾ In the leave-one-out method, regression relation is built by leaving one data point at a time. The model thus developed is used to determine the activity of the data that was left out and the square of the difference between the experimental and model value is calculated. This exercise is repeated for all the data points and the sum of square of the difference is estimated, which is known as PRESS. This method is also known as internal validation. While R^2 and R^2_{adj} are indication of the model fit, q^2 is an indication of the predictive capability of the model. A large F indicates that the model fit is not a chance occurrence. R^2 and R^2_{adj} above a value of 0.6 indicate good model fit, while q^2 above 0.50 indicates that the model has reasonably good predictive capability.

In order to further test the predictive capability of some of the models, the data set was divided into training and test/validation sets. This division is made in a random manner. The training set data was used for developing the regression model. This model was used to predict the activity of the test set data and compared with the actual values. This external validation technique definitely proves the predictive capability of models and it is superior to internal leave-one-out method.

Analysis of variance (ANOVA) on the activity data, cluster analysis and principal component analysis were performed using KyPlot[®] (U.S.A.). Cluster analysis is an unsupervised learning technique which aims at sorting different objects into groups in a way that the degree of similarity (or activity) between two objects is maximal if they belong to the same group and minimal otherwise.²⁸⁾ The forming of the groups is based on dissimilarity distant (*i.e.*, differences between the activities of individual compounds). If two compounds are in the same group it means that they may have similar structural features. Compounds within a cluster are more similar to each other than they are to compounds in other clusters. Principal Components Analysis can be used to identify patterns in data, and expressing the data in such a way as to highlight their similarities and differences. It is suited for high dimensional systems, where the number of dimensions can be reduced without much loss of information. Principal compo-

nent analysis is performed on the descriptor data to reduce it to a small set which can be analysed much more meaningfully.

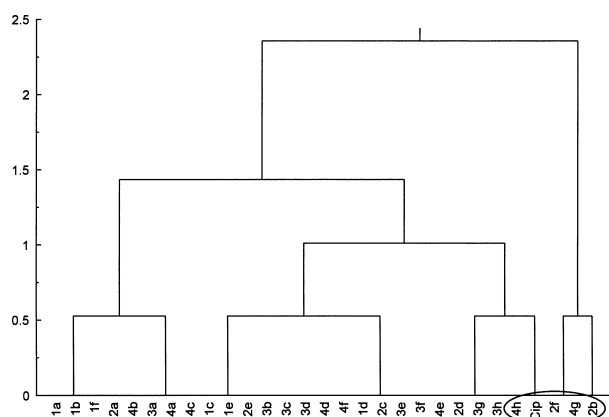
Antimicrobial Activity The twenty eight compounds (see Table 1 for compound details) were tested against four bacteria namely, *Staphylococcus aureus* (ATTC-25923), *Bacillus pumilus* (recultured), *Escherichia coli* (ATTC-25922) and *Proteus vulgaris* (recultured) and two fungi namely, *Aspergillus flavus* (NCIM No. 524), and *Aspergillus niger* (recultured). The activity was tested by the disc-diffusion method under standard conditions using Müller-Hinton agar medium as described by NCCLS.³⁰⁾ Standardized inoculum (5×10^5 cfu/ml) of each test bacterium was spread on to sterile Müller-Hinton agar plates so as to achieve a confluent growth. Discs measuring 9 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for 1 h. The test compounds (mol/ml) were dissolved in a mixture of EtOH/Me₂SO₄ mixture (1:1, v/v) and the discs were introduced on the agar medium. Subsequently, the test compounds were loaded on the filter paper discs so that the disc contained 100 or 200 µg of the compound. The discs only with EtOH/Me₂SO₄ mixture were used as solvents control. The antibiotics namely, ciprofloxacin and nystatin were used as standard drug to test the antibacterial and antifungal activities respectively. The plates were allowed to stand for 1 h or more for diffusion to take place and then incubated at 37 °C for 24 h. The zone of inhibition was recorded to the nearest mm. Experiments were repeated in triplicate and the average values are reported here with their standard deviation.

Results

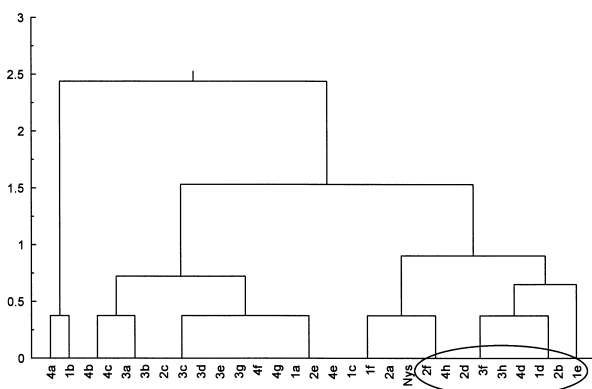
The antimicrobial activity of the benzimidazole derivatives is tested by the agar disc-diffusion method against the gram-positive and gram-negative bacteria and, the two fungi. The results of these studies are summarized in Table 2 with the corresponding standard deviation from the mean value. Two way ANOVA of the antimicrobial data at 100 and 200 µg/disc concentration levels for the four bacteria indicate (Tables are given in supporting material) that the compounds significantly differ in activity between themselves ($F=10.2$ and 17.1 at 100 and 200 µg/disc concentration levels respectively, $p<0.001^*$) and between the various microorganisms ($F=2.83$ and 5.83 for the two concentrations respectively, with corresponding probability values of $p<0.01^{**}$ and $p<0.05^{***}$ respectively). Compounds **2b**, with R as C₆H₄NO₂ and R' as SO₂C₆H₄-CH₃(p), **2f** with R as C₆H₄OCH₃ and R' as SO₂C₆H₄-CH₃(p), and **4h**, with R as CH₂C₆H₅ and R' as CH₂(CH₂)₉Cl exhibit comparable or higher antibacterial activity than ciprofloxacin against *S. aureus* and *E. coli* and, higher activity than Nystatin against *A. flavus*. The antibacterial activity of the compounds may be due to their direct binding to the double helix, thereby interfering with the DNA-associated enzymatic processes.³¹⁾ Compounds **2b** and **2f** have the same substituent in the R' position but have different groups in the R position. None of the synthesised compounds exhibit higher antibacterial activity than control against *B. pumilus* and *P. vulgaris*. The cluster analysis of the activities of the compounds against *S. aureus* at 100 µg/disc concentration is shown in Fig. 1. The

