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# Synthesis, antimicrobial activity and QSAR studies of new 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2*H*-indazoles

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ABSTRACT

Antimicrobial activity of synthesized 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2*H*-indazole derivatives indicated that 3-(4-chlorophenyl)-2-(4-nitrophenylsulfonyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (**6**) and 3-(4-fluorophenyl)-2-(4-nitrophenylsulfonyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (**20**) were the most active compounds. Further, the results of QSAR studies indicated the importance of topological parameters $^{2}\chi$ and $^{2}\chi^{v}$ in defining the antimicrobial activity of hexahydroindazoles.



The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of bacterial and fungal infections and drug side effects.[1–5] Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed towards the design of new agents.

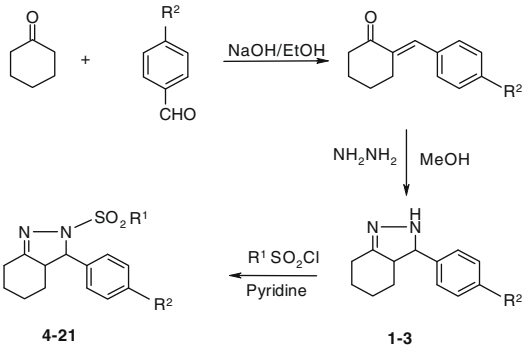
Hexahydroindazole, a novel heterocyclic compound has been reported to have antimicrobial[6,7] and anti-inflammatory activities.[8,9]

QSAR models are mathematical equations relating chemical structure to a wide variety of physical, chemical and biological properties.

In view of above, and as a part our composite programme of rational drug design[10–23] in the present study, we report the synthesis, antimicrobial and QSAR studies of hexahydroindazole derivatives.

The synthetic approach to obtain 3-substituted-3,3a,4,5,6,7-hexahydro-2*H*-indazoles (**1**–**3**) and 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2*H*-indazoles (**4**–**21**) followed the reaction shown in Scheme 1.

We accomplished the synthesis of substituted benzylidene cyclohexanones by the reaction of cyclohexanone with *p*-substituted benzaldehydes in the presence of ethanolic sodium hydrox-




**1)** $R^1$ = H, $R^2$ = Cl
**2)** $R^1$= H, $R^2$= F
**3** ) $R^1$= H, $R^2$= $OCH_3$
**4)** $R^1$= $C_6H_5$, $R^2$= Cl
**5)** $R^1$= $CH_3$, $R^2$= Cl
**6)** $R^1$= 4-$NO_2C_6H_4$, $R^2$= Cl
**7)** $R^1$= $C_{10}H_7$, $R^2$= Cl
**8)** $R^1$= 4-$ClC_6H_4$, $R^2$= Cl
**9)** $R^1$= 4-$CH_3C_6H_4$, $R^2$= Cl
**10)** $R^1$= $C_6H_5$, $R^2$= F
**11)** $R^1$= $CH_3$, $R^2$= F
**12)** $R^1$= $C_{10}H_7$, $R^2$= F
**13)** $R^1$= 4-$CH_3C_6H_4$, $R^2$= F
**14)** $R^1$= $C_6H_5$, $R^2$= $OCH_3$
**15)** $R^1$= $CH_3$, $R^2$= $OCH_3$
**16)** $R^1$= 4-$NO_2C_6H_4$, $R^2$= $OCH_3$
**17)** $R^1$= $C_{10}H_7$, $R^2$= $OCH_3$
**18)** $R^1$= 4-$ClC_6H_4$, $R^2$= $OCH_3$
**19** ) $R^1$= 4-$CH_3C_6H_4$, $R^2$= $OCH_3$
**20)** $R^1$= 4-$NO_2C_6H_4$, $R^2$= F
**21)** $R^1$= 4-$ClC_6H_4$, $R^2$ = F

