Synthesis, antimicrobial and QSAR studies of substituted anilides

Balasubramanian Narasimhan*, Rakesh Narang, Vikramjeet Judge, Ruchita Ohlan, and Sucheta Ohlan

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India
E-mail: naru2000us@yahoo.com

Abstract

A series of substituted anilides were synthesized and tested *in vitro* for antibacterial activity against Gram positive *B. subtilis, S. aureus* and Gram negative *E.coli* and as well as for antifungal activity against *C. albicans* and A. *niger*. The compounds having a nitro group showed better activity among different substituted anilides. QSAR investigation with linear regression analysis was applied to find correlation between various physicochemical parameters and antimicrobial activity. The QSAR results showed that antibacterial as well as antifungal activity could be modeled using molecular connectivity indices ($^0\chi$, $^0\chi^v$ and $^2\chi$). The predictive ability of models was cross validated by observation of the low residual activity values and appreciable cross validated r^2 values (q^2) obtained by leave one out (LOO) technique.

Keywords: Substituted anilides, antimicrobial activity, QSAR, topological descriptors

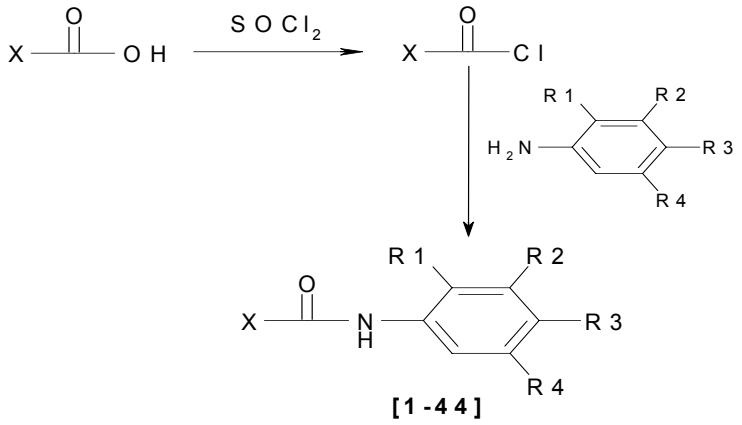
Introduction

The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by unsatisfactory status of present treatment of bacterial and fungal infections and drug side effects[1-3]. Therefore the development of new and different antimicrobial drugs is an important objective and much of research program efforts are directed towards the design of new agents. Recently substituted anilides have received considerable attention due to their wide range of biological activities *viz*. antibacterial, antifungal, anticonvulsant, anaesthetic, antiproliferative, antiplaque, antiplatelet-aggregation, antioxidant and potassium channel activating potentials[4-13].

QSAR analysis applies statistical methods to describe the relationship between chemical structure and biological activities of a series of analogs quantitatively[14]. In view of the above and in continuation to our work on QSAR studies in describing the biological activity[15-22], in the present study we report the synthesis and QSAR studies of substituted anilides.

Results and Discussion

A series of new substituted anilides (**1-44**) were synthesized by reaction of substituted anilines with different acid chlorides in moderate to good yield (Scheme 1). The intermediate acid chlorides were prepared by treatment of different organic acids with thionyl chloride. The IR and ^{1}H NMR spectral data of the synthesized compounds were found in agreement with the assigned molecular structures. The physicochemical parameters of synthesized anilide derivatives used in present study are given in Table 1.



Scheme 1. Scheme for synthesis of substituted anilide derivatives.

1-11, X = C_6H_5 ; **12-22,** X = $CH_3(CH_2)_9CH_2$; **23-33,** X = $CH_3(CH_2)_{11}CH_2$;
34-44, X = $CH_3(CH_2)_{15}CH_2$
1, 12, 23, **34**; R_1, R_2, R_3, R_4 = H
2, 13, 24, 35; R_2, R_3, R_4 = H, R_1= Cl
3, 14, 25, 36; R_1, R_3, R_4 = H, R_2= Cl
4, 15, 26, 37; R_1, R_2.,R_4 = H, R_3 = Cl
5, 16, 27, 38; R_1, R_3, R_4 = H, R_2 = CH_3
6, 17, 28, 39; R_1, R_2, R_4 = H, R_3 = CH_3
7, 18, 29, 40; R_2, R_3, R_4 = H, R_1 = OCH_3
8, 19, 30, 41; R_1, R_2, R_4 = H, R_3 = OCH_3
9, 20, 31, 42; R_1, R_3, R_4 = H, R_2 = NO_2
10, 21, 32, 43; R_1, R_2, R_4 = H, R_3 = NO_2
11, 22, 33, 44; R_1, R_2, R_4 = H, R_3 = Br

The newly obtained derivatives were evaluated for their *in vitro* antibacterial activity against Gram positive *Bacillus subtilis, Staphylococcus aureus,* Gram negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger.* Double strength nutrient broth IP and Sabouraud dextrose broth IP[23] were employed for bacterial and fungal growth

respectively. Minimal inhibitory concentration (MIC) was determined by means of standard serial dilution method[24] using ciprofloxacin and fluconazole as reference compounds in case of antibacterial and antifungal activity respectively and the pMIC values obtained are presented in Table 2. All the compounds showed appreciable *in vitro* antimicrobial activity against the microorganisms under study.

