# 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 auerus*, *Escherichia coli*, *Bacillus pumilus* and *Proteus vulgaris*) and two fungi (*Aspergillus flavus* and *Aspergilus niger*). Compounds with R as $C_6H_4NO_2$ and R′ as $SO_2C_6H_4$–$CH_3$(p), with R as $C_6H_4OCH_3$ and R′ as $SO_2C_6H_4$–$CH_3$(p), and with R as $CH_2C_6H_5$ and R′ as $CH_2(CH_2)_9Cl$ exhibited comparable or higher antibacterial activity than Ciprofloxacin against *S. auerus* 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 $CCl_3$ and R′ as $SO_2C_6H_4$–$CH_3$(p) or $COC_6H_5$ 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 $CH_2OC_6H_5$ and R′ as $CH_2(CH_2)_9Cl$ exhibited higher activity against both the fungii than the control Nystatin. Quantitative structure activity relationships (QSARs) developed were good for all the organisms ($R^2$=0.65 to 0.88; $R^2_{adj}$=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, antiamoebic, antiviral, antifungal,[1—4] anthelmintic,[5] anti-HIV,[6] antihistaminic,[7—9] antiulcer,[10,11] cardiotonic,[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 $C_2$- or $C_3$-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 pneumoniae*[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 Cerius$^2$ software® (Acceryls 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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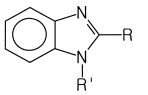
Table 1. Physical Property and Antimicrobial Activity of the Twenty Eight Compounds at Two Loadings ($\mu$g)[a]

| 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 | 200 | 100 | 200 | 100 | 200 | 100 | 200 | 100 | 200 |
| **1a** | $CCl_3$ | $COC_6H_5$ | 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** | $CCl_3$ | $SO_2C_6H_4$–$CH_3$(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** | $CH_2C_6H_5$ | $COC_6H_5$ | 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** | $CH_2C_6H_5$ | $SO_2C_6H_4$–$CH_3$(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** | $CH_2OC_6H_5$ | $COC_6H_5$ | 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** | $CH_2OC_6H_5$ | $SO_2C_6H_4$–$CH_3$(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** | $C_6H_5$ | $COC_6H_5$ | 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** | $C_6H_4NO_2$ | $SO_2C_6H_4$–$CH_3$(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** | $C_6H_4NH_2$ | $COC_6H_5$ | 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** | $C_6H_4CH_3$ | $SO_2C_6H_4$–$CH_3$(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** | $C_6H_4OH$ | $COC_6H_5$ | 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** | $C_6H_4OCH_3$ | $SO_2C_6H_4$–$CH_3$(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** | $CH_2OC_6H_5$ | $CH_2CH_2CH_2Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_3Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_4Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_5Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_6Cl$ | 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** | CH2OC6H5 | $CH_2(CH_2)_7Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_8Cl$ | 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** | $CH_2OC_6H_5$ | $CH_2(CH_2)_9Cl$ | 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** | $CH_2C_6H_5$ | $CH_2CH_2CH_2Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_3Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_4Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_5Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_6Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_7Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_8Cl$ | 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** | $CH_2C_6H_5$ | $CH_2(CH_2)_9Cl$ | 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 | | — | — | — |
| Nystatin | | | | | | | | | | | | | 16±0.045 | 17±0.032 | 20±0.063 | 22±0.063 |
| Control | | | | | | — | — | — | — | — | — | — | — | — | — | — |