**Scheme 1.** Scheme for syntheses of hexahydroindazole derivatives.

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**Table 1**
Physicochemical characteristics of hexahydroindazole derivatives

| Compd | Molecular formula | M.Wt. | mp (°C) | $R_f$ value* | Yield (%) |
|---|---|---|---|---|---|
| **1** | $C_{13}H_{15}ClN_2$ | 234 | 124–126 | 0.46 | 73 |
| **2** | $C_{13}H_{15}FN_2$ | 218 | 94–96 | 0.44 | 79 |
| **3** | $C_{14}H_{18}N_2O$ | 230 | 106–108 | 0.40 | 87 |
| **4** | $C_{19}H_{19}ClN_2O_2S$ | 374 | 162–164 | 0.68 | 60 |
| **5** | $C_{14}H_{17}ClN_2O_2S$ | 312 | 180–182 | 0.65 | 73 |
| **6** | $C_{19}H_{18}ClN_3O_4S$ | 419 | 185–187 | 0.73 | 57 |
| **7** | $C_{23}H_{21}ClN_2O_2S$ | 424 | 158–160 | 0.60 | 71 |
| **8** | $C_{19}H_{18}Cl_2N_2O_2S$ | 409 | 170–172 | 0.76 | 80 |
| **9** | $C_{20}H_{21}ClN_2O_2S$ | 388 | 154–156 | 0.70 | 65 |
| **10** | $C_{19}H_{19}FN_2O_2S$ | 358 | 172–174 | 0.80 | 60 |
| **11** | $C_{14}H_{17}FN_2O_2S$ | 296 | 186–188 | 0.59 | 68 |
| **12** | $C_{23}H_{21}FN_2O_2S$ | 408 | 190–192 | 0.74 | 72 |
| **13** | $C_{20}H_{21}FN_2O_2S$ | 372 | 179–181 | 0.69 | 59 |
| **14** | $C_{20}H_{22}N_2O_3S$ | 370 | 150–152 | 0.80 | 64 |
| **15** | $C_{15}H_{20}N_2O_3S$ | 308 | 188–190 | 0.73 | 71 |
| **16** | $C_{20}H_{21}N_3O_5S$ | 415 | 199–201 | 0.55 | 59 |
| **17** | $C_{24}H_{24}N_2O_3S$ | 420 | 196–198 | 0.61 | 60 |
| **18** | $C_{20}H_{21}ClN_2O_3S$ | 404 | 176–178 | 0.67 | 51 |
| **19** | $C_{21}H_{24}N_2O_3S$ | 384 | 174–176 | 0.50 | 62 |
| **20** | $C_{19}H_{18}FN_3O_4S$ | 403 | 193–195 | 0.66 | 62 |
| **21** | $C_{19}H_{18}ClFN_2O_2S$ | 392 | 164–166 | 0.84 | 56 |

* TLC mobile phase – $CHCl_3/CH_3OH$ (9.8:0.2).

ide.[24] Benzylidene cyclohexanones, on heating with hydrazine hydrate resulted in the formation of compounds **1–3**.[25] Further, the sulfonation of **1–3** with appropriate sulfonyl chlorides in pyridine yielded compounds **4–21**.[26,27] All compounds were characterized by IR, MS, elemental analysis and $^1$H NMR (Supplementary data). The physicochemical parameters and molecular structures of hexahydroindazole derivatives used in the present study are given in Table 1.

The synthesized hexahydroindazole derivatives were evaluated for their in vitro antimicrobial activity by tube dilution method[28–30] in duplicate, using ciprofloxacin and fluconazole as reference compounds for antibacterial and antifungal activities, respectively. Double strength nutrient broth-IP and Sabouraud dextrose broth-IP[31] have been employed as media for growth of bacterial and fungal cells, respectively.