The compound **42** showed significant antimicrobial activity against *B. subtilis, E. coli, S. aureus,* and *A. niger* with pMIC values 1.70, 1.35, 1.50 and 1.51 respectively. The compound **7** showed highest pMIC (pMICca = 1.75) value of all the compounds. Further the antibacterial activity of compounds **20, 21, 31, 35** and **42-44** were found to be more active against *B. subtilis* having pMIC value more than 1.59, than other synthesized substituted anilide derivatives. Compounds **21, 27, 28, 31, 32, 35-37, 39, 42-44** were found to be more active against *S. aureus* having pMICsa more than 1.39, than other synthesized substituted anilide derivatives. Compounds **20, 21, 31, 37, 44** were found to be more active against *E. coli* having pMICec more than 1.38, than other synthesized substituted anilide derivatives. Compounds **13-15, 19-22**, **31, 33-44** were found to be more active against *A. niger* having pMICan more than 1.38, than other synthesized substituted anilide derivatives.

Analysis of results indicates that the presence of an electron withdrawing NO_2 group (compounds **9, 10, 20, 21**, **31, 32, 42, 43**) leads to increase in activity in comparison to the presence of other groups. The importance of electron withdrawing group in enhancing the antimicrobial activity was supported by similar results observed by P. Sharma *et al*.[25]. The antimicrobial results also indicated that there is a significant increase in pMIC values in case of stearic acid derivatives as compared to other acid derivatives. It is also important to note that the range of pMIC value is increasing in each case as the chain length of acid derivatives increases. The positive contribution of chain length may be due to its more lipophilicity.

In general, the antimicrobial activity of tested compounds follows the pattern:

B. subtilis* > A niger > *S. aureus* > *E. coli

In an attempt to determine the role of structural features which appears to influence the observed activity of reported compounds, QSAR studies were undertaken using linear free energy relationship (LFER) model of Hansch and Fujita.[26] Biological activity data determined as MIC values were first transformed to pMIC on molar basis, which was used as dependent variable in QSAR study. These were correlated with different molecular descriptors like log of octanol–water partition coefficient (logP), molar refractivity (MR), Kier's molecular connectivity ($^0\chi^v$) and shape (κ_1,$\kappa\alpha_i$) topological indices, Randic topological index (R), Balban topological index (J), Wiener topological index (W), Total energy (Te), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment (μ), electronic energy (Ele.E) and nuclear energy (Nu.E)[26-33]. The values of selected descriptors used in regression analysis are presented in Table 3.

Table 1. Physicochemical characteristics of substituted anilides

Comp.	M. Formula	M. Wt.	M. p. (°C)	Rf value	% yield
1	$C_{13}H_{11}NO$	197	150-152	0.13	50.1
2	$C_{13}H_{10}NOCl$	231	81-83	0.39	41.2
3	$C_{13}H_{10}NOCl$	231	109-111	0.21	61.7
4	$C_{13}H_{10}NOCl$	231	182-184	0.25	26.5
5	$C_{14}H_{13}NO$	211	107-109	0.22	58.1
6	$C_{14}H_{13}NO$	211	118-120	0.10	68.3
7	$C_{14}H_{13}NO_2$	227	38-40	0.23	71.2
8	$C_{14}H_{13}NO_2$	227	140-142	0.12	55.4
9	$C_{13}H_{10}N_2O_3$	242	147-149	0.16	52.7
10	$C_{13}H_{10}N_2O_3$	242	146-148	0.12	22.2
11	$C_{13}H_{10}NOBr$	276	194-196	0.27	29.2
12	$C_{18}H_{29}NO$	275	52-54	0.46	43.6
13	$C_{18}H_{28}NOCl$	309	44-46	0.26	34.8
14	$C_{18}H_{28}NOCl$	309	127-129	0.33	67.3
15	$C_{18}H_{28}NOCl$	309	49-51	0.04*	23.9
16	$C_{19}H_{31}NO$	289	60-62	0.57*	32.5
17	$C_{19}H_{31}NO$	289	57-59	0.23	27.9
18	$C_{19}H_{31}NO_2$	305	46-48	0.31*	53.3
19	$C_{19}H_{31}NO_2$	305	50-52	0.26	40.0
20	$C_{18}H_{28}N_2O_3$	320	52-54	0.40	39.6
21	$C_{18}H_{28}N_2O_3$	320	41-43	0.11	68.8
22	$C_{18}H_{28}NOBr$	354	36-38	0.30	64.7
23	$C_{20}H_{33}NO$	303	49-51	0.34	42.2
24	$C_{20}H_{32}NOCl$	337	52-54	0.66	51.4
25	$C_{20}H_{32}NOCl$	337	44-46	0.54	93.1
26	$C_{20}H_{32}NOCl$	337	85-87	0.40	71.3
27	$C_{21}H_{35}NO$	317	59-61	0.43	31.9
28	$C_{21}H_{35}NO$	317	49-51	0.26	34.0
29	$C_{21}H_{35}NO_2$	333	53-55	0.41	42.8
30	$C_{21}H_{35}NO_2$	333	87-89	0.77	30.6
31	$C_{20}H_{32}N_2O_3$	348	57-59	0.22	32.6
32	$C_{20}H_{32}N_2O_3$	348	69-71	0.38	36.5
33	$C_{20}H_{32}NOBr$	382	74-76	0.50	50.8
34	$C_{24}H_{41}NO$	359	57-59	0.25**	26.4
35	$C_{24}H_{40}NOCl$	393	65-67	0.71	45.0
36	$C_{24}H_{40}NOCl$	393	50-52	0.61	51.7
37	$C_{24}H_{40}NOCl$	393	55-57	0.80	62.9
38	$C_{25}H_{43}NO$	373	44-46	0.34	72.7