Table 2. QSAR Models for Various Bacterial and Fungal Activities (Number of Data Points=28)

Microorganism	Concentration ($\mu\text{g/ml}$)	Regression model	R^2	R^2_{adj}	q^2	F	PRESS
<i>S. aureus</i>	100	$-2.793+0.0571 \text{ E-ADJ-mag} -0.0361 \text{ Shadow-xy} 3.971 \text{ A-type_O_58}$	0.75	0.719	0.62	23.98	36.1
	200	$-0.525+0.0427 \text{ E-ADJ-mag} +0.00897 \text{ Shadow-xy} -2.742 \text{ A type_O_58}$	0.68	0.64	0.52	17.2	48.1
<i>E. coli</i>	100	$-77.866+0.499 \text{ Molref} +12.780 \text{ S_aaN}-0.0596 \text{ Jurs-WPSA-1}$	0.871	0.854	0.83	53.8	24.72
	200	$-70.64+0.525 \text{ Molref} +10.759 \text{ S_aaN} -0.0532 \text{ Jurs-WPSA-1}$	0.881	0.866	0.83	59.26	32.68
<i>B. pumilus</i>	100	$6.4069+2.292 \text{ Kappa-2} -0.1318 \text{ MR}$	0.78	0.75	0.72	43.8	65.94
	200	$5.926+2.286 \text{ Kappa-2} -0.101 \text{ MR}$	0.653	0.626	0.58	23.6	146.9
<i>P. vulgaris</i>	100	$8.6+0.787 \text{ Sr} +0.0688 \text{ Shadow-xy} +0.26 \text{ LUMO}$	0.77	0.745	0.68	27.29	15.5
	200	$2.15+0.0965 \text{ Sr} +0.18 \text{ Shadow-xy} +0.45 \text{ LUMO}$	0.676	0.64	0.56	16.7	110.8
<i>A. flavus</i>	100	$12.489+1.042 \text{ A type_C_24} -61.295 \text{ Jurs-RNCG}$	0.609	0.58	0.53	19.489	95.399
	200	$12.5+1.16 \text{ A type_C_24} -61.02 \text{ Jurs-RNCG}$	0.66	0.63	0.58	24.53	84.15
<i>A. niger</i>	100	$3.305+1.831 \text{ A log p98} +0.391 \text{ S-ss_O}$	0.794	0.77	0.741	47.6	40.38
	200	$2.789+2.12 \text{ A log p98} +0.308 \text{ S-ss_O}$	0.744	0.72	0.67	36.4	67.4

Fig. 1. Cluster Analysis of Activity of *S. aureus* at a Concentration of 100 $\mu\text{g/ml}$

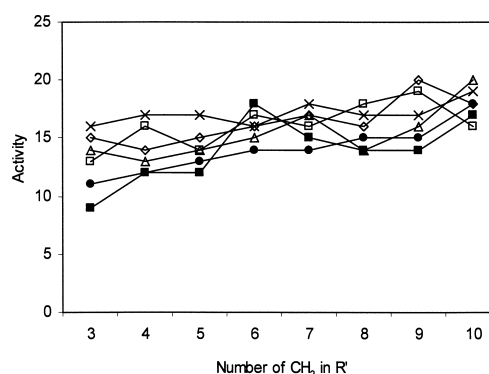
Clustering is based on group average.

Fig. 2. Cluster Analysis of Activity of *A. flavus* at a Concentration of 100 $\mu\text{g/ml}$

Clustering is based on group average.

compounds in the higher activity group are marked with a circle.

Compound **4a** with R as $\text{CH}_2\text{C}_6\text{H}_5$ and R' as $\text{CH}_2\text{CH}_2\text{-CH}_2\text{Cl}$ exhibits lowest antifungal activity in this series at both the concentration levels. Compound **3h**, with R as $\text{CH}_2\text{OC}_6\text{H}_5$ and R' as $\text{CH}_2(\text{CH}_2)_9\text{Cl}$ exhibits higher activity against both the fungi than the control Nystatin. Several compounds exhibit higher activity than the control against *A. flavus* (see cluster analysis—Fig. 2). In general, the activity

Fig. 3. Effect of Adding Methylene Group in the R' Position on the Antibacterial and Antifungal Activities (Compounds **4a** to **4h**, which Have $\text{R}=\text{CH}_2\text{C}_6\text{H}_5$)

◇, *S. aureus*; □, *E. coli*; △, *B. pumilus*; ×, *P. vulgaris*; ■, *A. flavus*; ◆, *A. niger*.

of the antifungal azoles is attributed to the presence of ergosterol in the fungal cell membrane. The synthesized compounds may block ergosterol synthesis by interfering with the demethylation of its precursor, lanosterol.³²⁾ Compounds **1b** with R as CCl_3 and R' as $\text{SO}_2\text{C}_6\text{H}_4\text{-CH}_3(\text{p})$ and **1a** with R as CCl_3 and R' as COC_6H_5 generally exhibit the lowest activity against all the organisms in this series. Cluster analyses for other microorganisms are given in the supporting material.