**Table 2**
Antimicrobial activity of hexahydroindazole derivatives

| Compd | pMIC (μmol/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *B. subtilis* | *E. coli* | *P. aeruginosa* | *C. albicans* | *A. niger* |
| *Training set* | | | | | | |
| **1** | 2.07 | 2.07 | 2.19 | 2.19 | 2.27 | 2.27 |
| **2** | 2.04 | 2.24 | 2.24 | 2.24 | 2.16 | 2.24 |
| **3** | 2.01 | 2.01 | 2.06 | 2.19 | 2.19 | 2.19 |
| **5** | 2.40 | 2.54 | 2.40 | 2.50 | 2.59 | 2.50 |
| **6** | 2.67 | 2.72 | 2.67 | 2.78 | 2.82 | 2.82 |
| **7** | 2.53 | 2.67 | 2.63 | 2.67 | 2.73 | 2.67 |
| **9** | 2.49 | 2.64 | 2.59 | 2.64 | 2.59 | 2.64 |
| **10** | 2.55 | 2.65 | 2.65 | 2.71 | 2.60 | 2.55 |
| **11** | 2.37 | 2.57 | 2.47 | 2.52 | 2.47 | 2.57 |
| **12** | 2.61 | 2.66 | 2.71 | 2.77 | 2.61 | 2.66 |
| **14** | 2.57 | 2.47 | 2.57 | 2.61 | 2.61 | 2.57 |
| **15** | 2.31 | 2.53 | 2.39 | 2.49 | 2.59 | 2.53 |
| **16** | 2.66 | 2.62 | 2.66 | 2.72 | 2.77 | 2.66 |
| **17** | 2.53 | 2.62 | 2.62 | 2.67 | 2.62 | 2.62 |
| **19** | 2.49 | 2.63 | 2.58 | 2.58 | 2.63 | 2.68 |
| **20** | 2.70 | 2.76 | 2.76 | 2.81 | 2.65 | 2.76 |
| **21** | 2.64 | 2.69 | 2.75 | 2.79 | 2.59 | 2.64 |
| SD[a] | 0.22 | 0.22 | 0.20 | 0.20 | 0.19 | 0.18 |
| *Test set* | | | | | | |
| **4** | 2.57 | 2.62 | 2.57 | 2.67 | 2.62 | 2.62 |
| **8** | 2.61 | 2.71 | 2.66 | 2.77 | 2.77 | 2.71 |
| **13** | 2.47 | 2.62 | 2.62 | 2.67 | 2.57 | 2.67 |
| **18** | 2.61 | 2.65 | 2.65 | 2.70 | 2.61 | 2.65 |
| Std. | 2.03* | 2.33* | 2.03* | 2.62* | 3.16** | 3.16** |

Reference: * ciporfloxacin; ** fluconazole; [a] standard deviation.

The compounds **6** and **20** were found to be the most effective ones against the representative fungal and bacterial species, respectively (Table 2). Analysis of antimicrobial results (Table 2) indicated that the electron withdrawing groups were generally more active than other derivatives. The importance of electron withdrawing groups in enhancing the antimicrobial activity is supported by similar results observed by *P.* Sharma et al.[32] From the analysis of the structures of most active compounds **6** and **20**, it may be concluded that among the electron withdrawing halo groups, the presence of a *p*-fluorophenyl group at 3rd position of hexahydroindazole (**20**) improved the antibacterial activity whereas the presence of *p*-chlorophenyl group at 3rd position of hexahydroindazole (**6**) improved the antifungal activity.

In an attempt to determine the role of structural features, QSAR studies were undertaken using the linear free energy relationship model (LFER) of Hansch and Fujita.[33] Biological activity data determined as MIC values was first transformed into pMIC values on molar basis, which was used as dependent variable in QSAR studies. These were correlated with different molecular descriptors like log of octanol-water partition coefficient (log *P*),[34] molar refractivity (MR),[35] Kiers molecular connectivity ($^2\chi^v$) and shape ($\kappa$, $\kappa\alpha$) topological indices,[36] Randic topological index (R),[37] Balaban topological index (J),[38] Wiener topological index (W),[39] Total energy (Te), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO).[40] The values of selected descriptors are presented in Table 3.

In the present work, a training set consisting of 17 molecules was used for linear regression model generation and a prediction set consisting of 4 molecules was used for the evaluation of generated linear regression model. Firstly, correlation analysis of various descriptors with antimicrobial activity was performed. The data presented in Table 4 shows that most of the parameters are highly correlated with antimicrobial activity. A correlation matrix (Table 5), constructed to find the interrelationship among the parameters demonstrated that each parameter selected in the study is highly correlated with the other ($r > 0.8$).

The topological parameters, especially the molecular connectivity indices ($^2\chi$ and $^2\chi^v$) have been found to exhibit best correlation and high statistical significance ($p < 0.01$). The resulting best-fit