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



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, $R^2$ ($=1-\mathrm{SSE/TSS}$), $R^2_{adj}$ ($=1-(n-1)(1-R^2)/(n-p-1)$), $q^2$ ($=1-\mathrm{PRESS/TSS}$), 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 component 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 ($5\times10^5$ 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/$Me_2SO_4$ 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 $\mu$g of the compound. The discs only with EtOH/$Me_2SO_4$ 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 $\mu$g/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 $\mu$g/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 $C_6H_4NO_2$ and R′ as $SO_2C_6H_4$–$CH_3$(p), **2f** with R as $C_6H_4OCH_3$ and R′ as $SO_2C_6H_4$–$CH_3$(p), and **4h**, with R as $CH_2C_6H_5$ and R′ as $CH_2(CH_2)_9Cl$ exhibit comparable or higher antibacterial activity than ciprofloxacin against *S. auerus* 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 substitutent 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 $\mu$g/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 ($\mu$g/ml) | Regression model | $R^2$ | $R^2_{adj}$ | $q^2$ | $F$ | PRESS |
|---|---|---|---|---|---|---|---|
| *S. aureus* | 100 | −2.793+0.0571 E-ADJ-mag −0.0361 Shadow-xy 3.971 A-type_O_58 | 0.75 | 0.719 | 0.62 | 23.98 | 36.1 |
| | 200 | −0.525+0.0427 E-ADJ-mag +0.00897 Shadow-xy −2.742 A type_O_58 | 0.68 | 0.64 | 0.52 | 17.2 | 48.1 |
| *E. coli* | 100 | −77.866+0.499 Molref +12.780 S_aaN−0.0596 Jurs-WPSA-1 | 0.871 | 0.854 | 0.83 | 53.8 | 24.72 |
| | 200 | −70.64+0.525 Molref +10.759 S_aaN −0.0532 Jurs-WPSA-1 | 0.881 | 0.866 | 0.83 | 59.26 | 32.68 |
| *B. pumilus* | 100 | 6.4069+2.292 Kappa-2 −0.1318 MR | 0.78 | 0.75 | 0.72 | 43.8 | 65.94 |
| | 200 | 5.926+2.286 Kappa-2 −0.101 MR | 0.653 | 0.626 | 0.58 | 23.6 | 146.9 |
| *P. vulgaris* | 100 | 8.6+0.787 Sr +0.0688 Shadow-xy +0.26 LUMO | 0.77 | 0.745 | 0.68 | 27.29 | 15.5 |
| | 200 | 2.15+0.0965 Sr+0.18 Shadow-xy+0.45 LUMO | 0.676 | 0.64 | 0.56 | 16.7 | 110.8 |
| *A. flavus* | 100 | 12.489+1.042 A type_C_24 −61.295 Jurs-RNCG | 0.609 | 0.58 | 0.53 | 19.489 | 95.399 |
| | 200 | 12.5+1.16 A type_C_24 −61.02 Jurs-RNCG | 0.66 | 0.63 | 0.58 | 24.53 | 84.15 |
| *A. niger* | 100 | 3.305+1.831 A log p98+0.391 S-ss_O | 0.794 | 0.77 | 0.741 | 47.6 | 40.38 |
| | 200 | 2.789+2.12 A log p98+0.308 S-ss_O | 0.744 | 0.72 | 0.67 | 36.4 | 67.4 |

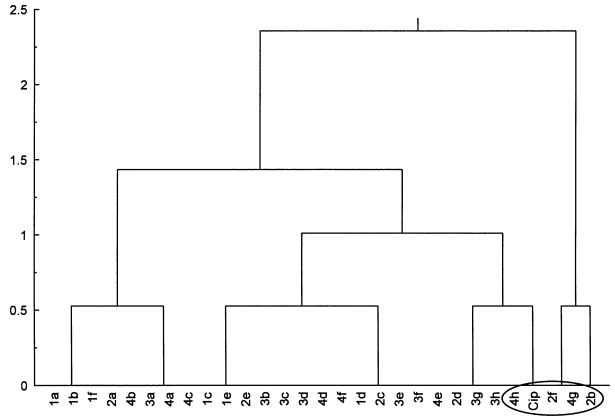



Fig. 1. Cluster Analysis of Activity of *S. aureus* at a Concentration of 100 $\mu$g/ml

Clustering is based on group average.

