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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial evaluation, ot-QSAR and mt-QSAR studies of 2-amino benzoic acid derivatives

Kuldeep Mahiwal · Pradeep Kumar · Balasubramanian Narasimhan

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Abstract A series of 2-amino benzoic acid derivatives (**1**–**28**) were synthesized and evaluated for their in vitro antimicrobial activity against the panel of Gram positive, Gram negative bacterial and fungal strains. The results of antimicrobial studies indicated that, in general, the synthesized compounds were found to be bacteriostatic and fungistatic in action. QSAR studies performed by the development of one target and multi target models indicated that multi-target model was effective in describing the antimicrobial activity as well demonstrated the effect of structural parameters viz. LUMO, $^{3}\chi^{v}$ and *W* on antimicrobial activity of 2-amino benzoic acid derivatives.

Keywords 2-Amino benzoic acid derivatives · Antibacterial · Antifungal · QSAR

Introduction

The incidence of invasive microbial infections caused by opportunistic pathogens, often lead to a large number of people to death every year. Over the past few years, patients that become severely immunocompromised, because of underlying diseases such as leukemia or recently acquired immunodeficiency syndrome or patients who undergo cancer chemotherapy or organ transplantation are particularly susceptible to opportunistic fungal infections.

The number of cases of multidrug resistant bacterial infections is increasing at an alarming rate. Clinicians have to become reliant on few antimicrobial drugs available in the market but that is not sufficient as microbial species are getting resistant very fastly. In order to meet these challenges there is need for the development of novel antimicrobial drugs to which the microbes have never been presented before (Emami *et al.*, 2008).

It has been well documented that 2-amino benzoic acid derivatives exhibit broad spectrum of activity including antiviral (Selvam *et al.*, 2008), anticancer (Cocco *et al.*, 2004), anti-alzheimer (Simons *et al.*, 2009), antiallergic (Inglis *et al.*, 2007), diuretic (Dambrosio *et al.*, 1965) and insecticidal (Raman *et al.*, 2008) activities.

Quantitative structure activity relationship (QSAR) is a methodology mostly used to correlate properties (such as biological activities) with chemical structures. It is a statistically validated mathematical model of correlation between the chemical structures and their activity profiles (Sperandio *et al.*, 2004).

In light of above facts and in continuation of our research efforts in development of novel antimicrobial drugs (Minu *et al.*, 2008; Narasimhan *et al.*, 2003, 2004, 2006a, b, 2007a, b, c), we hereby report the synthesis, antimicrobial evaluation and QSAR studies of a series of 2-amino benzoic acid derivatives.

Experimental

Starting materials were obtained from commercial sources (HiMedia Chemicals, Mumbai and LOBA Chemie, Mumbai) and were used without further purification. Reaction progress was observed by thin layer chromatography (TLC) making use of commercial silica gel plates (Merck). Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined by

K. Mahiwal · P. Kumar · B. Narasimhan (✉)
Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124001, India
e-mail: naru2000us@yahoo.com

Bruker Avance II 400 NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). NMR data are given as multiplicity (s singlet, d doublet, t triplet, m multiplet) and number of protons. Infrared (IR) spectra were recorded on a Perkin Elmer FTIR spectrometer.

General procedure for synthesis of amides/anilides derivatives of 2-amino benzoic acid (**1**–**20**)

The solution of corresponding amine/aniline (0.1 mol) in ether (50 ml) was added drop wise to a solution of 2-amino benzoyl chloride (0.1 mol) in ether (50 ml) maintained at 0–10°C temperature. The solution was stirred for 30 min and the precipitated amide was separated by filtration. The crude amide was recrystallized with alcohol. In case of anilides, the precipitated crude anilide was treated with 5% hydrochloric acid, 4% sodium carbonate and water to remove residual aniline and the resultant anilide was recrystallized with alcohol.

Procedure for synthesis of 8-hydroxy quinoline ester of 2-amino benzoic acid (**21**)

For the preparation of 2-amino benzoyl chloride, thionyl chloride (0.3 mol) was added gradually to 2-amino benzoic acid (0.25 mol) in a round bottom flask. After addition of thionyl chloride, the mixture was stirred for 4 h and heated to 80°C for 30 min in water bath. The excess of thionyl chloride was removed by distillation. A solution of 8-hydroxy quinoline (0.05 mol) in ether (50 ml) was added to a solution of 2-amino benzoyl chloride (0.05 mol) in ether (50 ml). The mixture was heated on a water bath until no further evolution of hydrogen chloride was observed and completion of reaction was checked by single spot TLC. The mixture was cooled to room temperature and evaporation of solvent yielded the crude product which was purified by recrystallization with alcohol.

General procedure for synthesis of ester derivatives of 2-amino benzoic acid (**22**–**28**)

A mixture of 2-amino benzoic acid (0.08 mol) and appropriate alcohol (0.74 mol) was heated under reflux in presence of sulphuric acid (Scheme 2) till the completion of reaction which was checked by single spot TLC. Then the reaction mixture was poured in 200 ml ice cold water, neutralized with sodium bicarbonate solution followed by the extraction of ester with ether (50 ml). The ether layer was separated, which on evaporation yielded the ester derivatives of 2-amino benzoic acid.

Compound 2: M.P. (°C) 258–260; Yield 34.45%; ^{1}H NMR (DMSO): 3.67 (s, 3H, OCH3), 7.17–8.05 (m, 8H of ArH); IR (KBr pellets, cm^{-1}): 1609.5 (C=O str., secondary amide), 3464.56 (N–H sym. str., amine), 1772.92 (C–H out of plane bending of Ar), 1290.77 (C–N str., N–H bending of sec., amide), 1388.83 (C–N str., aryl primary amine), 1511.41 (N–H in plane bending of sec., amide), 1021.08 (CH in plane bending of phenyl ring), 775.92 (C–H out of plane bending of 1,2 disubstituted benzene ring); **Compound 10**: M.P. (°C) 248–250; Yield 48.46%; ^{1}H NMR (DMSO): 5.21 (s, 2H, NH_2 of $ArNH_2$), 8.51 (s, 1H, H of CONH), 7.06–8.02 (m, 7H, H of ArH); IR (KBr pellets, cm^{-1}): 1768.64 (C–H out of plane bending of Ar), 1613.57 (C=O str., secondary amide), 1499.16 (N–H in plane bending of secondary amide), 1213.16 (C–F str.); **Compound 12**: M.P. (°C) 178–180; Yield 37.09%; ^{1}H NMR (DMSO): 7.17–8.63 (m, 4H, H of ArH), 3.07–4.70 (m, 8H of morpholine); IR (KBr pellets, cm^{-1}): 1772.92 (C–H out of plane bending of Ar), 1609.48 (C=O str. of tertiary amide), 1033.34 (ring str., of morpholine); 771.83 (C–H out of plane bending of 1,2 disubstituted benzene ring); **Compound 16**: M.P. (°C) 183–185; Yield 37.59%; ^{1}H NMR (DMSO): 3.40 (s, 2H, H of $ArNH_2$), 6.17–8.98 (m, 4H of ArH); IR (KBr pellets, cm^{-1}): 3460.47 (N–H asym. of primary amine), 3354.24 (N–H sym. str., of primary amine), 1768.84 (C–H out of plane bending of Ar), 1650.34(C=O str., primary amide), 1605.39 (N–H in plane bending of primary amine), 1572.71 (N–H in plane bending primary amide), 1507.33 (C=C str., skeletal of Ar), 1155.93 (C–H in plane bending of phenyl ring); **Compound 28**: B.P. (°C) 105–107; Yield 82.7%; ^{1}H NMR (DMSO): 3.46 (s, 2H, H of $ArNH_2$), 7.31–7.79 (m, 4H of ArH); IR (KBr pellets, cm^{-1}): 1654.43 (C=O str., ester), 1209.04 (C–N str., aryl primary amine), 1115.06 (C–O–C str., of aromatic ester), 1037.43 (sym. str., of aryl ester).