Figure 3 plots the effect of adding a methylene group in the R' position (compounds **4a** to **4h**), which have $\text{R}=\text{CH}_2\text{C}_6\text{H}_5$ on the antibacterial and antifungal activities. The antibacterial activity increases from 13–16 to 16–20 mm and the antifungal activity from 9–10 to 17–18 mm when the number of methylenes is increased from 3 to 10 in the R' position of the benzimidazole derivatives, indicating that increasing the hydrophobicity at the R' position favours activity. For the addition of the same number of methylene groups in the R' position the increase in the antifungal activity is higher than the increase in the antibacterial activity. Figure 4 plots the effect of adding a methylene group in the R' position (compounds **3a** to **3h**), which have $\text{R}=\text{CH}_2\text{OC}_6\text{H}_5$ on the antibacterial and antifungal activities. The antibacterial activity increases from 12–15 to 16–21 mm and the antifungal activity from 13–14 to 18–20 mm when number of methylenes is increased from 3 to 10 in the R' position of the benzimidazole derivatives. As ob-

served before, for the addition of the same number of methylene groups in the R' position the increase in antifungal activity is higher than the increase in the antibacterial activity. This observed increase in activity is higher for compounds which have $\text{CH}_2\text{OC}_6\text{H}_5$ in their R position, when compared to those which have $\text{CH}_2\text{C}_6\text{H}_5$ in their R position.

Figure 5 plots the effect of various electronegative functional groups in the R position ($=\text{CCl}_3$, $\text{CH}_2\text{C}_6\text{H}_5$, $\text{CH}_2\text{OC}_6\text{H}_5$, $\text{C}_6\text{H}_4\text{NO}_2$, $\text{C}_6\text{H}_4\text{CH}_3$ and $\text{C}_6\text{H}_4\text{OCH}_3$) when $\text{R}'=\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$ on the antibacterial activities of the benzimidazole derivatives. The antibacterial activity of all the microorganisms correlate well with the highest occupied molecular orbital energy (with correlation coefficient values of -0.62 , -0.44 , -0.67 , -0.72 for *S. aureus*, *E. coli*, *B. pumilus* and *P. vulgaris* respectively). More negative is the HOMO energy, lower is the activity. In the case of fungi, the activity does not correlate with HOMO but with a descriptor known as SIC (-0.79 and -0.73 for *A. flavus* and *A. niger* respectively), which describes the structural information. Increasing SIC (structural information content) decreases antifungal activity. In the case of *A. flavus*, the least active com-

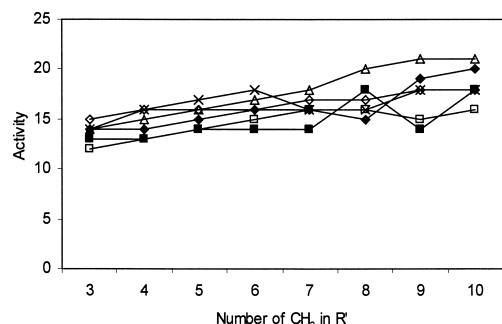


Fig. 4. Effect of Adding Methylene Group in the R' Position on the Antibacterial and Antifungal Activities (Compounds **3a** to **3h**, Which Have $\text{R}=\text{CH}_2\text{OC}_6\text{H}_5$)

◇, *S. aureus*; □, *E. coli*; △, *B. pumilus*; ×, *P. vulgaris*; ■, *A. flavus*; ◆, *A. niger*.

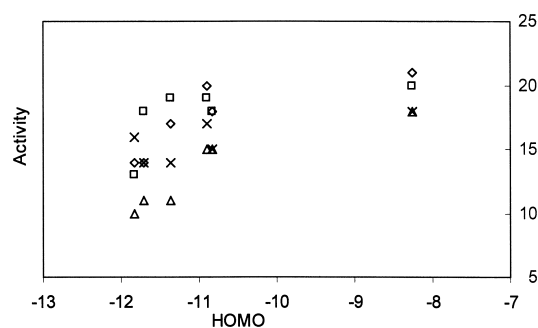


Fig. 5. Effect of Adding Different Functional Groups in the R Position on the Antibacterial Activity for Compounds with $\text{R}'=\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$ as a Function of HOMO

◇, *S. aureus*; □, *E. coli*; △, *B. pumilus*; ×, *P. vulgaris*.

pound has R equal to CCl_3 and the most active compound has R equal to $\text{C}_6\text{H}_4\text{CH}_3$. In the case of *A. niger*, the most active compound also has $\text{C}_6\text{H}_4\text{CH}_3$ group in the R position, while all other compounds have the same lower activity.

Table 2 lists the best QSAR models that were developed using the 28 experimental data to describe the observed activity against the four bacteria and the two fungi at 100 and 200 $\mu\text{g}/\text{disc}$ concentrations. Four of the linear regression relations have two and the remaining eight of the equations have three independent variables. The data fit for all the six microorganisms at both the concentrations are good with R^2 between 0.65 and 0.88 and R^2_{adj} between 0.63 and 0.86. Except for two cases, the predictive capability of the models developed for all the microorganisms are in the acceptable range ($q^2 > 0.56$). The q^2 for *A. flavus* at 100 $\mu\text{g}/\text{disc}$ is 0.53 and for *S. aureus* at 200 $\mu\text{g}/\text{disc}$ is 0.52. The F values in all cases are very high and are statistically significant indicating that the regression fit is not due to chance.