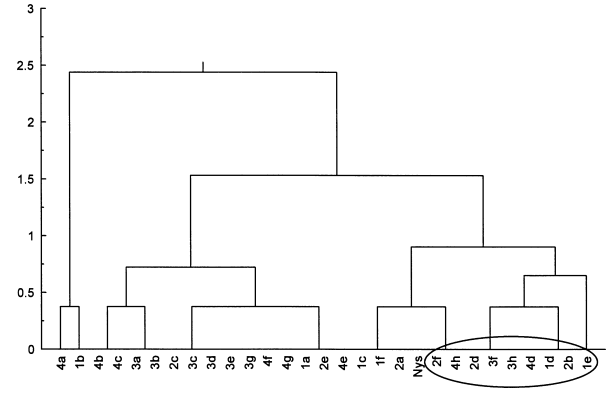



Fig. 2. Cluster Analysis of Activity of *A. flavus* at a Concentration of 100 $\mu$g/ml

Clustering is based on group average.

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

Compound **4a** with R as $CH_2C_6H_5$ and R′ as $CH_2CH_2$-$CH_2Cl$ exhibits lowest antifungal activity in this series at both the concentration levels. Compound **3h**, with R as $CH_2OC_6H_5$ and R′ as $CH_2(CH_2)_9Cl$ exhibits higher activity against both the fungii than the control Nystatin. Several compounds exhibit higher activity than the control against *A. flavus* (see cluster analysis—Fig. 2). In general, the activity

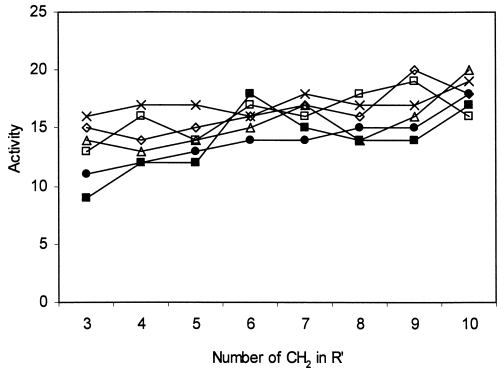



Fig. 3. Effect of Adding Methlyene Group in the R′ Position on the Antibacterial and Antifungal Activities (Compounds **4a** to **4h**, which Have R=$CH_2C_6H_5$)

◇, *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 $SO_2C_6H_4$–$CH_3$(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 R=$CH_2C_6H_5$ 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 R=$CH_2OC_6H_5$ 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 $CH_2OC_6H_5$ in their R position, when compared to those which have $CH_2C_6H_5$ in their R position.

Figure 5 plots the effect of various electronegative functional groups in the R position (=$CCl_3$, $CH_2C_6H_5$, $CH_2OC_6H_5$, $C_6H_4NO_2$, $C_6H_4CH_3$ and $C_6H_4OCH_3$) when R′=$SO_2C_6H_4$–$CH_3$(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 fungii, 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 compound has R equal to $CCl_3$ and the most active compound has R equal to $C_6H_4CH_3$. In the case of *A. niger*, the most active compound also has $C_6H_4CH_3$ group in the R position, while all other compounds have the same lower activity.

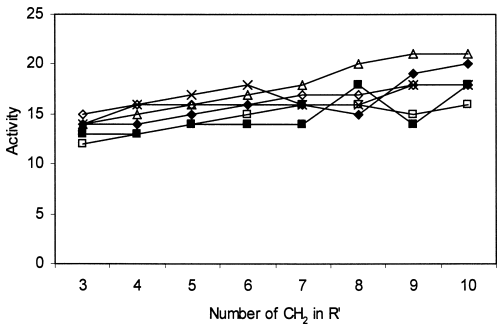



Fig. 4. Effect of Adding Methlyene Group in the R′ Position on the Antibacterial and Antifungal Activities (Compounds **3a** to **3h**, Which Have R=$CH_2OC_6H_5$)

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

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 μg/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 μg/disc is 0.53 and for *S. aureus* at 200 μg/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 μg/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