**Table 3**
Value of selected descriptors used in linear regression analysis

| Compd | log*P* | $^0\chi$ | $^0\chi^v$ | $^1\chi$ | $^1\chi^v$ | $^2\chi$ | $^2\chi^v$ | $\kappa\alpha1$ |
|---|---|---|---|---|---|---|---|---|
| *Training set* | | | | | | | | |
| **1** | 4.4 | 11.0 | 9.8 | 7.8 | 6.2 | 7.0 | 4.9 | 10.8 |
| **2** | 4.0 | 11.0 | 9.0 | 7.8 | 5.8 | 7.0 | 4.4 | 10.4 |
| **3** | 3.6 | 11.7 | 10.0 | 8.4 | 6.2 | 7.1 | 4.6 | 11.4 |
| **5** | 3.7 | 14.3 | 13.0 | 9.5 | 9.2 | 9.5 | 7.9 | 14.4 |
| **6** | 5.3 | 19.9 | 16.6 | 13.4 | 11.0 | 12.8 | 9.4 | 20.2 |
| **7** | 6.4 | 20.0 | 17.5 | 14.0 | 11.9 | 13.3 | 10.1 | 19.2 |
| **9** | 5.8 | 18.3 | 16.3 | 12.5 | 10.9 | 11.9 | 9.5 | 18.1 |
| **10** | 5.0 | 17.4 | 14.6 | 12.1 | 10.1 | 11.3 | 8.5 | 16.8 |
| **11** | 3.3 | 14.3 | 12.2 | 9.5 | 8.8 | 9.5 | 7.5 | 14.1 |
| **12** | 6.0 | 20.0 | 16.7 | 14.0 | 11.5 | 13.3 | 9.7 | 18.8 |
| **14** | 4.6 | 18.2 | 15.6 | 12.6 | 10.5 | 11.5 | 8.7 | 17.8 |
| **15** | 2.9 | 15.0 | 13.2 | 10.0 | 9.2 | 9.7 | 7.7 | 15.1 |
| **16** | 4.5 | 20.6 | 16.8 | 13.9 | 11.0 | 13.0 | 9.1 | 19.6 |
| **17** | 5.6 | 20.7 | 17.7 | 14.6 | 11.9 | 13.5 | 9.9 | 19.8 |
| **19** | 5.1 | 19.0 | 16.5 | 13.0 | 10.9 | 12.1 | 9.2 | 18.7 |
| **20** | 4.9 | 19.9 | 15.8 | 13.4 | 10.6 | 12.8 | 8.9 | 20.2 |
| **21** | 5.1 | 18.3 | 14.9 | 12.5 | 10.2 | 11.9 | 8.6 | 17.7 |
| *Test set* | | | | | | | | |
| **4** | 5.4 | 17.4 | 15.4 | 12.1 | 10.5 | 11.3 | 9.0 | 17.1 |
| **8** | 5.9 | 18.3 | 16.5 | 12.5 | 11.0 | 11.9 | 9.6 | 18.4 |
| **13** | 5.4 | 18.3 | 15.5 | 12.5 | 10.5 | 11.9 | 9.0 | 17.8 |
| **18** | 4.3 | 19.7 | 16.9 | 13.5 | 11.0 | 12.3 | 9.1 | 19.7 |

**Table 4**
Correlation of different molecular descriptors with antimicrobial activity of hexahydroindazole derivatives

| | pMICsa | pMICbs | pMICec | pMICpa | pMICca | pMICan |
|---|---|---|---|---|---|---|
| log *P* | 0.586 | 0.513 | 0.658 | 0.608 | 0.498 | 0.560 |
| MR | 0.859 | 0.782 | 0.850 | 0.848 | 0.851 | 0.847 |
| $^{0}\chi$ | 0.932 | 0.856 | 0.919 | 0.923 | 0.894 | 0.909 |
| $^{0}\chi^{v}$ | 0.893 | 0.837 | 0.872 | 0.878 | 0.902 | 0.893 |
| $^{1}\chi$ | 0.897 | 0.807 | 0.894 | 0.891 | 0.849 | 0.862 |
| $^{1}\chi^{v}$ | 0.910 | 0.888 | 0.894 | 0.904 | 0.920 | 0.913 |
| $^{2}\chi$ | 0.936 | 0.882 | 0.932 | 0.936 | 0.898 | 0.920 |
| $^{2}\chi^{v}$ | 0.913 | 0.909 | 0.898 | 0.909 | 0.929 | 0.926 |
| $\kappa_1$ | 0.936 | 0.855 | 0.917 | 0.923 | 0.900 | 0.913 |
| $\kappa\alpha 1$ | 0.943 | 0.873 | 0.917 | 0.929 | 0.915 | 0.940 |
| R | 0.897 | 0.807 | 0.894 | 0.891 | 0.849 | 0.862 |
| B | −0.307 | −0.168 | −0.373 | −0.331 | −0.211 | −0.243 |