39	$C_{25}H_{43}NO$	373	67-69	0.23**	36.3
40	$C_{25}H_{43}NO_2$	389	43-45	0.34	52.6
41	$C_{25}H_{43}NO_2$	389	54-56	0.36	66.6
42	$C_{24}H_{40}N_2O_3$	404	49-51	0.15	50.8
43	$C_{24}H_{40}N_2O_3$	404	42-44	0.21	69.4
44	$C_{24}H_{40}NOBr$	438	37-39	0.22	24.2

TLC mobile phase - **Toluene: chloroform (1:1), *ethyl acetate: hexane (1:3)

Table 2. The *in vitro* antimicrobial activity of synthesized anilide derivatives

Comp.	pMICbs	pMICsa	pMICec	pMICan	pMICca
1	1.45	1.30	1.09	1.25	1.25
2	1.32	1.22	1.16	1.27	1.32
3	1.39	1.06	1.06	1.32	1.36
4	1.40	1.22	0.97	1.16	1.27
5	1.42	1.12	1.18	1.07	1.23
6	1.40	1.16	1.10	1.23	1.23
7	1.51	1.26	1.01	1.26	1.75
8	1.42	1.10	1.06	1.26	1.32
9	1.44	1.24	1.18	1.29	1.29
10	1.54	1.25	1.24	1.29	1.34
11	1.34	1.14	1.14	1.30	1.44
12	1.50	1.30	1.14	1.18	1.44
13	1.50	1.30	1.20	1.40	1.69
14	1.54	1.30	1.20	1.39	1.49
15	1.50	1.25	1.09	1.39	1.45
16	1.50	1.26	1.21	1.32	1.66
17	1.56	1.20	1.06	1.32	0.68
18	1.44	1.25	1.18	1.34	1.49
19	1.44	1.20	1.18	1.39	1.49
20	1.60	1.35	1.40	1.41	1.51
21	1.60	1.50	1.41	1.41	1.41
22	1.48	1.15	1.15	1.45	1.45
23	1.48	1.30	1.25	1.34	1.39
24	1.51	1.20	1.13	1.38	1.43
25	1.43	1.38	1.19	1.38	1.49
26	1.49	1.30	1.27	1.38	1.49
27	1.46	1.40	1.10	1.36	1.46
28	1.46	1.40	1.16	1.35	1.40
29	1.50	1.29	1.10	1.29	1.40

30	1.50	1.20	1.14	1.30	1.40
31	1.60	1.42	1.40	1.40	1.45
32	1.45	1.40	1.24	1.35	1.50
33	1.49	1.20	1.24	1.38	1.49
34	1.51	1.33	1.30	1.46	0.78
35	1.60	1.50	1.29	1.45	1.50
36	1.50	1.45	1.25	1.55	1.55
37	1.64	1.40	1.39	1.39	1.50
38	1.48	1.27	1.27	1.48	0.73
39	1.48	1.40	1.27	1.43	1.48
40	1.55	1.39	1.25	1.44	1.55
41	1.49	1.29	1.29	1.39	1.55
42	1.70	1.50	1.35	1.51	1.51
43	1.60	1.50	1.35	1.51	0.83
44	1.60	1.44	1.39	1.55	0.86
Std.	3.33*	3.33*	3.33*	2.64**	2.64**

*Ciprofloxacin **Fluconazole bs – *Bacillus subtilis* ec – *Escherichia coli*
sa – *Staphylococcus aureus* an – *Aspergillus niger* ca- *Candida albican.*

In present studies a set of substituted anilides consisting of 44 molecules were used for linear regression model generation. The reference drugs were not included in model generation as they belong to different structural series. Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity against *B. subtilis* is presented in Table 4. The highest interrelationship was observed between $^{1}\chi$ and $^{0}\chi$ (r = 0.998), and in all the other cases r is greater than 0.8.

The data presented in Table 5 demonstrates the correlation of molecular descriptors of different substituted anilides with their corresponding antimicrobial activity. Generally good correlations were observed with the topological descriptors especially with molecular connectivity indices ($^{0}\chi$,$^{0}\chi^{v}$and $^{2}\chi$).

The linear regression equations developed using the highly correlated topological indices are reported in equations 1-4 together with statistical parameters of regression. It is important to note that all these models were developed by using the entire set (n = 44), since no outliers were identified.

The quality of the models is indicated by the following parameters: r- correlation coefficient; F- Fisher's statistics; and s- standard error of estimation, r^2_{cv}- cross-validated r^2 obtained by 'leave one out' (LOO) method.

QSAR model for antifungal activity against *A. niger*

$pMICan = 0.025\ {}^{0}\chi^{v} + 1.003$ (1)

$n = 44 \quad r = 0.835 \quad r^2 = 0.697 \quad F = 97.03 \quad s = 0.055 \quad r^2_{cv} = 0.662$

QSAR model for antibacterial activity against *E. coli*

$pMICec = 0.062\ {}^{2}\chi + 0.675$ (2)

$n = 44 \quad r = 0.767 \quad r^2 = 0.588 \quad F = 60.18 \quad s = 0.071 \quad r^2_{cv} = 0.554$

QSAR model for antibacterial activity against *S. aureus*

$pMICsa = 0.065\ {}^{2}\chi + 0.748$ (3)

$n = 44 \quad r = 0.765 \quad r^2 = 0.585 \quad F = 59.41 \quad s = 0.074 \quad r^2_{cv} = 0.542$

QSAR model for antibacterial activity against *B. subtilis*

$pMICbs = 0.017\ {}^{0}\chi + 0.762$ (4)

$n = 44 \quad r = 0.676 \quad r^2 = 0.457 \quad F = 35.38 \quad s = 0.057 \quad r^2_{cv} = 0.397$