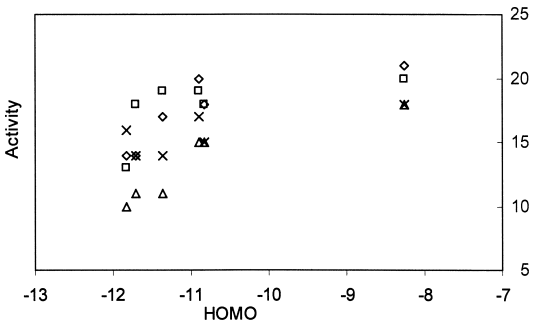



Fig. 5. Effect of Adding Different Functional Groups in the R Position on the Antibacterial Activity for Compounds with R′=$SO_2C_6H_4$–$CH_3$(p) as a Function of HOMO

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

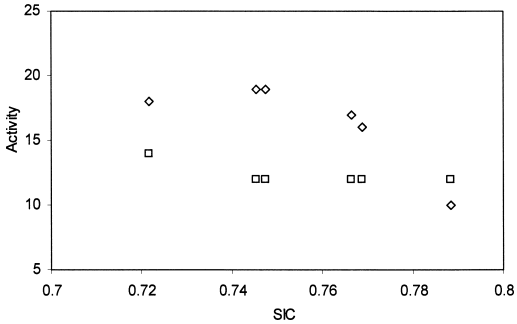



Fig. 6. Effect of Adding Different Functional Groups in the R Position on the Antifungal Activity for Compounds with R′=$SO_2C_6H_4$–$CH_3$(p) as a Function of SIC

◇, *A. flavus*; □, *A. niger*.

Table 3. QSAR Models for Three Bacterial Activities at a Concentration of 100 μg/disc Using Training Data set (Number of Data Points=23)

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

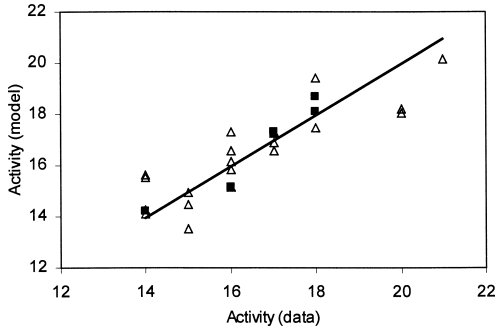



Fig. 7. Comparison of Activity Data and Model for *S. aureus* at a Concentration 100 $\mu$g/ml

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

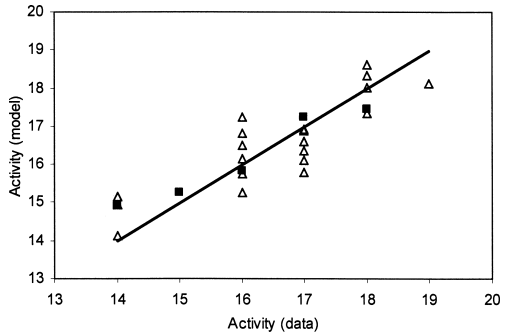



Fig. 9. Comparison of Activity Data and Model for *P. vulgaris* at a Concentration 100 $\mu$g/ml

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

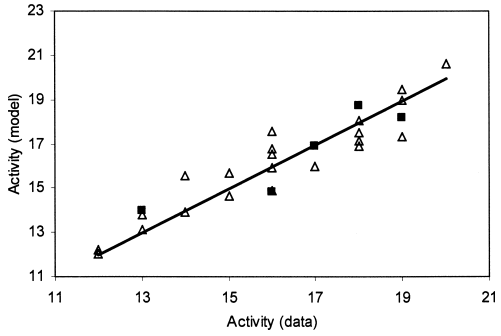



Fig. 8. Comparison of Activity Data and Model for *E. coli* at a Concentration 100 $\mu$g/ml