**Table 5**
Correlation matrix for pMICsa with selected molecular descriptors

| | $pMIC_{sa}$ | $^{0}\chi$ | $^{0}\chi^{v}$ | $^{1}\chi^{v}$ | $^{2}\chi$ | $^{2}\chi^{v}$ | $\kappa_1$ | $\kappa\alpha_1$ |
|---|---|---|---|---|---|---|---|---|
| $pMIC_{sa}$ | 1.000 | | | | | | | |
| $^{0}\chi$ | 0.932 | 1.000 | | | | | | |
| $^{0}\chi^{v}$ | 0.893 | 0.979 | 1.000 | | | | | |
| $^{1}\chi^{v}$ | 0.910 | 0.964 | 0.986 | 1.000 | | | | |
| $^{2}\chi$ | 0.936 | 0.994 | 0.978 | 0.976 | 1.000 | | | |
| $^{2}\chi^{v}$ | 0.913 | 0.943 | 0.973 | 0.995 | 0.962 | 1.000 | | |
| $\kappa_1$ | 0.936 | 0.999 | 0.976 | 0.958 | 0.988 | 0.936 | 1.000 | |
| $\kappa\alpha_1$ | 0.943 | 0.995 | 0.984 | 0.967 | 0.985 | 0.950 | 0.997 | 1.000 |

models are reported in Eqs. (1)–(6) together with statistical parameters of regression. It is important to note that all these models were developed by using the entire set of hexahydroindazoles ($n = 21$), since no outliers were identified.

*QSAR model for antibacterial activity against Staphylococcus aureus*

$$\text{pMICsa} = 0.063\kappa\alpha_1 + 1.403 \quad (1)$$

$n = 17$; $r = 0.943$; $F = 119.93$; $s = 0.076$; $q^2 = 0.860$

*QSAR model for antibacterial activity against Bacillus subtilis*

$$\text{pMICbs} = 0.109^{2}\chi^{v} + 1.650 \quad (2)$$

$n = 17$; $r = 0.909$; $F = 70.97$; $s = 0.094$; $q^2 = 0.760$

*QSAR model for antibacterial activity against Escherichia coli*

$$\text{pMICec} = 0.083^{2}\chi + 1.609 \quad (3)$$

$n = 17$; $r = 0.932$; $F = 98.60$; $s = 0.076$; $q^2 = 0.823$

*QSAR model for antibacterial activity against Pseudomonas aeruginosa*

$$\text{pMICpa} = 0.084^{2}\chi + 1.662 \quad (4)$$

$n = 17$; $r = 0.936$; $F = 105.62$; $s = 0.074$; $q^2 = 0.843$

*QSAR model for antifungal activity against Candida albicans*

$$\text{pMICca} = 0.094^{2}\chi^{v} + 1.791 \quad (5)$$

$n = 17$; $r = 0.929$; $F = 94.64$; $s = 0.071$; $q^2 = 0.830$

*QSAR model for antifungal activity against Aspergillus niger*

$$\text{pMICan} = 0.089^{2}\chi^{v} + 1.840 \quad (6)$$

$n = 17$; $r = 0.926$; $F = 90.16$; $s = 0.068$; $q^2 = 0.823$

The coefficient of $\kappa\alpha_1$ in the mono-parametric model in Eq. (1) is positive indicating thereby that antibacterial activity of hexahydroindazoles derivatives against *S. aureus* is directly proportional to the magnitude of $\kappa\alpha_1$. The antibacterial activity

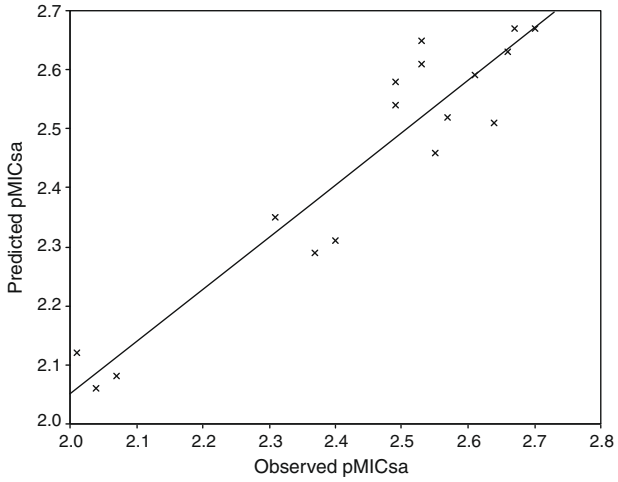


**Figure 1.** Plot of predicted pMICsa values against the experimental pMICsa values for the linear regression developed model by Eq. (1).