Antimicrobial evaluation

Antibacterial assay

A 24 h fresh culture was obtained by inoculation of respective bacteria in double strength nutrient broth-I.P. followed by incubation at 37 ± 1°C. The stock solution of synthesized 2-amino benzoic acid derivatives was serially diluted in tube containing 1 ml of sterile double strength nutrient broth-I.P. to get a concentration of 50–1.56 µg/ml and then inoculated with 100 µl of suspension (with a count of 10^5 cfu/ml) of respective microorganisms (Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative *Escherichia coli*) in sterile saline. The inoculated tubes were incubated at 37 ± 1°C for 24 h and minimum inhibitory concentration (MIC) was determined.

Antifungal assay

The antifungal activity of synthesized 2-amino benzoic acid derivatives against the fungal species *Candida albicans* and *Aspergillus niger* was determined by serial dilution method similar to antibacterial assay using Sabouraud dextrose broth-I.P. The inoculated tubes were incubated at 37 ± 1°C and 25 ± 1°C for a period of 2 and 7 days in case of *C. albicans* and *A. niger*, respectively, and minimum inhibitory concentration (MIC) was determined.

Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by subculturing on fresh medium 100 µl of culture from each tube that remained clear in the MIC determination. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction (Rodriguez-Arguelles *et al.*, 2005).

QSAR studies

The calculations of molecular descriptors as well as regression analysis were carried by using molecular package TSAR 3D version 3.3 (2000). The description of these descriptors is available in the literature (Hansch *et al.*, 1973; Kier and Hall, 1976; Randic, 1975, 1993; Balaban, 1982; Wiener, 1947).

The predictive powers of the equations were validated by leave one out (LOO) cross-validation method (Agrawal *et al.*, 2006), where a model is built with $N - 1$ compounds and Nth compound is predicted. Each compound is left out of the model derivation and predicted in turn. An indication of the performance is obtained from cross-validated r^2 method which is defined as

$$q^2 = 1 - \Sigma\left(Y_{\text{predicted}} - Y_{\text{actual}}\right)^2 / \Sigma\left(Y_{\text{actual}} - Y_{\text{mean}}\right)^2$$

where, $Y_{\text{predicted}}$, Y_{actual} and Y_{mean} are predicted, actual and mean values of target property (pMIC), respectively. $\Sigma(Y_{\text{predicted}} - Y_{\text{actual}})^2$ is predictive residual error sum of squares.

Results and discussion

A series of 2-amino benzoic acid derivatives (**1**–**28**) were synthesized by following the general pathway depicted in Schemes 1 and 2. The amide/anilide derivatives (**1**–**20**) of 2-amino benzoic acid were prepared by addition of amines/anilines to a solution of 2-amino benzoyl chloride. The ester derivative (**21**) having the quinoline nucleus was obtained by the reaction of 2-amino benzoyl chloride with 8-hydroxy quinoline. The esters of 2-amino benzoic acid (**22**–**28**) were prepared by reaction of 2-amino benzoic acid with appropriate alcohol in presence of sulphuric acid. The physiochemical characteristics of synthesized 2-amino benzoic acid derivatives (**1**–**28**) are presented in Table 1.

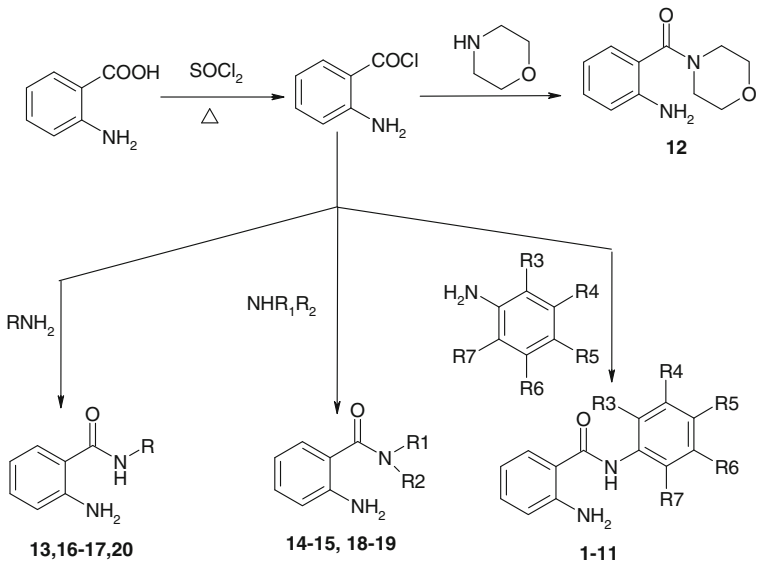
The synthesized compounds were characterized by their consistent IR and NMR spectral characteristics. The appearance of IR bands around 1630–1610 cm^{-1} indicates the formation of secondary amides and anilides. The appearance of aromatic ring is indicated by the IR bands at 775.92, 1021.08, 1772.92 cm^{-1} in case of compound **2**. The formation of anilides by the reaction of 3-chloro, 4-fluoro aniline is confirmed by the appearance of IR bands corresponding to C–F stretch around 1213.13 cm^{-1} apart from the appearance of C–H stretch of aromatic ring at 1768.84 cm^{-1} in case of compound **10**. The formation of tertiary amide containing morpholine (**12**) was confirmed by the appearance of IR peak at 1033.34 cm^{-1} which corresponds to morpholine ring in addition to IR peaks for tertiary amide and primary amino group of anthranilic acid. The formation of primary amide (**16**) was demonstrated by the shifting of IR band towards higher wave number 1650.34 cm^{-1} from 1630–1610 cm^{-1} appeared in case of secondary amide. Further, the formation of compound **16** was indicated by the appearance of IR peak at 1605.39 cm^{-1} and 3460.47 cm^{-1} which corresponds to NH in plane bending of primary amine and NH stretch of primary amide, respectively. The formation of aromatic esters is confirmed by the appearance of C–O–C stretch of aromatic esters at 1115.06 cm^{-1} along with the IR C=O stretch for esters.

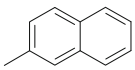
The ^{1}H NMR spectra of synthesized compounds showed a multiplet signal at δ 7.02–8.98 ppm corresponding to the presence of aromatic nucleus. The singlet signal for the methoxy group in compound **2** was found at δ 3.67 ppm. The presence of aromatic amino group in the synthesized compounds was evidenced by appearance of singlet signal around δ 3.40 ppm. A multiplet signal at δ 3.07–4.70 ppm demonstrated the presence of morpholine nucleus in compound **12**.

The synthesized 2-amino benzoic acid derivatives were screened in vitro for their antibacterial activity against Gram positive *S. aureus*, *B. subtilis* and Gram negative *E. coli* and in vitro antifungal activity against *C. albicans* and *A. niger* by tube dilution method (Cappucino and Sherman, 1999). Double strength Nutrient broth-I.P. and Sabouraud dextrose broth-I.P (Pharmacopoeia of India, 2007) have been employed as media for growth of bacterial and fungal species, respectively. The results of antimicrobial activity are presented in Table 2.