In order to test whether the linear regression equations developed for *S. aureus*, *E. coli* and *P. vulgaris* at 100 $\mu\text{g}/\text{disc}$ concentration were over fitted the data corresponding to those organisms were divided into training and test/validation sets containing 23 and 5 data points respectively. The training set data was used to develop the QSAR equations, which are listed in Table 3. Later, these equations were used to predict the antibacterial activity of the test set. Figures 7 to 9 compare the predicted and experimental activities for the test and training sets for all the three microorganisms. It is seen in all the cases the test set data is predicted very well indicating the predictive capability of the models developed (the tables comparing the predicted with the actual values for these three organisms are given in the supporting material).

Principal component analysis of the two hundred and forty nine descriptors led to the conclusion that at least eight principal components are required to describe 95% of the variance, with the first principal component accounting for about 45% of the variance. Figure 10 plots the activities against the

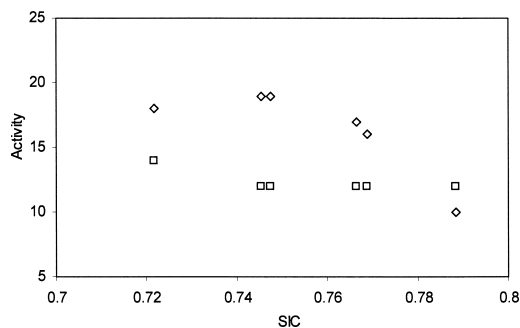


Fig. 6. Effect of Adding Different Functional Groups in the R Position on the Antifungal Activity for Compounds with $\text{R}'=\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$ as a Function of SIC

◇, *A. flavus*; □, *A. niger*.

Table 3. QSAR Models for Three Bacterial Activities at a Concentration of 100 $\mu\text{g}/\text{disc}$ Using Training Data set (Number of Data Points=23)

Microorganism	Regression model	R^2	R^2_{adj}	q^2	F	PRESS
<i>S. aureus</i>	$-3.735 + 0.0576 \text{ E-ADJ-mag} - 0.0278 \text{ Shadow-xy} - 3.992 \text{ A-type_O_58}$	0.734	0.693	0.64	1722	38.5
<i>E. coli</i>	$-70 + 0.487 \text{ Molref} + 11.185 \text{ S_aaN} - 0.0563 \text{ Jurs-WPSA-1}$	0.872	0.853	0.82	43.4	21.7
<i>P. vulgaris</i>	$8.72 + 0.732 \text{ Sr} + 0.0691 \text{ Shadow-xy} + 0.218 \text{ LUMO}$	0.732	0.69	0.67	17.3	16.6

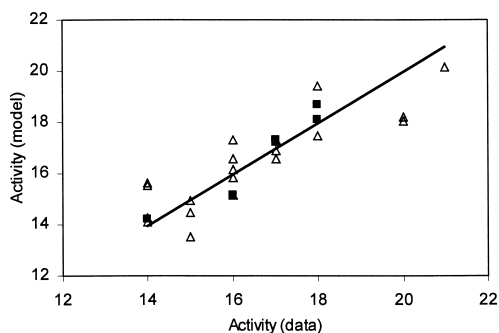


Fig. 7. Comparison of Activity Data and Model for *S. aureus* at a Concentration 100 µg/ml

△, training set data; ■, test set data.

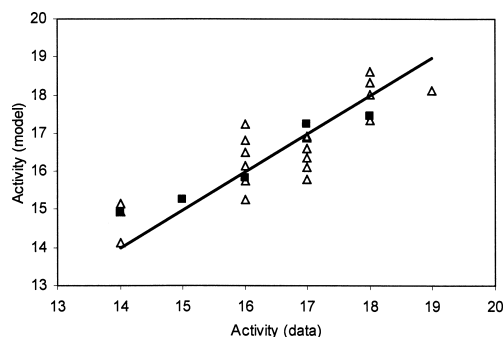


Fig. 9. Comparison of Activity Data and Model for *P. vulgaris* at a Concentration 100 µg/ml

△, training set data, ■, test set data.

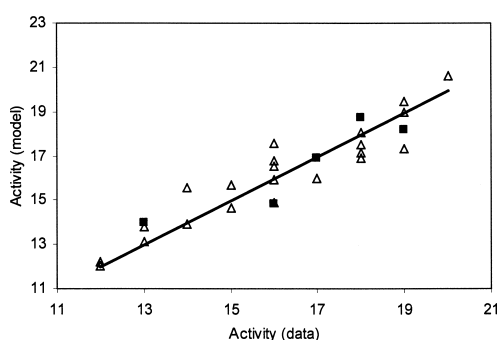


Fig. 8. Comparison of Activity Data and Model for *E. coli* at a Concentration 100 µg/ml

△, training set data; ■, test set data.

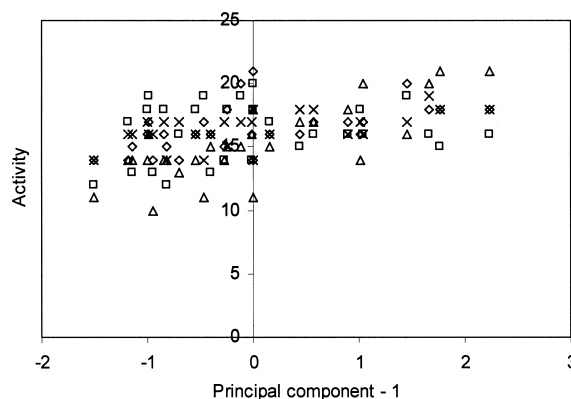


Fig. 10. Antibacterial Activities Plotted against First Principal Component

◇, *S. aureus*; □, *E. coli*; △, *B. pumilus*; ×, *P. vulgaris*.