increases with an increase in magnitude of $\kappa\alpha_1$. This is evidenced by the values of $\kappa\alpha_1$ in Table 3. The values of $\kappa\alpha_1$ for compounds **6** and **20** were 20.22 and 20.24, respectively. These are higher than the $\kappa\alpha_1$ value of other compounds, which make them to be the most active compounds against *S. aureus*. Similarly the compounds **1** and **2** having the minimum $\kappa\alpha_1$ values of 10.78 and 10.44 have minimum activity against *S. aureus*. Similar trend was observed in case of *B. subtilis, C. albicans* and *A. niger* with $^{2}\chi^{v}$, *E. coli* and *P. aeruginosa* with $^{2}\chi$.

In order to confirm our results, we have constituted a test set consisting of 4 hexahydroindazole derivatives viz. **4, 8, 13** and **18** and predicted their activities using the model expressed by Eqs. (1)–(6) and compared them with the observed values. We have also applied the same model to predict the activity of training set and the prediction results of QSAR models (Supplementary Table 1) indicated that the observed and the estimated activities are very close to each other evidenced by low values of residual activity. Further the plot of linear regression predicted pMICsa values against the observed pMICsa values also favors the model expressed by Eq. (1) (Fig. 1). In Figure 2, the propagation of the residuals on both sides of zero indicates that no systemic error exists in the development of linear regression model as suggested by Jalali-Heravi and Kyani.[41] The cross-validation of the models ($q^2 > 0.5$) was also done by leave one out (LOO) technique.[42]

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of antimicrobial activities of the synthesized compounds is within one order of magnitude. But it is important to note that the predictability of the QSAR models developed in the present study is highly evidenced by the low residual values (Supplementary Table 1). This is in accordance with results suggested by the Bajaj et al.,[43] who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range. Further, recent literature reveals that the QSAR has been applied to describe the relationship between narrow range of biological activity and physicochemical properties of the molecules.[5,21,44,45] When biological activity data lies in the narrow range, the presence of minimum standard deviation observed between the entire antimicrobial data against a particular species justifies its use in QSAR studies.[16,21] The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies it use in QSAR studies.

In conclusion, the present study revealed that the compounds **6** and **20** exhibited appreciable antimicrobial activity. Further, the

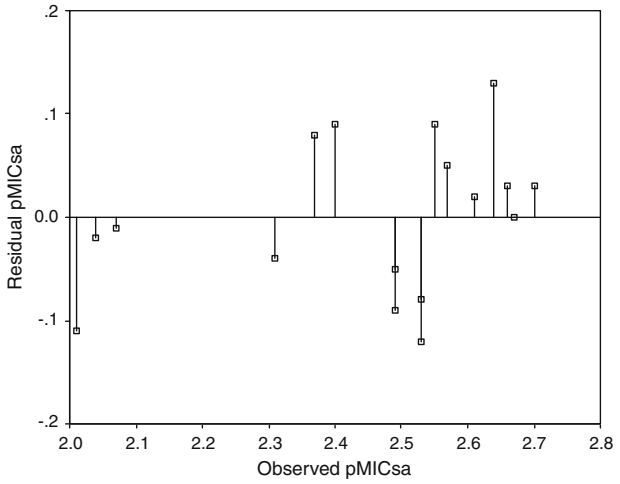


**Figure 2.** Plot of residual pMICsa values against the experimental pMICsa values for the linear regression developed model by Eq. (1).

results of QSAR studies indicated that the topological parameters, the second order molecular connectivity index and valence second order molecular connectivity index, $^{2}\chi$ and $^{2}\chi^{v}$ can be used successfully for modeling antimicrobial activity of hexahydroindazoles. The validity of models obtained by linear and multiple linear regressions are clearly evidenced by the high $q^2$ values obtained for the developed QSAR models.