The coefficient of ${}^{0}\chi^{v}$ in the mono-parametric model in Eq. 1 is positive indicating thereby that antifungal activity of different substituted anilides against *A. niger* is directly proportional to the magnitude of ${}^{0}\chi^{v}$. The antifungal activity increases with an increase in magnitude of ${}^{0}\chi^{v}$. This is evidenced by the values of ${}^{0}\chi^{v}$ in Table 3. The values of ${}^{0}\chi^{v}$ for compounds **33-44** lie in range of 17-19 which are higher than the ${}^{0}\chi^{v}$ values of other compounds. This makes them to be the most effective compounds against *A. niger*. Similar trend was observed in case of *E. coli*, *S. aureus*, and *B. subtilis* with ${}^{2}\chi$, ${}^{2}\chi$ and ${}^{0}\chi$ respectively.

In order to confirm our results we have predicted the activities of different substituted anilides using the model expressed by Eqs. 1–4 and compared them with the observed values. The data presented in Table 6 shows that the observed and the estimated activities are very close to each other evidenced by low values of residual activity [difference between experimentally observed pMIC and QSAR calculated pMIC].

The cross-validation of the models was also done by leave one out (LOO) technique.[34] The cross-validated correlation coefficient ($r^2_{cv}>0.5$) values obtained for the best QSAR models indicated their reliability in predicting the antimicrobial activity of different substituted anilides. In case of *B.subtilis* the r^2_{cv} value is less than 0.5 which appears that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh *et al.*[35] 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. The low residual activity values observed in case of *B. subtilis* (Table 6) justify the selection of the linear regression model expressed by Eq 4. Further the plot of linear regression predicted pMICan values against the observed pMICan values also favors the model expressed by Eq. 1 (Fig. 1). In case of *C. albicans* the MLR attempt to derive QSAR models failed to give statistically significant QSAR equation.

Table 3. Values of selected descriptors used in the linear regression analysis

Comp.	log P	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$
1	2.81	59.31	10.51	8.18	7.36	4.78	6.12	3.19
2	3.33	64.12	11.38	9.30	7.77	5.29	6.63	3.74
3	3.33	64.12	11.38	9.30	7.75	5.28	6.75	3.80
4	3.33	64.12	11.38	9.30	7.75	5.28	6.74	3.80
5	3.28	64.35	11.38	9.10	7.75	5.19	6.75	3.69
6	3.28	64.35	11.38	9.10	7.75	5.19	6.74	3.69
7	2.56	65.78	12.09	9.51	8.31	5.30	6.82	3.52
8	2.56	65.78	12.09	9.51	8.29	5.30	6.91	3.55
9	2.76	66.64	12.96	9.37	8.66	5.27	7.65	3.63
10	2.76	66.64	12.96	9.37	8.66	5.27	7.64	3.62
11	3.60	66.94	11.38	10.10	7.75	5.69	6.74	4.26
12	5.09	85.18	14.47	12.87	9.83	8.18	7.46	5.49
13	5.61	89.98	15.34	13.98	10.24	8.69	7.98	6.05
14	5.61	89.98	15.34	13.98	10.22	8.68	8.08	6.11
15	5.61	89.98	15.34	13.98	10.22	8.68	8.08	6.11
16	5.56	90.22	15.34	13.79	10.22	8.59	8.10	6.00
17	5.56	90.22	15.34	13.79	10.22	8.59	8.08	5.99
18	4.84	91.64	16.05	14.20	10.77	8.70	8.17	5.83
19	4.84	91.64	16.05	14.20	10.76	8.70	8.25	5.86
20	5.04	92.50	16.92	14.05	11.13	8.67	8.99	5.93
21	5.04	92.50	16.92	14.05	11.13	8.67	8.98	5.93
22	5.88	92.80	15.34	14.79	10.22	9.09	8.08	6.57
23	5.88	94.38	15.88	14.28	10.83	9.18	8.17	6.20
24	6.40	99.18	16.75	15.40	11.24	9.69	8.69	6.75
25	6.40	99.18	16.75	15.40	11.22	9.68	8.79	6.81
26	6.40	99.18	16.75	15.40	11.22	9.68	8.79	6.81
27	6.35	99.42	16.75	15.20	11.22	9.59	8.80	6.70
28	6.35	99.42	16.75	15.20	11.22	9.59	8.80	6.70
29	5.09	85.18	14.47	12.87	9.83	8.18	7.46	5.49
30	5.09	85.18	14.47	12.87	9.83	8.18	7.46	5.49
31	5.84	101.70	18.33	15.47	12.13	9.67	9.70	6.64
32	5.84	101.70	18.33	15.47	12.13	9.67	9.69	6.64
33	6.67	102.00	16.75	16.20	11.22	10.09	8.79	7.28
34	7.47	112.78	18.71	17.11	12.83	11.18	9.58	7.61
35	7.99	117.59	19.58	18.23	13.24	11.69	10.10	8.17
36	7.99	117.59	19.58	18.23	13.22	11.68	10.22	8.23
37	7.99	117.59	19.58	18.23	13.22	11.68	10.21	8.23
38	7.94	117.82	19.58	18.03	13.22	11.59	10.22	8.12
39	7.94	117.82	19.58	18.03	13.22	11.59	10.21	8.11
40	7.22	119.25	20.29	18.44	13.77	11.70	10.29	7.95
41	7.22	119.25	20.29	18.44	13.76	11.70	10.37	7.98
42	7.42	120.11	21.16	18.30	14.13	11.67	11.12	8.06
43	7.42	120.11	21.16	18.30	14.13	11.67	11.10	8.05
44	8.26	120.41	19.58	19.03	13.22	12.09	10.21	8.69