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

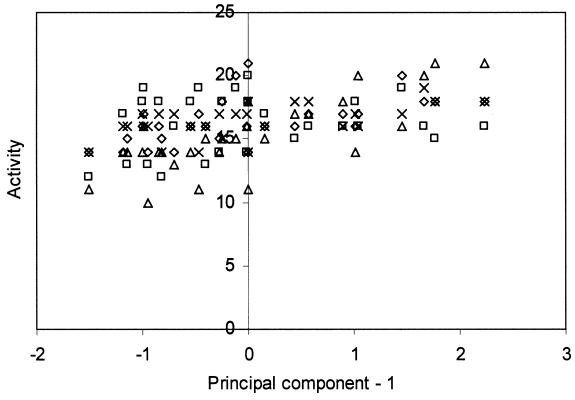



Fig. 10. Antibacterial Activities Plotted against First Principal Component

◇, *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 liphophylic–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 $C_6H_4NO_2$ and R′ as $SO_2C_6H_4$–$CH_3$(p), **2f** with R as $C_6H_4OCH_3$ and R′ as $SO_2C_6H_4$–$CH_3$(p), and **4h**, with R as $CH_2C_6H_5$ and R′ as $CH_2(CH_2)_9Cl$ exhibited comparable or higher antibacterial activity than Ciprofloxacin against *S. auerus* 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 $CCl_3$ and R′ as $SO_2C_6H_4$–$CH_3$(p) and, **1a** with R as $CCl_3$ and R′ as $COC_6H_5$ 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 $CHCl_3$ : $CH_3OH$ (3 : 1 v/v), and the structures were confirmed by FTIR (in KBr pellet) using Perkin Elmer double beam Infra red spectrometer and $^1$H-, $^{13}$C-NMR (90 MHz) using EM-390-spectrometer. The mass spectra were recorded on Shimadu 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$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.53—6.85 (m, 11H (Ar)), 4.78—4.34 (m, 6H $(CH_2)_3$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{17}H_{17}N_2OCl$: 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$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.64—7.35 (m, 11H (Ar)), 4.85—4.79 (m, 8H $(CH_2)_4$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{18}H_{19}N_2OCl$: 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$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.89—7.59 (m, 11H (Ar)), 4.85—4.79 (m, 10H $(CH_2)_5$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{19}H_{21}N_2OCl$: 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$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.93—7.62 (m, 11H (Ar)), 4.90—4.62 (m, 12H $(CH_2)_6$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{20}H_{23}N_2OCl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.00—7.71 (m, 11H (Ar)), 4.96—4.62 (m, 14H $(CH_2)_7$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{21}H_{25}N_2OCl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.09—7.71 (m, 11H (Ar)), 4.99—4.63 (m, 16H $(CH_2)_8$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{22}H_{27}N_2OCl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.12—7.79 (m, 11H (Ar)), 5.09—4.69 (m, 18H $(CH_2)_9$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{23}H_{29}N_2OCl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.18—7.80 (m, 11H (Ar)), 5.15—4.72 (m, 20H $(CH_2)_{10}$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{24}H_{31}N_2OCl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.60—7.20 (m, 11H (2-benzylbenzimidazole)), 4.82—4.72 (m, 6H $(CH_2)_3$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{17}H_{17}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.50—7.00 (m, 11H (2-benzylbenzimidazole)), 4.91—4.74 (m, 8H $(CH_2)_4$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{18}H_{19}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.64—6.91 (m, 11H (2-benzylbenzimidazole)), 4.93—4.71 (m, 10H $(CH_2)_5$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{19}H_{21}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.84—6.84 (m, 11H (2-benzylbenzimidazole)), 4.95—4.74 (m, 10H $(CH_2)_6$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{20}H_{23}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.89—6.86 (m, 11H (2-benzylbenzimidazole)), 4.96—4.78 (m, 10H $(CH_2)_7$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{21}H_{25}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.92—6.84 (m, 11H (2-benzylbenzimidazole)), 4.98—4.80 (m, 10H $(CH_2)_8$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{22}H_{27}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.00—8.00 (m, 11H (2-benzylbenzimidazole)), 4.98—4.82 (m, 10H $(CH_2)_9$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{23}H_{29}N_2Cl$: 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 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$: 7.00—8.00 (m, 11H (2-benzylbenzimidazole)), 5.01—4.82.(m, 10H $(CH_2)_{10}$) ppm; $^{13}$C-NMR $\delta$: 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 $C_{24}H_{31}N_2Cl$: C, 75.39, H, 8.11, N, 7.32%. Found: C, 75.43, H, 8.13, N, 7.34%.

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