In case of *S. aureus* compound **21** was found to be active having pMIC value of 1.93 (Table 2). Against *B. subtilis* compounds **10**, **14** and **20** emerged as most active ones with

Scheme 1 Scheme for synthesis of amide/anilide derivatives of 2-aminobenzoic acid



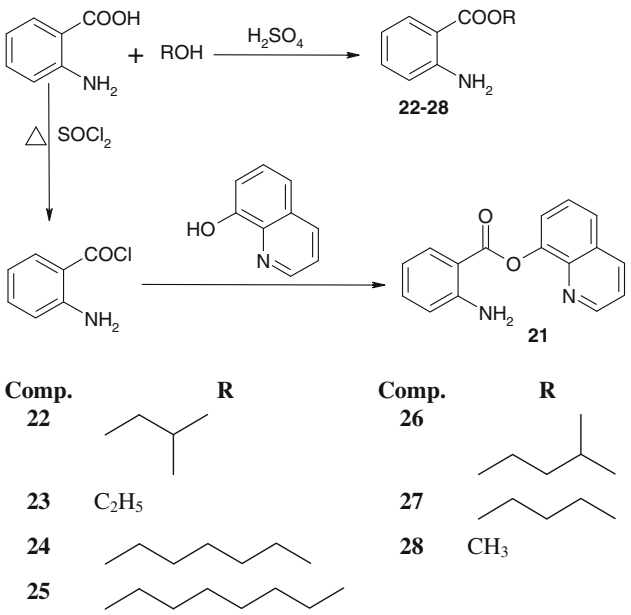
Comp.	R	R1	R2	R3	R4	R5	R6	R7
1	-	-	-	CH_3	H	H	H	H
2	-	-	-	H	H	OCH_3	H	H
3	-	-	-	H	CH_3	H	H	H
4	-	-	-	OCH_3	H	H	H	H
5	-	-	-	H	H	CH_3	H	H
6	-	-	-	CH_3	H	H	CH_3	H
7	-	-	-	CH_3	H	CH_3	H	H
8	-	-	-	H	H	H	H	H
9	-	-	-	H	Cl	H	H	H
10	-	-	-	H	Cl	F	H	H
11	-	-	-	F	H	H	H	H
13	C_3H_7	-	-	-	-	-	-	-
14	-	C_6H_5	C_6H_5	-	-	-	-	-
15	-	C_2H_5	C_2H_5	-	-	-	-	-
16	H	-	-	-	-	-	-	-
17	C_4H_9	-	-	-	-	-	-	-
18	-	C_4H_9	C_4H_9	-	-	-	-	-
19	-	CH_3	CH_3	-	-	-	-	-
20		-	-	-	-	-	-	-

pMIC values of 2.23, 2.27 and 2.23 (Table 2), respectively. Compounds **24** and **25** demonstrated high antibacterial activity against the Gram negative bacteria, *E. coli* with pMIC values 1.85 and 1.88 (Table 2), respectively.

For antifungal activity against *C. albicans*, compounds **21** and **25** exhibited their antifungal potential at pMIC values 1.63 and 1.58, respectively, which is high in comparison to the antifungal activity of other synthesized 2-amino benzoic acid derivatives. In case of antifungal activity against *A. niger* compounds **23** and **24** were found to be active ones with pMIC values 1.72 and 1.85, respectively. In general, the compound **21**, which has 8-hydroxyquinoline nucleus, has shown appreciable antimicrobial activity against all the microorganisms under test. The idea of coupling heterocyclic moiety to anthranilic acid gave appreciable antimicrobial potential to the anthranilic acid.

The minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) (Table 3) determination results revealed that, in general, the synthesized 2-amino benzoic acid derivatives were bacteriostatic/fungistatic in action except in case of *B. subtilis* where most of the synthesized derivatives were found to be bactericidal in action (a drug is considered to be bacteriosatic/fungistatic when its MBC and MFC values are 3-fold higher than its MIC values) (Emami *et al.*, 2004).

From the aforementioned antimicrobial activity results following structure–activity relationship (SAR) of 2-amino benzoic acid derivatives can be deduced:



Scheme 2 Scheme for synthesis of ester derivatives of 2-aminobenzoic acid

1. Compound **21** (2-amino-benzoic acid quinolin-8-yl ester) was found to be active against *S. aureus.* This indicates that heterocyclic nucleus quinoline is essential for an anthranilic acid derivative to be effective against *S. aureus.*
2. In contrast to *S. aureus*, the esters of saturated alcohols, 2-amino-benzoic acid hexyl ester (**24**) and 2-amino-benzoic acid heptyl ester (**25**) are effective against *E. coli.*
3. In case of antifungal activity against *C. albicans* and *A. niger*, the anthranilic acid requires esterification.
4. The bulky aromatic substitution in the anilide portion is essential for antibacterial activity of anthranilic acid derivatives against *B. subtilis.*
5. It is important to note here that compound **10** that has a single phenyl group attached to amide nitrogen is as equally active as compound **14** and **20** with bulky aromatic group against *B. subtilis.* This higher activity of compound **10** may be attributed to the presence of electron withdrawing group, i.e. F and Cl in compound **10**. The role of electron withdrawing groups improving antibacterial activity is supported by the findings of Sharma *et al.* (2004).
6. In general the esters are more active than the amides and anilides against the tested panel of microorganisms. The anilides were active against *B. subtilis*.
7. The amide formation does not improve the antimicrobial profile of 2-amino benzoic acid as none of the synthesized amides were found to be active.
8. The aforementioned facts demonstrated that different compounds are active against different microorganisms. This clearly indicates that different structural requirements are essential for a compound to be effective against different microorganisms which is similar to the results of Sortino *et al.* (2007).

The above findings are summarized in Fig. 1.

QSAR studies

Development of one-target QSAR model

In order to understand the experimental antimicrobial data on theoretical basis, we established a quantitative structure activity relationship (QSAR) between the in vitro antimicrobial activity of 2-amino benzoic acid derivatives and descriptors coding for lipophilic, electronic, steric and topological parameters of the molecules under consideration using the linear free energy relationship model (LFER) described by Hansch and Fujita (1964). Biological activity data determined as MIC values was first transformed into pMIC values on molar basis, which was used as dependent variable in QSAR study. The different molecular descriptors (independent variables) calculated as: log of octanol − water partition coefficient (log P), molar refractivity (MR), Kier's molecular connectivity ($^0\chi$, $^0\chi^v$, $^1\chi$, $^1\chi^v$, $^2\chi$, $^2\chi^v$) and shape (κ_1, $\kappa\alpha_1$) topological indices, Randic topological index (R), Balaban topological index (J), Wiener topological index (W), Total energy (T_e), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment (μ), electronic energy (Ele.E), nuclear energy (Nu.E) and molecular surface area (SA) (Hansch *et al.*, 1973; Kier and Hall, 1976; Randic, 1975, 1993; Balaban, 1982; Wiener, 1947). The structures of 2-aminobenzoic acid derivatives are first pre-optimized with the Eigen-vector following procedure included in TSAR for Windows, and the resulting geometries are further refined by means of the semiempirical method AM-1 (Hamiltonian type). We chose a time limit of 3600 s for the geometry optimization and the electronic parameters T_e, HOMO, LUMO, dipole moment, electronic energy (Ele.E) and nuclear energy (Nu.E) were calculated. The values of selected molecular descriptors used in the QSAR study are presented in Table 4.

In the present study, a data set of 28 synthesized compounds was subjected to linear free energy regression analysis for model generation. Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity against *S. aureus* is presented in Table 5. The high interrelationship was observed between $^1\chi$ and W ($r = 0.989$) and low interrelationship was observed between LUMO and μ as well as $\kappa\alpha_3$ and $^3\chi^v$ ($r = 0.008$).