Table 4. Correlation matrix for substituted anilides against *B. subtilis*

	pMICbs	log P	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$	κ_1	$\kappa\alpha_1$
pMICbs	1.000										
log P	0.562	1.000									
MR	0.626	0.975	1.000								
$^{0}\chi$	0.676	0.930	0.986	1.000							
$^{0}\chi^{v}$	0.613	0.979	0.997	0.977	1.000						
$^{1}\chi$	0.664	0.935	0.989	0.998	0.978	1.000					
$^{1}\chi^{v}$	0.615	0.984	0.996	0.973	0.998	0.975	1.000				
$^{2}\chi$	0.675	0.897	0.960	0.987	0.944	0.984	0.936	1.000			
$^{2}\chi^{v}$	0.597	0.991	0.992	0.962	0.997	0.963	0.996	0.930	1.000		
κ_1	0.672	0.943	0.990	0.997	0.984	0.996	0.983	0.973	0.971	1.000	
$\kappa\alpha_1$	0.656	0.959	0.994	0.991	0.993	0.990	0.992	0.959	0.983	0.997	1.000

Table 5. Correlation of pMIC with molecular descriptors

Parameter	pMICbs	pMICsa	pMICec	pMICan
log P	0.562	0.657	0.620	0.800
MR	0.626	0.708	0.686	0.829
$^{0}\chi$	0.676	0.753	0.738	0.831
$^{0}\chi^{v}$	0.613	0.684	0.670	0.835
$^{1}\chi$	0.664	0.748	0.727	0.827
$^{1}\chi^{v}$	0.615	0.689	0.663	0.824
$^{2}\chi$	0.675	0.765	0.767	0.828
$^{2}\chi^{v}$	0.597	0.673	0.658	0.832
κ_1	0.672	0.744	0.722	0.827
$\kappa\alpha_1$	0.656	0.724	0.701	0.830

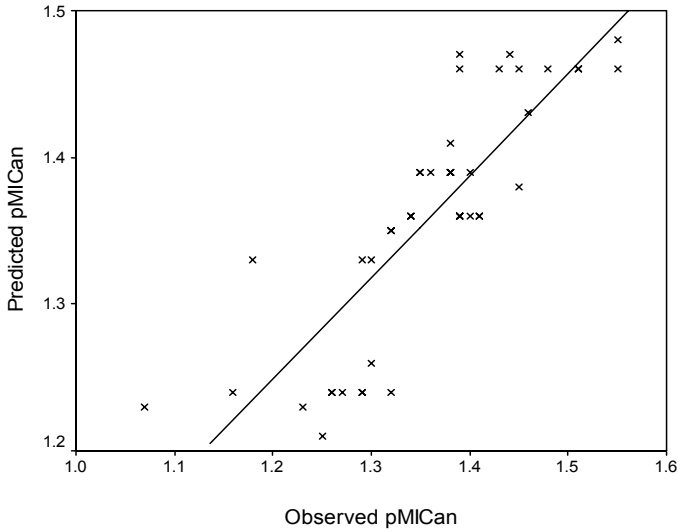


Figure 1. Plot of predicted pMICan activity values against the experimental pMICan values for the QSAR model by Eq. 1 for *A. niger*.

Table 6. Observed and predicted antibacterial activity of substituted anilides against *B.subtilis, S. aureus, E. coli,* and *A. niger* using the best QSAR models

	pMICbs using Eq. 1			pMICsa using Eq. 2			pMICec using Eq. 3			pMICan using Eq. 4		
Com.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.45	1.40	0.05	1.30	1.14	0.16	1.09	1.06	0.03	1.25	1.21	0.04
2	1.32	1.42	-0.10	1.22	1.18	0.04	1.16	1.09	0.07	1.27	1.24	0.03
3	1.39	1.42	-0.03	1.06	1.18	-0.12	1.06	1.10	-0.04	1.32	1.24	0.08
4	1.40	1.42	-0.02	1.22	1.18	0.04	0.97	1.09	-0.12	1.16	1.24	-0.08
5	1.42	1.42	0.00	1.12	1.18	-0.06	1.18	1.10	0.08	1.07	1.23	-0.16
6	1.40	1.42	-0.02	1.16	1.18	-0.02	1.10	1.09	0.01	1.23	1.23	0.00
7	1.51	1.43	0.08	1.26	1.19	0.07	1.01	1.10	-0.09	1.26	1.24	0.02
8	1.42	1.43	-0.01	1.10	1.19	-0.09	1.06	1.11	-0.05	1.26	1.24	0.02
9	1.44	1.44	0.00	1.24	1.24	0.00	1.18	1.15	0.03	1.29	1.24	0.05
10	1.54	1.44	0.10	1.25	1.24	0.01	1.24	1.15	0.09	1.29	1.24	0.05
11	1.34	1.42	-0.08	1.14	1.18	-0.04	1.14	1.09	0.05	1.30	1.26	0.04
12	1.50	1.47	0.03	1.30	1.23	0.07	1.14	1.14	0.00	1.18	1.33	-0.15
13	1.50	1.49	0.01	1.30	1.26	0.04	1.20	1.17	0.03	1.40	1.36	0.04
14	1.54	1.49	0.05	1.30	1.27	0.03	1.20	1.18	0.02	1.39	1.36	0.03
15	1.50	1.49	0.01	1.25	1.27	-0.02	1.09	1.18	-0.09	1.39	1.36	0.03
16	1.50	1.49	0.01	1.26	1.27	-0.01	1.21	1.18	0.03	1.32	1.35	-0.03
17	1.56	1.49	0.07	1.20	1.27	-0.07	1.06	1.18	-0.12	1.32	1.35	-0.03
18	1.44	1.50	-0.06	1.25	1.28	-0.03	1.18	1.18	0.00	1.34	1.36	-0.02
19	1.44	1.50	-0.06	1.20	1.28	-0.08	1.18	1.19	-0.01	1.39	1.36	0.03