Table 4. Details of Descriptors Used in the Models Listed in Table 2

Descriptor	Type	Description
A log p98	Thermodynamic	Log P (the octanol/water partition coefficient) is related to the hydrophobic character of the molecule
A type-C-24	Atom type thermodynamic	Type of bonds attached to a carbon atom
A type-O-58	Atom type thermodynamic	Type of bonds attached to an oxygen atom (=O)
E-ADJ-mag	Information-content	Entropy of edge adjacency matrix
Jurs -RNCG	Shape and electronic information	Charge of most negative atom divided by the total negative charge
Jurs-WPSA-1	Shape and electronic information	Surface-weighted charged partial surface areas
Kappa-2	Topological	Kier's Shape index
LUMO	Electronic	Lowest unoccupied molecular orbital
Molref	Thermodynamic	Molar refractivity
MR	Fragment constants	Molecular refractivity
S_aaN	Electrotopological	N with 2 aromatic bonds
SC-1	Topological	Number of the 1st order subgraphs
Shadow-xy	Shape	Area of the molecular shadow in the XY plane
Sr	Electronic descriptor	Captures changes in electronic distribution and it is an index indicating the reactivity of aromatic hydrocarbons
S-ss_O	Electrotopological	O with two single bonds

four bacteria as a function of the first principal component. There seems to be a positive correlation between the antibacterial activity and the first principal component.

The description of the various descriptors that were used in the QSAR is tabulated in Table 4. The descriptors used in the model for *S. aureus* are E-ADJ-mag, Shadow-xy and A-type_O_58. The first descriptor is positively correlated and the other two are negatively correlated with activity. The first two descriptors relate to the molecular size and area. Hydrophobicity of organic molecules is represented in terms of

Log P (the logarithm of 1-octanol/water partition coefficient). The C log P is one such method which makes use of Ghose-Crippen atom counts descriptors (A type).^{33,34)} A type_O_58 is such a descriptor which represents a double bonded oxygen in the form of =O. Turker *et al.* observed that a group which possesses hydrogen accepting capability improves activity.²¹⁾

The descriptors used in the QSAR for *E. coli* are Molref, S_aaN and Jurs-WPSA-1. The first two descriptors have a positive and the last one has a negative correlation with ac-

tivity. Jurs descriptor is a combination of surface area and surface charge of the molecule. Molref describes the molar refractivity and S_aaN captures the number of aromatic nitrogens in the compound. The descriptors used in the QSAR for *B. pumilus* are Kappa-2 and MR. MR represents the molecular refractivity and is negatively correlated with the anti bacterial activity and Kappa-2 is positively correlated with the activity. Kappa represents the shape of the molecule and Kumar *et al.*, similar to the current findings, also observed positive contribution of Kier-shape index towards activity.³⁵⁾

The descriptors used in the QSAR for *P. vulgaris* are Sr, Shadow-xy and LUMO, and all are positively correlated with activity. Shadow-xy relates to the shape of the molecule and contribution of shape in QSAR for predicting activity against antimicrobials, specifically against *Mycobacterium tuberculosis* has been earlier reported by our research group.^{26,36)} Sr and LUMO relate the electronic features of the compound. Sener *et al.* found that activity of similar compounds against *C. albicans* is highly correlated with LUMO.²⁰⁾

The descriptors used in the QSAR for *A. flavus* are A type_C_24 and Jurs -RNCG. A type_C_24 represents the carbon atom which is connected to two R groups on either side though aromatic single bond.^{33,34)} Such groups are present in the present set of structures. Jurs -RNCG is negatively correlated while the other descriptor is positively correlated with activity. The Jurs descriptor is a combination of shape and charge. The descriptors used in the QSAR for *A. niger* are A log p98 and S-ss_O and both are positively correlated with activity. The importance of Log P as a descriptor against *Bacillus subtilis* has been pointed out by others.¹⁷⁾ S-ss_O relates to the electronic environment around the oxygen atom.

The models have identified several independent variables or descriptors suitable for QSAR. They pertain to the size, shape and area of the molecule, electronic parameters such as Sr and LUMO, and lipophylic-hydrophilic balance. The descriptors short listed in the present work match with those that were found by several other researchers.^{14,15,18)}

The present study included the synthesis of twenty eight analogues of benzimidazoles which were tested for their antimicrobial activity against four bacteria namely *S. aureus*, *E. coli*, *B. pumilus* and *P. vulgaris* and two fungi namely *A. flavus* and *A. niger*. The test compound solutions were prepared at two concentrations at constant molarity (mol/ml). Detailed characterization data of sixteen of the compounds are reported in this paper since they have not been mentioned anywhere. Compounds **2b**, with R as C₆H₄NO₂ and R' as SO₂C₆H₄-CH₃(p), **2f** with R as C₆H₄OCH₃ and R' as SO₂C₆H₄-CH₃(p), and **4h**, with R as CH₂C₆H₅ and R' as CH₂(CH₂)₉Cl exhibited comparable or higher antibacterial activity than Ciprofloxacin against *S. aureus* and *E. coli* and, higher activity than Nystatin against *A. flavus*. Several other compounds showed better activity than the standard antibiotic for *E. coli*. None of the synthesised compounds exhibited higher antibacterial activity than control against *B. pumilus* and *P. vulgaris*. A few compounds exhibited better activity than the standard drug towards the fungus, *A. flavus*. Compounds **1b** with R as CCl₃ and R' as SO₂C₆H₄-CH₃(p) and, **1a** with R as CCl₃ and R' as COC₆H₅ generally exhibited the lowest activity against all the organisms in this series. Addition of methylene groups in the R' position increased anti-infective activity. QSAR models were developed

relating their observed activities with molecular descriptors. All the models had between two to three independent variables. A genetic algorithm technique was used to select the descriptors from a large pool so as to arrive at the best QSAR models. The QSARs developed were good for all the cases ($R^2=0.65$ to 0.88 ; $R^2_{adj}=0.63$ to 0.86). Except for two cases, the predictive capability of the developed models was in acceptable range ($q^2>0.56$). In order to further test the predictive capabilities of the models that had three independent variables, the activity data for those cases were divided into training (23 values) and test/validation (5 values) sets. QSARs were developed with the training data set and these equations were used to predict the test data. In all the three cases the models were able to predict the external test data well. The best molecular descriptors identified relate to polar surface area, log P, electronic parameters, molecular size, shape and area.