Table 1 Physicochemical characteristics of 2-amino benzoic acid derivatives

Comp.	Mol. formula	Mol. weight	M.P./ B.P.* (°C)	R_f value (Benzene)	%Yield
1	$C_{14}H_{14}N_2O$	226.28	250–252	0.80	81.39
2	$C_{14}H_{14}N_2O_2$	242.28	258–260	0.71	34.45
3	$C_{14}H_{14}N_2O$	226.28	228–230	0.78	71.37
4	$C_{14}H_{14}N_2O_2$	242.28	240–242	0.68	35.06
5	$C_{14}H_{14}N_2O$	226.28	242–244	0.67	50.48
6	$C_{15}H_{16}N_2O$	240.31	255–257	0.81	61.64
7	$C_{15}H_{16}N_2O$	240.31	230–232	0.77	55.91
8	$C_{13}H_{12}N_2O$	212.35	256–258	0.79	63.26
9	$C_{13}H_{11}ClN_2O$	246.70	244–246	0.89	19.34
10	$C_{13}H_{10}FClN_2O$	264.90	248–250	0.64	48.46
11	$C_{13}H_{11}FN_2O$	230.24	249–251	0.77	25.50
12	$C_{11}H_{14}N_2O_2$	206.25	178–180	0.83	37.09
13	$C_{10}H_{14}N_2O$	178.24	159–161	0.90	35.69
14	$C_{19}H_{16}N_2O$	288.00	141–143	0.75	41.57
15	$C_{11}H_{16}N_2O$	192.00	177–179	0.81	36.56
16	$C_7H_8N_2O$	136.15	183–185	0.78	37.59
17	$C_{11}H_{16}N_2O$	192.15	168–170	0.69	24.71
18	$C_{15}H_{24}N_2O$	248.40	238–240	0.80	25.19
19	$C_9H_{12}N_2O$	164.21	173–175	0.69	55.92
20	$C_{17}H_{14}N_2O$	262.31	208–210	0.73	71.38
21	$C_{16}H_{12}N_2O_2$	264.29	96–98	0.93	69.11
22	$C_{11}H_{15}NO_2$	193.25	135–137	0.83	67.97
23	$C_9H_{11}NO_2$	165.19	134–136	0.80	78.97
24	$C_{13}H_{19}NO_2$	221.30	150–152*	0.70	67.79
25	$C_{14}H_{21}NO_2$	235.23	174–176*	0.68	69.46
26	$C_{12}H_{17}NO_2$	207.27	190–192*	0.85	76.05
27	$C_{11}H_{15}NO_2$	193.25	165–167*	0.88	29.13
28	$C_8H_9NO_2$	151.17	105–107*	0.87	82.70

* Boiling point

The correlations of different molecular descriptors with antimicrobial activity are presented in Table 6. For *S. aureus* the QSAR model (Eq. 1) was developed with topological parameter, first order molecular connectivity index, $^1\chi$ ($r = 0.723$, Tables 5, 6).

ot-QSAR model for antibacterial activity against S. aureus

$$\text{pMIC}_{sa} = 0.094\,{}^1\chi + 0.512 \quad n = 28 \quad r = 0.723$$
$$q^2 = 0.393 \quad s = 0.129 \quad F = 28.53 \qquad (1)$$

Here and thereafter, n is the number of data points, r is the correlation coefficient, q^2 is the cross validated r^2 obtained by LOO method, s is the standard error of the estimate and F is the Fischer statistics.

Being the coefficient of $^1\chi$ in Eq. 1 is positive, the antibacterial activity against *S. aureus* will increase with increase in value of $^1\chi$. This is clearly evident that compounds **14** and **21** having high $^1\chi$ values 10.77 and 9.75, respectively (Table 4) have high pMIC values 1.36 and 1.93, respectively (Table 2). Similarly, compound **19** having low $^1\chi$ value (Table 4) has shown minimum antibacterial activity against *S. aureus.*

In order to improve value of regression coefficient (r), we coupled topological parameter, first order molecular connectivity index, $^1\chi$ with electronic parameter, dipole moment, based on their low inter-relationship between them ($r = 0.365$, Table 5), resulted in marginal improvement in r-value from 0.723 to 0.767 in the developed QSAR model (Eq. 2).

ot-QSAR model for antibacterial activity against S. aureus

$$\text{pMIC}_{sa} = 0.107\,{}^1\chi - 0.050\mu + 0.522 \quad n = 28 \quad r = 0.767$$
$$q^2 = 0.329 \quad s = 0.123 \quad F = 17.82 \qquad (2)$$

Further in search of a better QSAR model, we coupled topological parameter, first order molecular connectivity

Table 2 Antimicrobial activity of 2-amino benzoic acid derivatives

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	1.26	1.86	1.26	1.56	1.56	1.46	1.56	1.50
2	1.29	2.19	1.29	1.29	1.29	1.59	1.29	1.47
3	1.26	2.16	1.26	1.26	1.26	1.56	1.26	1.44
4	1.29	1.59	1.20	1.29	1.29	1.36	1.29	1.33
5	1.26	2.16	1.56	1.26	1.26	1.66	1.26	1.50
6	1.28	2.19	1.28	1.30	1.28	1.58	1.29	1.47
7	1.28	2.19	1.28	1.28	1.28	1.58	1.28	1.46
8	1.23	2.13	1.23	1.23	1.23	1.53	1.23	1.41
9	1.30	2.20	1.60	1.30	1.30	1.70	1.30	1.54
10	1.33	2.23	1.33	1.33	1.02	1.63	1.18	1.45
11	1.27	2.17	1.27	1.27	1.27	1.57	1.27	1.45
12	1.22	1.22	1.52	1.22	1.22	1.32	1.22	1.28
13	1.15	2.06	1.46	1.12	1.15	1.56	1.14	1.39
14	1.36	2.27	1.66	1.36	1.66	1.76	1.51	1.66
15	1.19	2.09	1.19	1.19	1.49	1.49	1.34	1.43
16	1.04	1.94	1.04	1.04	1.64	1.34	1.34	1.34
17	1.20	1.49	1.19	1.19	1.20	1.29	1.20	1.25
18	1.30	2.20	1.60	1.30	1.60	1.70	1.45	1.60
19	0.82	2.02	1.42	0.82	1.42	1.42	1.12	1.30
20	1.32	2.23	1.62	1.32	1.62	1.72	1.47	1.62
21	1.93	1.93	1.80	1.63	1.63	1.89	1.63	1.78
22	0.89	1.79	1.51	0.59	1.06	1.4	0.83	1.17
23	1.12	1.91	1.72	1.12	1.72	1.58	1.42	1.52
24	1.25	2.15	1.85	1.25	1.85	1.75	1.55	1.67
25	1.27	1.88	1.88	1.58	1.58	1.68	1.58	1.64
26	1.22	2.12	1.82	1.22	1.22	1.72	1.22	1.52
27	1.20	2.09	1.79	1.19	1.30	1.69	1.25	1.51
28	1.08	1.99	1.69	1.08	1.08	1.59	1.08	1.38
SD	0.18	0.25	0.24	0.20	0.22	0.15	0.17	0.14
Std.	3.33[a]	3.33[a]	3.33[a]	2.64[b]	2.64[b]	–	–	–

SD standard deviation

[a] Norfloxacin

[b] Fluconazole

index, $^1\chi$ with electronic parameter, LUMO and the change resulted in Eq. 3 having value of regression coefficient 0.794.

ot-QSAR model for antibacterial activity against S. aureus

$$pMIC_{sa} = 0.071\,{}^1\chi - 0.437\ \mathrm{LUMO} + 0.648 \quad n = 28$$
$$r = 0.794 \quad q^2 = 0.339 \quad s = 0.116 \quad F = 21.27 \qquad (3)$$

As coupling of topological parameter, first order molecular connectivity index, $^1\chi$ with electronic parameters, μ and LUMO resulted in improvement in value of regression coefficient (Eqs. 2, 3), we finally coupled both μ and LUMO with $^1\chi$ in order to achieve the best QSAR model with highest value of regression coefficient (Eq. 4).

ot-QSAR model for antibacterial activity against S. aureus

$$pMIC_{sa} = 0.083\,{}^1\chi - 0.383\,\mathrm{LUMO} - 0.038\mu + 0.639 \quad n = 28$$
$$r = 0.816 \quad q^2 = 0.393 \quad s = 0.113 \quad F = 15.96 \qquad (4)$$

The developed QSAR model (Eq. 4) was cross validated by q^2 value ($q^2 = 0.393$) obtained by LOO method. The value of q^2 less than 0.5 indicated that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha who have recently reported that the only way to estimate the true predictive power of a model is to test their ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other (Table 7), the QSAR model for *S. aureus* (Eq. 4) is a valid one (Golbraikh and Tropsha, 2002). The comparison of observed and predicted antibacterial activities is presented in Table 7. It can be seen