20	1.60	1.51	0.09	1.35	1.33	0.02	1.40	1.24	0.16	1.41	1.36	0.05
21	1.60	1.51	0.09	1.50	1.33	0.17	1.41	1.23	0.18	1.41	1.36	0.05
22	1.48	1.49	-0.01	1.15	1.27	-0.12	1.15	1.18	-0.03	1.45	1.38	0.07
23	1.48	1.49	-0.01	1.30	1.28	0.02	1.25	1.18	0.07	1.34	1.36	-0.02
24	1.51	1.51	0.00	1.20	1.31	-0.11	1.13	1.22	-0.09	1.38	1.39	-0.01
25	1.43	1.51	-0.08	1.38	1.32	0.06	1.19	1.22	-0.03	1.38	1.39	-0.01
26	1.49	1.51	-0.02	1.30	1.32	-0.02	1.27	1.22	0.05	1.38	1.39	-0.01
27	1.46	1.51	-0.05	1.40	1.32	0.08	1.10	1.22	-0.12	1.36	1.39	-0.03
28	1.46	1.51	-0.05	1.40	1.32	0.08	1.16	1.22	-0.06	1.35	1.39	-0.04
29	1.50	1.47	0.03	1.29	1.23	0.06	1.10	1.14	-0.04	1.29	1.33	-0.04
30	1.50	1.47	0.03	1.20	1.23	-0.03	1.14	1.14	0.00	1.30	1.33	-0.03
31	1.60	1.54	0.06	1.42	1.37	0.05	1.40	1.28	0.12	1.40	1.39	0.01
32	1.45	1.54	-0.09	1.40	1.37	0.03	1.24	1.28	-0.04	1.35	1.39	-0.04
33	1.49	1.51	-0.02	1.20	1.32	-0.12	1.24	1.22	0.02	1.38	1.41	-0.03
34	1.51	1.54	-0.03	1.33	1.37	-0.04	1.30	1.27	0.03	1.46	1.43	0.03
35	1.60	1.56	0.04	1.50	1.40	0.10	1.29	1.30	-0.01	1.45	1.46	-0.01
36	1.50	1.56	-0.06	1.45	1.41	0.04	1.25	1.31	-0.06	1.55	1.46	0.09
37	1.64	1.56	0.08	1.40	1.41	-0.01	1.39	1.31	0.08	1.39	1.46	-0.07
38	1.48	1.56	-0.08	1.27	1.41	-0.14	1.27	1.31	-0.04	1.48	1.46	0.02
39	1.48	1.56	-0.08	1.40	1.41	-0.01	1.27	1.31	-0.04	1.43	1.46	-0.03
40	1.55	1.57	-0.02	1.39	1.41	-0.02	1.25	1.32	-0.07	1.44	1.47	-0.03
41	1.49	1.57	-0.08	1.29	1.42	-0.13	1.29	1.32	-0.03	1.39	1.47	-0.08
42	1.70	1.58	0.12	1.50	1.47	0.03	1.35	1.37	-0.02	1.51	1.46	0.05
43	1.60	1.58	0.02	1.50	1.46	0.04	1.35	1.37	-0.02	1.51	1.46	0.05
44	1.60	1.56	0.04	1.44	1.41	0.03	1.39	1.31	0.08	1.55	1.48	0.07

Conclusions

From the results and discussion made above we conclude that the different substituted anilide derivatives are effective against the microbial species tested. The results obtained from present investigation of *in vitro* antimicrobial activity studies indicate that the stearic-*m*-nitroanilide **(42),** stearic-o-chloroanilide **(35)** and stearic-p-chloroanilide **(37)** are the most effective ones. Further, a general trend showed that the presence of electron-withdrawing group (NO_2) leads to increase in the activity in comparison to the presence of other groups. The topological parameters especially, the molecular connectivity indices ($^0\chi$, $^2\chi$ and $^0\chi^v$) can be used successfully for modeling antimicrobial activity of different substituted anilides against the microbial species included in the present study. Contribution of topological descriptors in describing the antimicrobial activity of different substituted anilides was further evidenced by the results of our

previous studies[16-17]. The low residual activity and cross-validated r^2values (r^2_{cv}) observed indicated the predictive ability of the developed QSAR models.

Experimental Section

General Procedures. All chemicals used were of Ranbaxy Laboratories Ltd., Delhi; Qualigens, Mumbai and S.D. Fine Chemicals, Mumbai. Melting points in degree Celsius were determined with Elico melting point apparatus and are uncorrected. The FTIR spectra were recorded in KBr pellets on Perkin Elmer IR spectrophotometer. The ^{1}H-NMR were recorded on Brucker Avance II 400 NMR spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard (chemical shift in δ ppm). The purity of compounds was checked by thin layer chromatography (TLC) on silica gel plates. The spots were detected by exposure to iodine vapours.