Melting points were determined in open glass capillary using Bells India melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded with Shimadzu 8400S-FTIR spectrophotometer in KBr discs. The $^{1}$H NMR spectra in $CDCl_3$ were recorded on Bruker-DPX 300 NMR spectrophotometer using TMS as an internal standard. Mass spectra were measured on a Shimadzu 2010A spectrophotometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer and values were within the acceptable limits of the calculated values (within ± 0.4%). The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on Silica Gel-G coated aluminum plates, visualized by iodine vapour and UV light. Developing solvent was chloroform/methanol (9.8:0.2).

*General procedure for synthesis of 3-substituted-3,3a,4,5,6,7-hexahydro-2H-indazoles* (**1**–**3**). The solution of hydrazine hydrate (0.03 mol) and appropriate *p*-substituted benzylidene cyclohexanones (0.01 mol) in methanol (200 ml) was refluxed for 2–3 h. The above reaction mixture was cooled and kept at 0 °C for 24 h. The precipitated product was filtered, washed with methanol and recrystallized from a mixture of methanol–hydrazine hydrate.

*General procedure for the synthesis of 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2H-indazoles* (**4**–**21**). To the solution of **1–3** (0.004 mol) in pyridine (20 mL) was added an equimolar quantity of appropriate sulfonyl chlorides, and the mixture was heated on a water bath for 2–4 h. Then the reaction mixture was cooled, poured into dilute HCl and the solid thus obtained was filtered, washed with water and recrystallized from alcohol.

*Evaluation of antimicrobial activity*. The synthesized compounds were evaluated for their in vitro antimicrobial activity using tube dilution method.[28–30] In this method, 1 mL of 10 μg/mL of test solution in DMSO was transferred to a sterile test tube containing 1 mL of sterile nutrient media and serially diluted to give a concentration of 5, 2.5, 1.25, 0.625, 0.312 μg/mL. To all the tubes, 0.1 mL of microbial suspension in saline was added and the tubes were incubated at 37 °C for 24 h (bacteria) and 48 h for *C. albicans* and at 25 °C for 7 d in case of *A. niger* (fungi). After the incubation period the tubes were observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity. From the MIC values observed, the intermediate concentrations between MIC values were prepared and the accurate MIC values were determined.

*QSAR analysis*. The calculation[34–40] of molecular descriptors of hexahydroindazole derivatives as well as the regression analysis was carried out by using the molecular package TSAR 3D version 3.3.[46]

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.052.