Table 3 MBC/MFC values of 2-amino benzoic acid derivatives

Comp.	*S. aureus* MBC	*B. subtilis* MBC	*E. coli* MBC	*C. albicans* MFC	*A. niger* MFC
1	>50	3.12	12.5	>50	12.5
2	12.5	25	12.5	>50	50
3	12.5	12.5	50	>50	50
4	>50	50	>50	50	50
5	>50	1.56	6.25	12.5	50
6	50	1.56	12.5	50	50
7	50	1.56	12.5	>50	50
8	>50	1.56	>50	>50	50
9	12.5	1.56	12.5	12.5	25
10	50	6.25	12.5	>50	>50
11	50	1.56	12.5	12.5	50
12	>50	12.5	50	25	50
13	>50	1.56	50	50	25
14	>50	1.56	50	>50	25
15	>50	1.56	25	25	50
16	>50	1.56	12.5	>50	25
17	>50	6.25	12.5	>50	50
18	>50	25	6.25	>50	50
19	>50	1.56	6.25	50	25
20	>50	1.56	12.5	>50	25
21	6.25	25	12.5	>50	50
22	50	3.12	50	>50	>50
23	25	6.25	6.25	>50	12.5
24	12.5	1.56	3.12	>50	12.5
25	25	3.12	3.12	50	50
26	50	25	3.12	25	50
27	50	1.56	3.12	50	25
28	>50	3.12	12.5	>50	50

from the results that the observed and predicted antimicrobial activities lie close to each other as evidenced by their low residual values. The plot of predicted $pMIC_{sa}$ against observed $pMIC_{sa}$ (Fig. 2) also favours the developed model expressed by Eq. 4. Further, the plot of observed $pMIC_{sa}$ versus residual $pMIC_{sa}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero (Kumar *et al.*, 2007).

Equations 5–7 were developed to predict the antimicrobial activity of synthesized 2-amino benzoic acid derivatives against the *B. subtilis*, *E. coli* and *C. albicans*, respectively.

ot-QSAR model for antibacterial activity against B. subtilis

$$pMIC_{bs} = 0.214\ \log P - 0.125\kappa\alpha_3 + 1.927$$
$$n = 28 \quad r = 0.617 \quad q^2 = 0.187 \quad s = 0.202 \quad F = 7.71 \tag{5}$$

ot-QSAR model for antibacterial activity against E. coli

$$pMIC_{ec} = 0.012\ Te + 0.073\ \log P - 0.496\ LUMO + 1.425$$
$$n = 28 \quad r = 0.752 \quad q^2 = 0.301 \quad s = 0.169 \quad F = 10.44 \tag{6}$$

ot-QSAR model for antifungal activity against C. albicans

$$pMIC_{ca} = 0.118\ {}^1\chi - 0.571\ {}^3\chi^v + 0.532$$
$$n = 28 \quad r = 0.757 \quad q^2 = 0.385 \quad s = 0.138 \quad F = 16.82 \tag{7}$$

In case of *B. subtilis*, the developed QSAR model (Eq. 5) indicated the predominance of lipophilic parameter, log *P* and topological parameter Kier's alpha third order shape index, $\kappa\alpha_3$ in describing the antibacterial activity. The coefficient of log *P* is positive which shows that the antibacterial activity will increase with the increase in log *P* value of the synthesized compounds, which is clearly evident from the results of antibacterial activity against *B. subtilis* (Table 2) and values of log *P* presented in Table 4.

For antibacterial activity against *E. coli*, the developed QSAR model (Eq. 6) describes the importance of total energy (T_e), log *P* and LUMO. In this case a positive correlation was observed between T_e and antibacterial

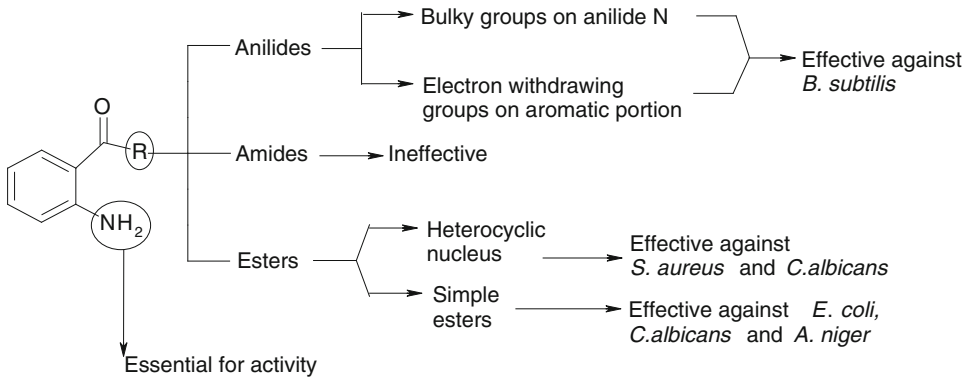


Fig. 1 SAR for antimicrobial activity of 2-amino benzoic acid derivatives

Table 4 Value of QSAR descriptors of 2-amino benzoic acid derivatives used in the present study

Comp.	T_e (eV)	log *P*	MR (m^3 mol^{-1})	$^1\chi$	$^3\chi^v$	$\kappa\alpha_3$	*W*	LUMO (eV)	HOMO (eV)	μ (Debye)
1	−24.16	2.49	69.05	8.18	0.42	2.58	532.00	−0.11	−8.63	2.73
2	−23.31	1.77	70.48	8.70	0.36	3.03	662.00	−0.10	−8.38	3.30
3	−27.77	2.49	69.05	8.16	0.46	2.78	542.00	−0.11	−8.65	1.91
4	−22.27	1.77	70.48	8.72	0.34	2.83	622.00	−0.05	−8.46	2.16
5	−26.97	2.49	69.05	8.16	0.46	2.78	552.00	−0.11	−8.54	2.01
6	−28.16	2.96	74.10	8.58	0.59	2.84	620.00	−0.10	−8.59	2.22
7	−27.50	2.96	74.10	8.58	0.59	2.84	629.00	−0.10	−8.49	2.38
8	−23.59	2.03	64.01	7.77	0.29	2.55	459.00	−0.13	−8.68	2.43
9	−27.98	2.54	68.82	8.16	0.49	2.91	542.00	−0.24	−8.78	3.73
10	−24.18	2.68	69.03	8.58	0.52	2.94	638.00	−0.39	−8.82	4.98
11	−24.75	2.16	64.23	8.18	0.33	2.55	532.00	−0.22	−8.73	1.34
12	4.04	0.25	58.21	7.29	0.31	2.23	362.00	0.05	−8.74	2.27
13	−13.56	1.15	53.51	6.25	0.21	2.65	262.00	0.08	−8.61	1.98
14	9.98	3.95	88.69	10.77	0.41	2.98	984.00	−0.05	−8.66	4.88
15	2.72	1.28	58.63	6.70	0.31	2.41	300.00	0.15	−8.63	2.27
16	−28.73	0.10	39.34	4.72	0.21	1.41	114.00	0.05	−8.64	2.42
17	−13.52	1.55	58.11	6.75	0.21	3.24	336.00	0.08	−8.61	2.05
18	4.34	3.00	76.88	8.70	0.31	4.55	638.00	0.15	−8.63	2.25
19	4.73	0.59	49.13	5.63	0.42	1.91	192.00	0.11	−8.64	2.18
20	−23.19	3.03	80.46	9.74	0.46	2.62	853.00	−0.35	−8.40	2.24
21	11.79	2.11	77.58	9.75	0.40	2.60	813.00	−0.56	−8.62	0.74
22	−7.12	2.21	56.03	6.61	0.61	3.24	325.00	−0.11	−8.53	1.54
23	−5.65	1.34	47.03	5.75	0.20	2.15	201.00	−0.11	−8.52	1.34
24	−4.93	2.99	65.36	7.75	0.20	4.48	527.00	−0.11	−8.53	1.26
25	−4.93	3.39	69.96	8.25	0.20	5.11	646.00	−0.14	−8.60	1.29
26	−7.38	2.53	60.71	7.11	0.61	3.81	412.00	−0.10	−8.52	1.21
27	−4.80	2.20	56.16	6.75	0.20	3.24	336.00	−0.11	−8.53	1.28
28	−4.40	0.99	42.29	5.25	0.20	1.63	152.00	−0.13	−8.55	1.57