Preparation of anilides. General procedure
Acid chloride was prepared by the reaction of 0.15 mol of corresponding organic acids [benzoic acid (**1-11)**, lauric acid **(12-22)**, myristic acid **(23-33)** and stearic acid **(33-44)**] with thionyl chloride. Anilides were prepared by dropwise addition of a solution of corresponding substituted aniline (0.1 mol) in ether (50 mL) to a solution of respective acid chloride (0.05 mol) in ether (50 mL). An immediate reaction took place and the mixture was stirred for 30 min at room temperature which resulted in the precipitation of crude anilide. The resulting mixture was washed successively with 5% Hydrochloric acid and water to remove excess of aniline. The crude anilide was recrystallized from ethanol. Structures of the synthesized . compounds were confirmed on the basis of spectroanalytical data.

1. Yield-50% ; mp – 150-152^{0}C; IR: 3342 (NH str., 2^0 amide), 3045 (CH str., aromatic), 1656 (C=O str., 2^0 amide), 1529 (C=C str., aromatic); ^{1}H NMR δ: 7.13-7.17 (t, 1H), 7.34-7.38 (t, 2H), 7.45-7.48 (t, 2H), 7.52-7.53 (t, 1H), 7.85-7.87 (d, 2H), 7.63-7.65 (d, 2H), 7.91 (s, 1H).
2. Yield-41% ; mp – 81-83^{0}C; IR: 3228 (NH str., 2^0 amide), 3051 (CH str., aromatic), 1652 (C=O str., 2^0 amide), 1522 (C=C str., aromatic); ^{1}H NMR δ: 7.07-7.11 (t, 1H), 7.26 (d, 1H), 7.32 (t, 1H), 7.34-7.36 (d, 1H), 7.50-7.55 (t, 2H), 7.57-7.61 (m, 1H), 7.91-7.94 (d, 2H), 8.45 (s, 1H).
8. Yield-55% ; mp – 140-142^{0}C; IR: 3330 (NH str., 2^0 amide), 3049 (CH str., aromatic), 2837 (CH str., OCH_3) 1647 (C=O str., 2^0 amide), 1515 (C=C str., aromatic); ^{1}H NMR δ: 3.81 (s, 3H), 6.89-7.44 (m, 9H), 7.81 (s, 1H).
12. Yield-44% ; mp – 50-54^{0}C; IR: 3307 (NH str., 2^0 amide), 2920 (CH str., aliphatic, antisym.), 2851 (CH str, aliphatic, sym), 1656 (C=O str., 2^0 amide), 1542 (C=C str., aromatic); ^{1}H NMR δ: 0.86-0.89 (t, 3H), 1.25-1.34 (m, 16H), 1.50-1.70 (m, 2H), 2.32-2.37 (t, 2H), 7.08-7.52 (m, 5H).
15. Yield-24% ; mp – 49-51^{0}C; IR: 3309 (NH str., 2^0 amide), 3101 (CH str., aromatic), 2919 (CH str., aliphatic, antisym.) 2850 (CH str., aliphatic, sym.), 1659 (C=O str., 2^0 amide), 1524 (C=C

str., aromatic); ^{1}H NMR δ: 0.85-0.90 (t, 3H), 1.22-1.29 (q, 20H), 1.65-1.72 (m, 2H), 2.29-2.35 (t, 2H), 7.83 (s, 1H), 7.23-7.48 (m, 4H).

28. Yield-34% ; mp – 49-51^{0}C; IR: 3296 (NH str., 2^0 amide), 2918 (CH str., aliphatic, antisym.), 2850 (CH str, aliphatic, sym), 1659 (C=O str., 2^0 amide), 1537 (C=C str., aromatic); ^{1}H NMR δ: 0.86-0.89 (t, 3H), 1.25-1.31 (t, 18H), 1.61-1.65 (m, 2H), 1.67-1.75 (t, 2H), 2.31 (s, 1H), 7.12-7.39 (m, 4H).

30. Yield-31% ; mp – 87-89^{0}C; IR: 3306 (NH str., 2^0 amide), 2919 (CH str., aliphatic, antisym.), 2850 (CH str, aliphatic, sym), 1654(C=O str., 2^0 amide), 1547 (C=C str., aromatic); ^{1}H NMR δ: 0.86-0.89 (t, 3H), 1.25-1.36 (t, 20H), 1.67-1.74 (m, 2H), 2.02-2.34 (t, 2H), 3.33 (s, 3H), 6.83-6.86 (d, 2H), 7.15 (s, 1H), 7.31-7.41(d, 2H).

34. Yield-27% ; mp – 57-59^{0}C; IR: 3309 (NH str., 2^0 amide), 3100 (CH str., aromatic), 2917 (CH str., aliphatic, antisym.), 2849 (CH str., aliphatic, sym.), 1657 (C=O str., 2^0 amide), 1541 (C=C str., aromatic); ^{1}H NMR δ: 0.86-0.89 (t, 3H), 1.21-1.23 (m, 2H), 1.60-1.73 (m, 2H) 2.30-2.36 (m, 2H), 7.28-7.52 (s, 5H), 7.26 (s, 1H).

Antimicrobial evaluation

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against Gram positive *S. aureus, B. subtilis,* Gram negative *E. coli* and also against fungi *C. albicans* and A. *niger*. The MIC (μg/ml) was determined by serial dilution technique[24] using double strength nutrient broth IP and Sabouraud dextrose broth IP as media for bacterial and fungal growth respectively. Ciprofloxacin and Fluconazole were used as reference compounds in case of antibacterial and antifungal activity respectively. The compounds were dissolved in dimethyl sulfoxide to give a concentration of 100 μg/mL, which was serially diluted to give concentrations of 50.0, 25.0, 12.5, 6.25, 3.125 μg/mL in culture tubes containing 1 ml of nutrient medium. To all the tubes including standards and controls, 0.1 mL of fresh inoculum was added and the tubes were incubated at 37 ± 1°C for 24 h (bacteria) and 25°C for 7 d (*A. niger*). The MIC was recorded in each case as the minimum concentration of compound, which inhibited the growth of tested microorganism. From the MIC observed, the intermediate concentrations between MIC values were prepared by suitable dilution of stock solution and the accurate MIC values were determined.