Experimental

The melting point of the synthesized compounds (Table 1) was determined in open capillaries using Toshniwal melting point apparatus. Purity of the compounds was tested by TLC using an eluent mixture of CHCl₃:CH₃OH (3:1 v/v), and the structures were confirmed by FTIR (in KBr pellet) using Perkin Elmer double beam Infra red spectrometer and ¹H-, ¹³C-NMR (90 MHz) using EM-390-spectrometer. The mass spectra were recorded on Shimadzu QP-5000 mass spectrometer. Elemental analysis was recorded on Perkin-Elmer 240 CHN analyzer. The reagents and solvents were purchased from Ranbaxy, E-Merck, Loba and Sisco chemicals, India.

General Procedure A mixture of substituted benzimidazoles (1.0 g, 0.0042 mol) and appropriate dichloroalkanes (2.24 g, 0.15 mol) in aqueous sodium hydroxide (5%, 20 ml) was stirred for 1–2 h at room temp. and heated under reflux for 1–4 h. The reaction mixture was allowed to stand to attain room temperature. A solid precipitate that separated out was filtered off, washed with little cold water, dried in vacuum and recrystallized from THF. Detailed characterization data is given below for compounds **3a–3h** and **4a–4h**. The data for other compounds had been reported earlier by us and are not reported here.²⁹⁾

N-1-(Chloropropyl)-2-(phenoxymethyl)benzimidazole (**3a**): IR (KBr): 2968, 2818, 1635, 1602, 1476, 1446, 1206, 1069 and 734 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ : 7.53–6.85 (m, 11H (Ar)), 4.78–4.34 (m, 6H (CH₂)₃) ppm; ¹³C-NMR δ : 149.64, 144.02, 141.24, 133.64, 131.06, 129.83, 129.31, 128.83, 127.45, 120.61, 110.15, 108.83 and 100.31 ppm; MS (FAB) *m/z*: 300 (M⁺, 80%), 301 (M⁺+1, 40%), 299 (M⁺-1, 12%), 165 (16%), 115 (18%). Anal. Calcd for C₁₇H₁₇N₂OCl: C, 68.00, H, 5.66, N, 9.33%. Found: C, 68.06, H, 5.71, N, 9.37%.

N-1-(Chlorobutyl)-2-(phenoxymethyl)benzimidazole (**3b**): IR (KBr): 2907, 2717, 1642, 1612, 1486, 1426, 1208, 1066 and 736 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ : 7.64–7.35 (m, 11H (Ar)), 4.85–4.79 (m, 8H (CH₂)₄) ppm; ¹³C-NMR δ : 144.87, 142.22, 134.13, 129.78, 129.30, 128.87, 128.72, 128.37, 127.09, 124.24, 115.99, 113.29 and 102.23 ppm; MS (FAB) *m/z*: 314 (M⁺, 80%), 315 (M⁺+1, 40%), 313 (M⁺-1, 12%), 165 (16%), 115 (18%). Anal. Calcd for C₁₈H₁₉N₂OCl: C, 68.78, H, 6.05, N, 8.91%. Found: C, 68.83, H, 6.09, N, 8.97%.

N-1-(Chloropentyl)-2-(phenoxymethyl)benzimidazole (**3c**): IR (KBr): 2957, 2836, 1675, 1607, 1445 1408, 1208, 1069 and 740 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ : 7.89–7.59 (m, 11H (Ar)), 4.85–4.79 (m, 10H (CH₂)₅) ppm; ¹³C-NMR δ : 147.68, 146.89, 146.34, 135.70, 129.47, 128.46, 127.72, 121.58, 118.52, 115.58, 109.45, 104.40 and 101.58 ppm; MS (FAB) *m/z*: 328 (M⁺, 80%), 329 (M⁺+1, 40%), 327 (M⁺-1, 12%), 165 (16%), 115 (18%). Anal. Calcd for C₁₉H₂₁N₂OCl: C, 69.51, H, 6.40, N, 8.53%. Found: C, 69.55, H, 6.46, N, 8.57%.

N-1-(Chlorohexyl)-2-(phenoxymethyl)benzimidazole (**3d**): IR (KBr): 2982, 2811, 1660, 1593, 1449, 1400, 1212, 1605 and 736 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ : 7.93–7.62 (m, 11H (Ar)), 4.90–4.62 (m, 12H (CH₂)₆) ppm; ¹³C-NMR δ : 143.23, 134.65, 130.94, 130.50, 129.58, 129.04, 128.37, 128.08, 127.84, 127.54, 127.11, 110.94 and 104.65 ppm; MS (FAB) *m/z*: 342 (M⁺, 80%), 343 (M⁺+1, 40%), 340 (M⁺-1, 12%), 165 (16%), 115 (18%). Anal. Calcd for C₂₀H₂₃N₂OCl: C, 70.17, H, 6.72, N, 8.18%. Found: C, 70.22, H, 6.77, N, 8.23%.