Table 5 Correlation matrix for antibacterial activity of 2-amino benzoic acid derivatives against *S. aureus*

	$pMIC_{sa}$	T_e	log *P*	MR	$^1\chi$	$^3\chi^v$	$\kappa\alpha_3$	*W*	LUMO	μ
pMICsa	1.000									
T_e	0.078	1.000								
log *P*	0.425	−0.093	1.000							
MR	0.671	−0.056	0.817	1.000						
$^1\chi$	0.723	−0.056	0.771	0.985	1.000					
$^3\chi^v$	0.086	−0.285	0.398	0.439	0.402	1.000				
$\kappa\alpha_3$	0.209	0.177	0.674	0.460	0.391	0.008	1.000			
W	0.713	−0.051	0.794	0.974	0.989	0.384	0.414	1.000		
LUMO	−0.634	0.182	−0.389	−0.385	−0.481	−0.305	−0.009	−0.508	1.000	
μ	0.028	−0.217	0.218	0.360	0.365	0.280	−0.125	0.363	−0.008	1.000

activity against *E. coli* which reveals that decrease in T_e value (Table 4) will decrease the antibacterial activity against *E. coli.*

The model described by Eq. 7 depicted the importance of first order molecular connectivity index, $^1\chi$ and valence third order molecular connectivity index, $^3\chi^v$ in describing

Table 6 Correlation of molecular descriptors with antimicrobial activity of 2-amino benzoic acid derivatives

	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{an}$	$pMIC_{ca}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
T_e	0.078	−0.235	0.565	−0.071	0.328	0.212	0.167	0.215
log *P*	0.425	0.537	0.348	0.487	0.205	0.658	0.417	0.638
MR	0.671	0.348	0.119	0.644	0.211	0.530	0.511	0.601
$^1\chi$	0.723	0.308	0.126	0.668	0.192	0.534	0.513	0.604
$^3\chi^v$	0.086	0.294	−0.119	−0.057	−0.320	0.132	−0.238	−0.029
$\kappa\alpha_3$	0.209	0.136	0.472	0.318	0.195	0.419	0.312	0.428
W	0.713	0.350	0.173	0.667	0.250	0.579	0.549	0.651
LUMO	−0.634	−0.264	−0.313	−0.440	0.003	−0.580	−0.257	−0.500
HOMO	−0.071	0.047	0.174	−0.104	0.166	0.089	0.043	0.078
μ	0.028	0.266	−0.327	0.122	−0.130	−0.023	−0.010	−0.016

Table 7 Observed and predicted antibacterial activity of 2-amino benzoic acid derivatives against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* using the best ot-QSAR models

Comp.	pMIC sa			pMIC bs			pMIC ec			pMIC ca		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.26	1.26	0.00	1.86	2.14	−0.28	1.26	1.38	−0.12	1.56	1.26	0.30
2	1.29	1.28	0.01	2.19	1.93	0.26	1.29	1.33	−0.04	1.29	1.36	−0.07
3	1.26	1.29	−0.03	2.16	2.11	0.05	1.26	1.33	−0.07	1.26	1.24	0.02
4	1.29	1.30	−0.01	1.59	1.95	−0.36	1.20	1.31	−0.11	1.29	1.37	−0.08
5	1.26	1.29	−0.03	2.16	2.11	0.05	1.56	1.35	0.21	1.26	1.24	0.02
6	1.28	1.31	−0.03	2.19	2.20	−0.01	1.28	1.36	−0.08	1.30	1.21	0.09
7	1.28	1.30	−0.02	2.19	2.20	−0.01	1.28	1.37	−0.09	1.28	1.21	0.07
8	1.23	1.25	−0.02	2.13	2.04	0.09	1.23	1.36	−0.13	1.23	1.29	−0.06
9	1.30	1.27	0.03	2.20	2.11	0.09	1.60	1.40	0.20	1.30	1.22	0.08
10	1.33	1.31	0.02	2.23	2.13	0.10	1.33	1.53	−0.20	1.33	1.25	0.08
11	1.27	1.36	−0.09	2.17	2.07	0.10	1.27	1.40	−0.13	1.27	1.31	−0.04
12	1.22	1.14	0.08	1.22	1.70	−0.48	1.52	1.47	0.05	1.22	1.22	0.00
13	1.15	1.06	0.09	2.06	1.84	0.22	1.46	1.31	0.15	1.12	1.16	−0.04
14	1.36	1.37	−0.01	2.27	2.40	−0.13	1.66	1.86	−0.20	1.36	1.58	−0.22
15	1.19	1.06	0.13	2.09	1.90	0.19	1.19	1.48	−0.29	1.19	1.15	0.04
16	1.04	0.92	0.12	1.94	1.77	0.17	1.04	1.07	−0.03	1.04	0.97	0.07
17	1.20	1.10	0.10	1.49	1.85	−0.36	1.19	1.34	−0.15	1.19	1.22	−0.03
18	1.30	1.22	0.08	2.20	2.00	0.20	1.60	1.62	−0.02	1.30	1.39	−0.09
19	0.82	0.99	−0.17	2.02	1.81	0.21	1.42	1.47	−0.05	0.82	0.96	−0.14
20	1.32	1.50	−0.18	2.23	2.25	−0.02	1.62	1.54	0.08	1.32	1.43	−0.11
21	1.93	1.64	0.29	1.93	2.05	−0.12	1.80	1.99	−0.19	1.63	1.46	0.17
22	0.89	1.17	−0.28	1.79	1.99	−0.20	1.51	1.56	−0.05	0.59	0.97	−0.38
23	1.12	1.11	0.01	1.91	1.94	−0.03	1.72	1.51	0.21	1.12	1.10	0.02
24	1.25	1.28	−0.03	2.15	2.01	0.14	1.85	1.64	0.21	1.25	1.34	−0.09
25	1.27	1.33	−0.06	1.88	2.01	−0.13	1.88	1.68	0.20	1.58	1.40	0.18
26	1.22	1.23	−0.01	2.12	1.99	0.13	1.82	1.57	0.25	1.22	1.03	0.19
27	1.20	1.20	0.00	2.09	1.99	0.10	1.79	1.58	0.21	1.19	1.22	−0.03
28	1.08	1.07	0.01	1.99	1.93	0.06	1.69	1.51	0.18	1.08	1.04	0.04

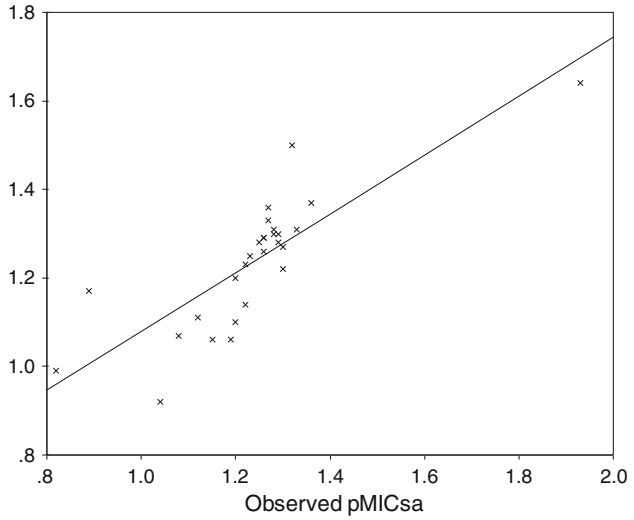


Fig. 2 Plot of predicted $pMIC_{sa}$ against the observed pMICsa for the regression model developed by Eq. 4

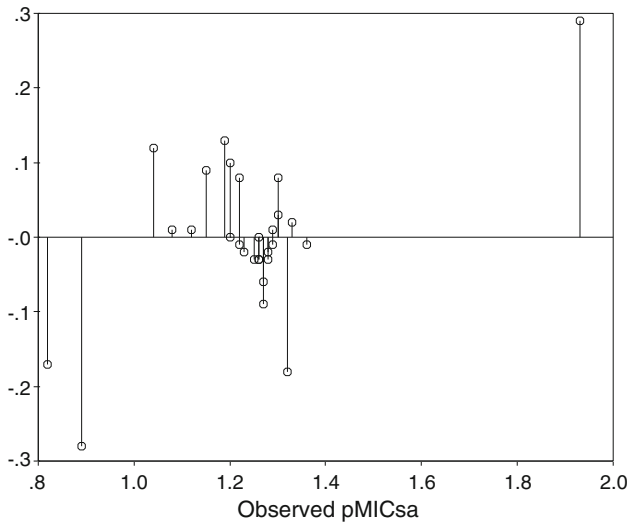


Fig. 3 Plot of residual $pMIC_{sa}$ values against the experimental $pMIC_{sa}$ values

the antifungal activity against *C. albicans*. It is important to note that no significant correlation was found between molecular descriptors of the synthesized compounds and their antifungal activity against *A. niger*.