QSAR Analysis

The calculations of molecular descriptors of anilides as well as regression analysis were carried by using molecular package TSAR 3D version 3.3.[36] The description of these descriptors are available in the literature[26-33] and therefore they are not described again here.

Cross validation

The predictive powers of the equations were validated by leave one out (LOO) cross validation method,[37] 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 (or predictive q^2) method which is defined as

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

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

References and Footnotes

1. Fiddler, D. F. *Emerge. 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. Z. *Farmaco.* **2001**, *56*, 41.
4. Ozawa, I.; Takeuchi, I.; Yamomoto, K.; Hamada, Y.; Ito, T.; Kuwahara, M.; Takagaki, T. *Chem. Pharm. Bull. (TOKYO).* **1984**, *32(1)*, 305.
5. Narasimhan, B.; Belsare, D.; Pharande, D.; Mourya, V.; Dhake, A. *Eur. J. Med. Chem.* **2004,** *39*, 827.
6. Schultz, H. W. *J. Pharm. Sci*. **2006**, *52(5),* 503.
7. Niewiadomy, A.; Matisiak, J.; Macik-Niewiadomy, G. *Eur. J. Pharm. Sci.* **2001**, *13,* 243.
8. Ragavendran, J. V.; Sriram, D.; Patel, S.K.; Reddy, I. V.; Bharathwazan, N.; Stables, J.; Yogeeswari, P. *Eur. J. Med. Chem.* **2007**, *42*, 146.
9. Biagi, G.; Giorgi, I.; Livi, O.; Nardi, A.; Calderone, V.; Martelli, A.; Martinotti, E.; LeRoy, S. O. *Eur. J. Med. Chem.* **2004,** *39(6) ,*491.
10. Aydin, O. N.; Eyigor, M.; Aydin, N. *Eur. J. Anaesthesiol*. **2001**, *18,* 687.
11. Bouchain, G.; Delorme, D. *Current Medicinal Chemistary*. **2003**, *10,* 2359.
12. Clark, M. T.; Coburn, R. A.; Evans, R. T.; Genco, R. J. *J. Med. Chem.* **1986**, *29*, 25.
13. Hung, C.; Tsia, W.; Kuo, L.; Kuo, Y. *Bioorg. Med. Chem*. **2004**, *13,* 1791.
14. Wang, X.; Tang, Y.; Xie, Q.; Qiu, Z. *Eur.J. Med Chem.* **2006**, *41*, 226.
15. Narasimhan, B.; Kothawade, U. R.; Pharande, D. S.; Mourya, V. K.; Dhake, A. S. *Indian J. Chem*. **2003,** *42B*, 2828.
16. Narasimhan, B.; Mourya, V.; Dhake, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3023.
17. Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Khim.-Farm. Zh.* **2007,** *3*, 120.
18. Gangwal, N. A.; Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Indian J. Het. Chem.* **2003,** *12*, 201.
19. Narasimhan, B.; Kumari, M.; Jain, N.; Dhake, A.; Sundaravelan, C, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4951.
20. Narasimhan, B.; Kumari, M.; Dhake, A.; Sundaravelan, C. *Arkivoc* **2006,** *xiii*, 73.
21. Kumar, A.; Narasimhan, B.; Kumar, D. *Bioorg. Med. Chem.* **2007,** *15*, 4113.
22. Narasimhan, B.; Mourya, V. K.; Dhake, A. S. *Arkivoc* **2007,** *i*, 189.
23. Pharmacopoeia of India; Ministry of Health Department: Govt. of India: New Delhi, **1996**, Vol. II, pA-88.
24. Cappucino, J. G.; Sherman, *N. Microbiology—A Laboratory Manual; Addison Wesley: California*, **1999**, pp 263.

25. Sharma, P.; Rane, N.; Gurram, V.K. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 4185.
26. Hansch, C.; Fujita, *T. J. Am. Chem. Soc.* **1964**, *86*, 1616.
27. Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani,D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
28. Kier, L. B.; Hall, L. H. *Molecular Connectivity in Chemistry and Drug Research; Academic Press: New York,* **1976,** pp 129–145.
29. Randic, M. J. *Am. Chem. Soc.* **1975**, *97*, 6609.
30. Balban, A. T. *Chem. Phys. Lett.* **1982**, *89*, 399.
31. Wiener, H. *J. Am. Chem. Soc.* **1947**, *69*, 17.
32. Sharma, P.; Rane, N.; Gurram, V. K. *Bioorg. Med. Chem.Lett.* **2004**, *14*, 4185.
33. Randic, M. Croat. *Chem. Acta.* **1993**, *66*, 289.
34. Wold, S.; Erikkson, L. *Statistical validation of QSAR results. In Chemometrics Methods in Molecular Design; Water, H. V., Ed.;* VCH: Weinheim, 1995; pp 309–318.
35. Golbraikh, A.; Tropsha, *A. J. Mol. Graph. Model.* **2002**, *20*, 209.
36. TSAR 3D Version 3.3, Oxford Molecular Limited, 2000.
37. Agrawal, V. K.; Singh, J.; Mishra, K. C.; Khadikar, P. V.; Jaliwala, Y. A. *Arkivoc* **2006**, *ii*, 162.