N-1-(Chloroheptyl)-2-(phenoxymethyl)benzimidazole (**3e**): IR (KBr):

2989, 2797, 1660, 1594, 1450, 1401, 1211, 1602 and 739 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 8.00–7.71 (m, 11H (Ar)), 4.96–4.62 (m, 14H (CH₂)₇) ppm; ¹³C-NMR δ: 145.83, 134.14, 133.44, 130.20, 128.62, 127.22, 126.86, 124.84, 119.05, 116.82, 112.77, 109.05 and 104.72 ppm; MS (FAB) *m/z*: 356 (M⁺, 80%), 357 (M⁺+1.40%), 355 (M⁺–1, 12%), 165 (16%), 115 (18%). *Anal.* Calcd for C₂₁H₂₅N₂OCl: C, 70.78, H, 7.02, N, 7.86%. Found: C, 70.82, H, 7.07, N, 7.91%.

N-1-(Chlorooctyl)-2-(phenoxymethyl)benzimidazole (**3f**): IR (KBr): 2973, 2871, 1662, 1604, 1450, 1402, 1211, 1067 and 741 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 8.09–7.71 (m, 11H (Ar)), 4.99–4.63 (m, 16H (CH₂)₈) ppm; ¹³C-NMR δ: 147.86, 140.94, 134.86, 133.05, 132.23, 130.88, 129.93, 129.40, 129.23, 129.05, 128.70, 127.86, 124.58 and 109.74 ppm; MS (FAB) *m/z*: 370 (M⁺, 80%), 371 (M⁺+1, 40%), 369 (M⁺–1, 12%), 165 (16%), 115 (18%). *Anal.* Calcd for C₂₂H₂₇N₂OCl: C, 71.35, H, 7.29, N, 7.56%. Found: C, 71.39, H, 7.29, N, 7.55%.

N-1-(Chlorononyl)-2-(phenoxymethyl)benzimidazole (**3g**): IR (KBr): 2987, 2868, 1669, 1600, 1493 1449, 1214, 1069 and 740 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 8.12–7.79 (m, 11H (Ar)), 5.09–4.69 (m, 18H (CH₂)₉) ppm; ¹³C-NMR δ: 144.02, 141.24, 133.64, 131.06, 129.83, 129.31, 128.83, 127.45, 126.90, 121.78, 120.61, 112.15 and 110.44 ppm; MS (FAB) *m/z*: 384 (M⁺, 80%), 385 (M⁺+1, 40%), 383 (M⁺–1, 12%), 165 (16%), 115 (18%). *Anal.* Calcd for C₂₃H₂₉N₂OCl: C, 71.87, H, 7.55, N, 7.30%. Found: C, 71.88, H, 7.55, N, 7.30%.

N-1-(Chlorodecyl)-2-(phenoxymethyl)benzimidazole (**3h**): IR (KBr): 2975, 2888, 1642, 1612, 1444, 1215, 1407, 1071 and 742 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 8.18–7.80 (m, 11H (Ar)), 5.15–4.72 (m, 20H (CH₂)₁₀) ppm; ¹³C-NMR δ: 144.47, 134.22, 134.13, 129.78, 129.30, 128.87, 128.72, 128.37, 127.09, 124.24, 115.99, 105.94 and 102.23 ppm; MS (FAB) *m/z*: 296 (M⁺, 100%), 297 (M⁺+1, 52%), 295 (M⁺–1, 13%), 193 (6%), 178 (13%), 165 (50%), 115 (10%) and 105 (13%). MS (FAB) *m/z*: 398 (M⁺, 80%), 399 (M⁺+1.40%), 397 (M⁺–1, 12%), 165 (16%), 115 (18%). *Anal.* Calcd for C₂₄H₃₁N₂OCl: C, 71.87, H, 7.78, N, 7.03%. Found: C, 71.91, H, 7.82, N, 7.03%.

N-1-(Chloropropyl)-2-benzylbenzimidazole (**4a**): IR (KBr): 2967, 2852, 1660, 1594, 1479, 1449 and 735 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.60–7.20 (m, 11H (2-benzylbenzimidazole)), 4.82–4.72 (m, 6H (CH₂)₃) ppm; ¹³C-NMR δ: 147.92, 146.26, 132.37, 132.02, 130.72, 129.56, 129.41, 129.10, 128.92, 128.66, 128.34, 127.77 and 126.48 ppm; MS (FAB) *m/z*: 284 (M⁺, 100%), 285 (M⁺+1, 17%), 283 (M⁺–1, 8%), 296 (4%), 194 (33%), 178 (21%), 165 (6%), 115 (31%) and 103 (25%). *Anal.* Calcd for C₁₇H₁₇N₂Cl: C, 71.83, H, 5.98, N, 9.85%. Found: C, 71.84, H, 5.97, N, 9.89%.

N-1-(Chlorobutyl)-2-benzylbenzimidazole (**4b**): IR (KBr): 2969, 2863, 1631, 1606, 1486 1400 and 737 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.50–7.00 (m, 11H (2-benzylbenzimidazole)), 4.91–4.74 (m, 8H (CH₂)₄) ppm; ¹³C-NMR δ: 141.30, 139.83, 134.22, 134.13, 129.78, 129.30, 128.87, 128.37, 127.09, 124.24, 123.26 115.99, 113.29 and 105.94 ppm; MS (FAB) *m/z*: 298 (M⁺, 100%), 299 (M⁺+1, 19%), 297 (M⁺–1, 12%), 297 (2%), 194 (6%), 165 (13%), 115 (22%), and 103 (21%). *Anal.* Calcd for C₁₈H₁₉N₂Cl: C, 72.48, H, 6.37, N, 9.39%. Found: C, 72.52, H, 6.41, N, 9.43%.