The validity and predictability of the QSAR models, i.e. Eqs. 5–7 is indicated by high values of their correlation coefficient (r) as well as the low residual values (Table 7).

Topological indices are numerical quantifier of molecular topology and are sensitive to bonding pattern, symmetry, content of heteroatom as well as degree of complexity of atomic neighbourhood (Lather and Madan, 2005). The molecular connectivity topological index, χ signifies the degree of branching, connectivity of atoms and the unsaturation in the molecule which accounts for variation in activity (Gupta *et al.*, 2003).

In the matter of QSAR, the total energy plays important role. Total energy of a molecular system is the sum of the total electronic energy, and the energy of internuclear repulsion (Pasha *et al.*, 2006).

The electronic parameter LUMO, which denotes the energy of lowest unoccupied molecular orbital directly relates to the electron affinity and characterizes the sensibility of the molecule towards an attack by nucleophile. The contribution of LUMO in describing antifungal activity may be attributed to the interaction of 2-amino benzoic acid derivatives with nucleophilic amino acid residue like cysteine of fungi (Kumar *et al.*, 2008).

The importance of dipole moment in modulating antibacterial activity against *S. aureus* may be due to the presence of carbonyl group (C^{+}–O^{-}) where permanent polarization is seen due to electro negativity difference between the atoms. The carbonyl oxygen of substituted benzimidazoles may involve in making fruitful binding interactions with amino acid present at the target site, through hydrogen bonding. The molecular property dipole moment plays a critical role in modulating antibacterial profile of this class of compounds (Pillai *et al.*, 2005).

It is important to note that Eqs. 2–7 were derived using the entire data set as there were no outliers in the data set. In multivariate statistics, it is common to define three types of outliers (Furusjo *et al.*, 2006).

1. *X*/*Y* relation outliers are substances for which the relationship between the descriptors (*X* variables) and the dependent variables (*Y* variables) is not the same as in the (rest of the) training data.
2. *X* outliers are substances whose molecular descriptors do not lie in the same range as the (rest of the) training data.
3. *Y* outliers are only defined for training or test samples. They are substances for which the reference value of response is invalid.

The sample size and the 'rule of thumb' allowed us to go for development of tetra-parametric model in multiple linear regression analysis. The 'rule of thumb' gives information about the number of parameters to be selected for regression analysis in QSAR based on the number of compounds (Narasimhan *et al.*, 2007c).

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. This is in accordance with results suggested by the Bajaj *et al.*, who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range (Bajaj *et al.*, 2005). Further, recent literature reveals that the QSAR have been applied to describe the relationship between narrow range of biological activity

Table 8 Observed and predicted antibacterial, antifungal and antimicrobial activities of 2-amino benzoic acid derivatives using the best mt-QSAR models

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.46	1.61	−0.15	1.56	1.29	0.27	1.50	1.47	0.03
2	1.59	1.55	0.04	1.29	1.41	−0.12	1.47	1.54	−0.07
3	1.56	1.61	−0.05	1.26	1.28	−0.02	1.44	1.46	−0.02
4	1.36	1.53	−0.17	1.29	1.40	−0.11	1.33	1.52	−0.19
5	1.66	1.61	0.05	1.26	1.28	−0.02	1.50	1.46	0.04
6	1.58	1.64	−0.06	1.29	1.24	0.05	1.47	1.44	0.03
7	1.58	1.65	−0.07	1.28	1.24	0.04	1.46	1.44	0.02
8	1.53	1.58	−0.05	1.23	1.34	−0.11	1.41	1.49	−0.08
9	1.70	1.66	0.04	1.30	1.26	0.04	1.54	1.48	0.06
10	1.63	1.73	−0.10	1.18	1.30	−0.12	1.45	1.54	−0.09
11	1.57	1.63	−0.06	1.27	1.35	−0.08	1.45	1.53	−0.08
12	1.32	1.37	−0.05	1.22	1.26	−0.04	1.28	1.40	−0.12
13	1.56	1.44	0.12	1.14	1.27	−0.13	1.39	1.39	0.00
14	1.76	1.71	0.05	1.51	1.58	−0.07	1.66	1.64	0.02
15	1.49	1.42	0.07	1.34	1.23	0.11	1.43	1.35	0.08
16	1.34	1.36	−0.02	1.34	1.18	0.16	1.34	1.33	0.01
17	1.29	1.47	−0.18	1.20	1.32	−0.12	1.25	1.42	−0.17
18	1.70	1.56	0.14	1.45	1.43	0.02	1.60	1.49	0.11
19	1.42	1.38	0.04	1.12	1.09	0.03	1.30	1.27	0.03
20	1.72	1.74	−0.02	1.47	1.46	0.01	1.62	1.65	−0.03
21	1.89	1.74	0.15	1.63	1.48	0.15	1.78	1.70	0.08
22	1.40	1.59	−0.19	0.83	1.05	−0.22	1.17	1.31	−0.14
23	1.58	1.52	0.06	1.42	1.24	0.18	1.52	1.41	0.11
24	1.75	1.65	0.10	1.55	1.44	0.11	1.67	1.55	0.12
25	1.68	1.69	−0.01	1.58	1.51	0.07	1.64	1.60	0.04
26	1.72	1.61	0.11	1.22	1.10	0.12	1.52	1.35	0.17
27	1.69	1.59	0.10	1.25	1.32	−0.07	1.51	1.47	0.04
28	1.59	1.50	0.09	1.08	1.21	−0.13	1.38	1.40	−0.02

and physicochemical properties of the molecules (Narasimhan *et al.*, 2007c; Sharma *et al.*, 2006; Hatya *et al.*, 2006; Kumar *et al.*, 2006). When biological activity data lies in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies (Kumar *et al.*, 2007; Narasimhan *et al.*, 2007c). The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies its use in QSAR studies.

The value of q^2 less than 0.5 indicated that some of the developed models are invalid ones. 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. This is in accordance with the recommendations of Golbraikh and Tropsha, who have recently reported that the only way to estimate the true predictive power of a model is to test their ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other (Tables 7, 8), the developed QSAR models are valid ones (Golbraikh and Tropsha, 2002).

Even though the sample size and the 'rule of thumb' allowed us to go for development of penta-parametric model in multiple linear regression analysis, the high colinearity among the parameters restricted us to go for mono-parametric model in most cases. The multi-colinearity occurs when two independent variables are correlated with each other. One should note that the change in signs of the coefficients, a change in the values of previous coefficient, change of significant variable into insignificant one or an increase in standard error of the estimate on addition of an additional parameter to the model are indications of high interrelationship among descriptors (Kumar *et al.*, 2007).