## References and notes

1. Fidler, D. F. *Emerg. Infect. Dis.* **1998**, *4*, 169.
2. Oren, I.; Temiz, O.; Yalcin, I.; Sener, E.; Altanlar, N. *Eur. J. Pharm. Sci.* **1998**, *7*, 153.
3. Hong, C. Y. J. *Farmaco* **2001**, *56*, 41.
4. Macchiarulo, A.; Constantino, G.; Fringuelli, D.; Vecchiarelli, A.; Schiaffella, F.; Fringuelli, R. *Bioorg. Med. Chem.* **2002**, *10*, 3415.
5. Hatya, S. A.; Aki-sener, E.; Tekiner-Gulbas, B.; Yildiz, I.; Temiz-Arpaci, O.; Yalcin, I.; Altanlar, N. *Eur. J. Med. Chem.* **2006**, *41*, 1398.
6. Raikova, S. V.; Shub, G. M.; Luneva, I. O.; Golikov, A. G.; Bugaev, A. A.; Kriven'ko, A. P. *Antibiot. Khimioter.* **2005**, *50*, 18.
7. Raikova, S. V.; Shub, G. M.; Golikov, A. G.; Kriven'ko, A. P. *Antibiot. Khimioter.* **2004**, *49*, 21.
8. Chuanmin, Q.; Yulan, L.; Zhizhong, J. *Zhongguo Yaowu Huaxue Zazhi* **1997**, *7*, 88.
9. Nasr, M. N. A.; Said, S. A. *Archiv der Pharmazie* **2003**, *336*, 551.
10. Minu, M.; Thangadurai, A.; Wakode, S. R.; Agrawal, S. S.; Narasimhan, B. *Archiv der Pharmazie* **2008**, *341*, 231.
11. Narasimhan, B.; Kothwade, U. R.; Pharande, D. S.; Mourya, V. K.; Dhake, A. S. *Indian J. Chem.* **2003**, *42*, 2828.
12. Narasimhan, B.; Belsare, D.; Pharnde, D.; Mourya, V.; Dhake, A. *Eur. J. Med. Chem.* **2004**, *39*, 827.
13. Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3023.
14. Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Pharm. Chem. J.* **2007**, *41*, 133.
15. Narasimhan, B.; Rakesh, N.; Vikramjeet, J.; Ruchita, O.; Sucheta, O. *ARKIVOC* **2007**, *XV*, 112.
16. Narasimhan, B.; Vikramjeet, J.; Rakesh, N.; Ruchita, O.; Sucheta, O. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5836.
17. Gangwal, N. A.; Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Indian J. Het. Chem.* **2003**, *12*, 201.
18. Narasimhan, B.; Ansari, A. H.; Singh, N.; Mourya, V. K.; Dhake, A. S. *Chem. Pharm. Bull.* **2006**, *54*, 1067.
19. Narasimhan, B.; Kumari, M.; Jain, N.; Dhake, A. S.; Sundaravelan, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4951.
20. Narasimhan, B.; Kumari, M.; Dhake, A. S.; Sundaravelan, C. *ARKIVOC* **2006**, *xiii*, 73.
21. Kumar, A.; Narasimhan, B.; Kumar, D. *Bioorg. Med. Chem.* **2007**, *15*, 4113.
22. Narasimhan, B.; Dhake, A. S.; Mourya, V. K. *ARKIVOC* **2007**, *1*, 189.
23. Ruchita, O.; Sucheta, O.; Vikramjeet, J.; Rakesh, N.; Ahuja, M.; Narasimhan, B. *ARKIVOC* **2007**, *XIV*, 172.
24. Kabli, R. A.; Kaddah, A. M.; Khalil, A. M.; Khalaf, A. A. *Indian J. Chem.* **1986**, *25*, 152.
25. Khalaf, A. A.; El-Shafei, A. K.; El-Sayed, A. M. *J. Heterocycl. Chem.* **1982**, *19*, 609.
26. Ozbach, G. Y.; Scab, D. *Acta Chim. (Budapast)* **1975**, *86*, 449.
27. Sayed, G. H. *Indian J. Chem.* **1980**, *19*, 364.
28. Nester, W. E.; Roberts, C. E.; Nester, M. T. In *Microbiology—A Human Perspective*; Wm. C. Brown Communications Inc: Dubuque, 1995; pp 655–656.
29. Collins, C. H.; Lyne, P. M. In *Mirobiol. Methods*, 3rd ed.; Butterworth: London, 1970; pp 414–427.
30. Cappucino, J. G.; Sherman, N. In *Microbiology—A Laboratory Manual*, 4th ed.; Addison Wesley Longman Inc.: California, 1999; pp 263–265.
31. Pharmacopoeia of India; Ministry of Health Department: Govt. of India: New Delhi, 1996; Vol. II, pp A-100-124.
32. Sharma, P.; Rane, N.; Gurram, V. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4185.
33. Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.

34. Pinheiro, A. A. C.; Borges, R. S.; Santos, L. S.; Alves, C. N. *J. Struct. Mol. (Theochem)* **2004**, *672*, 215.
35. Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
36. Kier, L. B.; Hall, L. H. In *Molecular Connectivity in Chemistry and Drug Research*; Academic Press: New York, 1976; pp 129–145.
37. Randic, M. *J. Am. Chem. Soc.* **1975**, *97*, 6609.
38. Balaban, A. T. *Chem. Phys. Lett.* **1982**, *89*, 399.
39. Wiener, H. *J. Am. Chem.* **1947**, *69*, 17.
40. Randic, M. *Croat. Chem. Acta.* **1993**, *66*, 289.
41. Jalali-Heravi, M.; Kyani, A. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1328.
42. Wold, S.; Erikkson, L. *Statistical validation of QSAR results, Chemometrics Methods in Molecular Design*; VCH Publishers: New York, 1995. pp 309–312.
43. Bajaj, S.; Sambi, S. S.; Madan, A. K. *Croat. Chem. Acta.* **2005**, *78*, 165.
44. Sharma, P.; Kumar, A.; Sharma, M. *Eur. J. Med. Chem.* **2006**, *41*, 833.
45. Kumar, A.; Sharma, P.; Gurram, V. K.; Rane, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2484.
46. TSAR 3D Version 3.3, Oxford Molecular Limited, 2000.