N-1-(Chloropentyl)-2-benzylbenzimidazole (**4c**): IR (KBr): 2974, 2856, 1660, 1628, 1479, 1414 and 741 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.64–6.91 (m, 11H (2-benzylbenzimidazole)), 4.93–4.71 (m, 10H (CH₂)₅) ppm; ¹³C-NMR δ: 149.45, 147.68, 146.89, 146.34, 135.70, 129.47, 128.46, 127.72, 127.23, 121.58, 118.52, 115.58, 109.45, 105.58, 100.46 and 21.53 ppm; MS (FAB) *m/z*: 312 (M⁺, 100%), 313 (M⁺+1, 12%), 311 (M⁺–1, 7%), 297 (29%), 194 (25%), 178 (18%), 165 (88%), 115 (17%), and 105 (23%). *Anal.* Calcd for C₁₉H₂₁N₂Cl: C, 73.07, H, 6.73, N, 8.97%. Found: C, 73.11, H, 6.72, N, 8.97%.

N-1-(Chlorohexyl)-2-benzylbenzimidazole (**4d**): IR (KBr): 3065, 2858, 1638, 1602, 1479, 1414 and 739 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.84–6.84 (m, 11H (2-benzylbenzimidazole)), 4.95–4.74 (m, 10H (CH₂)₆) ppm; ¹³C-NMR δ: 145.62, 135.51, 131.32, 129.59, 129.50, 128.36, 127.32, 126.72, 125.20, 123.16, 114.10, 104.12 and 104.20 ppm; MS (FAB) *m/z*: 326 (M⁺, 100%), 327 (M⁺+1, 21%), 325 (M⁺–1, 10%), 295 (21%), 194 (8%), 178 (8%), 165 (8%), and 106 (16%). *Anal.* Calcd for C₂₀H₂₃N₂Cl: C, 73.61, H, 7.05, N, 8.58%. Found: C, 73.65, H, 7.09, N, 8.62%.

N-1-(Chloroheptyl)-2-benzylbenzimidazole (**4e**): IR (KBr): 2989, 2883, 1624, 1602, 1483, 1443 and 731 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.89–6.86 (m, 11H (2-benzylbenzimidazole)), 4.96–4.78 (m, 10H (CH₂)₇) ppm; ¹³C-NMR δ: 145.64, 144.09, 129.43, 129.33, 128.91, 128.68, 127.31, 124.78, 115.44, 114.77, 110.31 and 105.42 ppm; MS (FAB) *m/z*: 340 (M⁺, 80%), 341 (M⁺+1, 16%), 339 (M⁺–1, 5%), 178 (4%), 165 (19%) and 104 (19%). *Anal.* Calcd for C₂₁H₂₅N₂Cl: C, 74.11, H, 7.35, N, 8.23%. Found: C, 74.13, H, 7.32, N, 8.26%.

N-1-(Chlorooctyl)-2-benzylbenzimidazole (**4f**): IR (KBr): 2968, 2889, 1642, 1611, 1444, 1403, and 736 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.92–6.84 (m, 11H (2-benzylbenzimidazole)), 4.98–4.80 (m, 10H (CH₂)₈) ppm; ¹³C-NMR δ: 147.86, 140.94, 134.86, 132.23, 130.88, 129.93, 129.40, 129.23, 129.05, 128.70, 127.86 122.86 and 115 ppm; MS (FAB) *m/z*: 354 (M⁺, 100%), 355 (M⁺+1, 15%), 353 (M⁺–1, 10%), 296 (13%), 194 (4%), 178 (4%), 165 (18%), 115 (6%), and 105 (25%). *Anal.* Calcd for C₂₂H₂₇N₂Cl: C, 74.57, H, 7.62, N, 7.90%, 2%). Found: C, 74.59, H, 7.67, N, 7.86%.

N-1-(Chlorononyl)-2-benzylbenzimidazole (**4g**): IR (KBr): 2993, 2878, 1696, 1660, 1525, 1497, 1411 and 729 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.00–8.00 (m, 11H (2-benzylbenzimidazole)), 4.98–4.82 (m, 10H (CH₂)₉) ppm; ¹³C-NMR δ: 149.20, 142.32, 133.07, 130.04, 129.22, 128.77, 127.58, 123.45, 116.80, 110.26, 105.58, and 104 ppm; MS (FAB) *m/z*: 368 (M⁺, 100%), 369 (M⁺+1, 20%), 367 (M⁺–1, 8%), 297 (29%), 178 (25), 165 (56%), 115 (38%), and 105 (60%). *Anal.* Calcd for C₂₃H₂₉N₂Cl: C, 75.00, H, 7.88, N, 7.60%. Found: C, 75.03, H, 7.91, N, 7.65%.

N-1-(Chlorodecyl)-2-benzylbenzimidazole (**4h**): IR (KBr): 3037, 2887, 1648, 1601, 1488 1461 and 738 cm⁻¹; ¹H-NMR (DMSO-*d*₆) δ: 7.00–8.00 (m, 11H (2-benzylbenzimidazole)), 5.01–4.82(m, 10H (CH₂)₁₀) ppm; ¹³C-NMR δ: 148.92, 146.26, 133.41, 132.37, 132.02, 130.72, 129.41, 129.10, 128.92, 128.66, 128.34, 127.77, and 110.72 ppm; MS (FAB) *m/z*: 382 (M⁺, 100%), 383 (M⁺+1, 18%), 381 (M⁺–1, 9%), 194 (16%), 178 (15%), 165 (75%), 115 (15%), and 105 (38%). *Anal.* Calcd for C₂₄H₃₁N₂Cl: C, 75.39, H, 8.11, N, 7.32%. Found: C, 75.43, H, 8.13, N, 7.34%.

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