Development of multi-target QSAR model

According to ot-QSAR models one should use five different equations with different errors to predict the activity of a new compound against the five microbial species. However, very recently the interest has been increased in

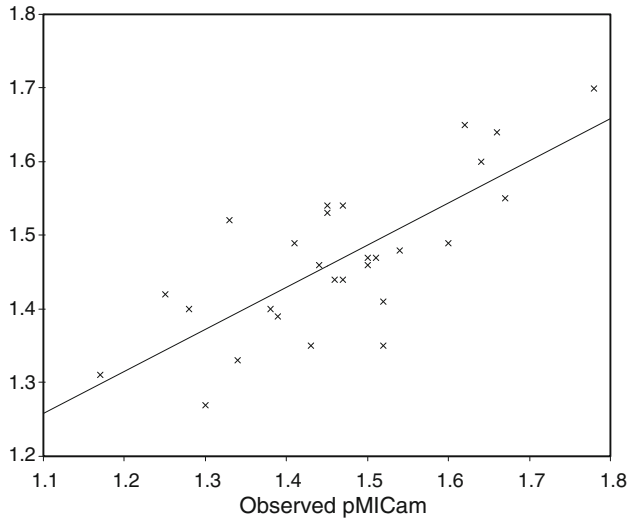


Fig. 4 Plot of predicted $pMIC_{am}$ against the observed $pMIC_{am}$ for the regression model developed by Eq. 10

development of multi-target QSAR (mt-QSAR) models. In opposition to ot-QSAR, the mt-QSAR model is a single equation that considers the nature of molecular descriptors which are common and essential for describing the antimicrobial activity (Prado-Prado *et al.*, 2008; Gonzalez-Diaz *et al.*, 2007, 2008; Cruz-Monteagudo *et al.*, 2007).

In the present study, we have attempted to develop three different types of mt-QSAR models viz. mt-QSAR model for describing antibacterial activity of synthesized compounds against *S. aureus*, *B. subtilis* and *E. coli*, mt-QSAR model for describing antifungal activity of synthesized compounds against *C. albicans* and *A. niger* as well a common mt-QSAR model for describing the antimicrobial activity of 2-amino benzoic acid derivatives against all the aforementioned microorganisms.

In order to develop mt-QSAR models, initially we have calculated the average antibacterial activity, antifungal activity and antimicrobial activity of substituted 2-amino benzoic acid derivatives which are presented in Table 2. These average activity values were correlated with the molecular descriptors of synthesized compounds (Table 4).

The mt-QSAR model of antibacterial activity displayed the importance of log *P* and LUMO (Table 6), in describing the antibacterial activity of 2-amino benzoic acid derivatives.

mt-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.081\ \log P - 0.362\ LUMO + 1.370$$
$$n = 28 \quad r = 0.746 \quad q^2 = 0.336 \quad s = 0.102 \quad F = 15.69 \tag{8}$$

The mt-QSAR model for antifungal activity reveals the importance of Wiener topological index (*W*) and valence third order molecular connectivity index, $^3\chi^v$ in describing antifungal activity.

mt-QSAR model for antifungal activity

$$pMIC_{af} = -0.656\,{}^3\chi^v + 0.0006\ W + 1.251$$
$$n = 28 \quad r = 0.733 \quad q^2 = 0.401 \quad s = 0.122 \quad F = 14.55 \tag{9}$$

The mt-QSAR model of antimicrobial activity (Eq. 10) depicted the importance of Wiener topological index (*W*), valence third order molecular connectivity index, $^3\chi^v$ and LUMO in describing the antimicrobial activity of synthesized 2-amino benzoic acid derivatives.

mt-QSAR model for antimicrobial activity

$$pMIC_{am} = -0.361\,{}^3\chi^v + 0.0004W - 0.248\ LUMO + 1.372$$
$$n = 28 \quad r = 0.756 \quad q^2 = 0.359 \quad s = 0.096 \quad F = 10.71 \tag{10}$$

The Wiener index (*W*) was introduced by Wiener to demonstrate correlations between physicochemical properties of organic compounds and the topological structure of their molecular graphs in terms of sum of distances between any two carbon atoms in the molecules, in terms of carbon–carbon bonds (Wiener, 1947).

The data presented in Table 8 and the plot of observed versus predicted antimicrobial activity (Fig. 4) obtained by the mt-QSAR model (Eq. 10) indicated their predictability as both were close to each other. Further the plot of observed activity and residual antimicrobial activity (Fig. 5) indicated that no systemic error was involved in

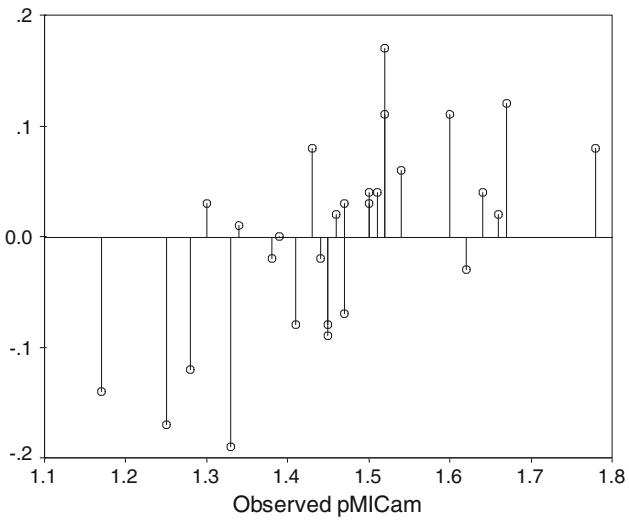


Fig. 5 Plot of residual $pMIC_{am}$ values against the experimental $pMIC_{am}$ values

Table 9 Regression analysis and quality of correlation for modeling antibacterial and antifungal activity of synthesized 2-amino benzoic acid derivatives

S. no.	QSAR model (pMIC=)	*n*	*r*	q^2	*s*	*F*
B. subtilis						
1.	0.141 log *P* + 1.719	28	0.536	0.120	0.212	10.52
E. coli						
2.	0.010 T_e + 1.608	28	0.565	0.217	0.204	12.18
3.	0.011 T_e + 0.104 log *P* + 1.397	28	0.693	0.336	0.182	11.57
4.	0.011 T_e − 0.665 LUMO + 1.561	28	0.705	0.207	0.178	12.41
C. albicans						
5.	0.0961 $^1\chi$ + 0.496	28	0.668	0.352	0.154	20.99
Antibacterial activity						
6.	0.104 log *P* + 1.356	28	0.658	0.373	0.114	19.84
Antifungal activity						
7.	0.0004*W* + 1.089	28	0.549	0.192	0.147	11.53
Antimicrobial activity						
8.	0.0004*W* + 1.262	28	0.650	0.340	0.107	19.10
9.	−0.325 $^3\chi^v$ + 0.0005 *W* + 1.342	28	0.717	0.380	0.100	13.24
10.	0.0005*W* − 0.040 μ + 1.317	28	0.704	0.386	0.101	12.33
11.	0.0003*W* − 0.2011 LUMO + 1.279	28	0.678	0.289	0.105	10.75

the development of mt-QSAR model as the propagation of residuals was found on both the sides of zero.

It was observed from mt-QSAR models (Eqs. 8–10) that the antibacterial activity, antifungal activity and overall antimicrobial activity of synthesized 2-amino benzoic acid derivatives is governed by the structural parameters especially the $^3\chi^v$, LUMO and *W*. The other ot-QSAR and mt-QSAR models derived for antimicrobial activity of 2-amino benzoic acid derivatives are presented in Table 9.

Conclusion

In the present study, 2-amino benzoic acid derivatives (**1**–**28**) were synthesized and evaluated for their in vitro antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *C. albicans* and *A. niger*. The results of antimicrobial studies indicated that, in general, synthesized compounds were found to be bacteriostatic and fungistatic in action. Further, the antimicrobial results of synthesized 2-amino benzoic acid derivatives indicated that different structural requirements are essential for a compound to be selected as antibacterial or antifungal agent. Further, QSAR investigation was performed by development of one target and multi target models. The multi-target model was found to be effective in describing the antimicrobial activity of 2-amino benzoic acid derivatives in comparison to the one target models and indicated the importance of the $^3\chi^v$, LUMO and *W* in describing the antimicrobial activity.

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