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Synthesis, antimicrobial evaluation and QSAR studies of *p*-amino benzoic acid derivatives

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Abstract A series of Schiff bases (**1-16**) and esters (**17-27**) of *p*-amino benzoic acid (PABA) was synthesized and its *in vitro* antimicrobial potential was evaluated by using tube dilution method. Compound **11** was found to be most promising antibacterial agent ($pMIC_{bs}$ = 2.11 µM/ml) having antibacterial potential comparable to standard drug norfloxacin ($pMIC_{bs}$ = 2.61 µM/ml) and may be taken as a lead compound for the development of novel antibacterial agents. QSAR analysis indicated that electronic parameters, total energy (Te) and energy of lowest unoccupied molecular orbital (LUMO) were found dominant in explaining the antimicrobial activity of synthesized compounds.

Keywords: PABA; Synthesis; Antimicrobial; mt-QSAR; LOO method

1. INTRODUCTION

The emerging problem of resistance to conventional antimicrobial agents has stimulated interest in the development of new antimicrobials. Newly characterized molecules have inspired molecular designs for the creation of therapeutics, and will continue to do so as more are discovered, because these are based on antimicrobial strategies that have proven efficacious over millennia (Zasloff, 2002).

p-Aminobenzoic acid (PABA) is a cyclic amino acid and belongs to the vitamin B group and used as a protective drug against UV irradiation and in diagnostic tests for gastrointestinal tract. PABA is also having anti-virulent properties and therapeutic effect against typhoid and rickettsial diseases (Akberova *et al*, 2002). Schiff bases show various biological properties including antibacterial, antifungal antitumor, analgesic and anti-inflammatory activity (Chhonker *et al*, 2009).

Quantitative structure activity relationship (QSAR) studies provide medicinal chemists valuable information that is useful for drug design and prediction of drug activity. QSAR models are mathematical equations which

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construct a relationship between chemical structures and their biological activities as a linear regression model (Sabet *et al,* 2010).

So, in the present study, we hereby report the synthesis, antimicrobial evaluation and QSAR studies of PABA derivatives.

2. MATERIALS AND METHODS

2.1Materials and Instruments used

Starting materials were obtained from M/s. Himdea Chemicals Pvt. Ltd. and were used without further purification. Reaction progress was observed by thin layer chromatography making use of commercial silica gel plates (Merck), Silica gel F254 on aluminum sheets. Melting points were determined in open capillary tubes on a Sonar melting point apparatus. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined by AV 300 spectrometer in appropriate deuteriated solvents and were 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 Schimazdu 8400S FTIR spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer.

2.2 General procedure for the synthesis of Schiff's bases of PABA (1-16)

p-Amino benzoic acid (0.01 mol) and benzaldehyde (0.01 mol) were refluxed in ethanol for 3-4 h and the contents were then poured on crushed ice. The precipitated crude product was filtered, dried and recrystallized from ethanol. The later (0.08 mol) was refluxed with ethanol (0.74 mol) in presence of sulphuric acid for 3-4 h. The reaction mixture was then added to 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 ethyl ester of PABA. The synthesized ester (0.01mol) was treated with hydrazine hydrate (0.02mol) in presence of ethanol and refluxed for 2-3 h. The reaction mixture was then cooled and resultant precipitate was filtered off and recrystallized from ethanol. Finally, a mixture of above synthesized hydrazide (0.1mol) and corresponding aromatic aldehyde (0.1mol) in ethanol was refluxed in presence of catalytic amount of glacial acetic acid for 2-3 h. The mixture was then cooled and poured in ice cold water. The Schiff's base obtained was filtered and recrystallized from ethanol.

2.3 General procedure for the synthesis of esters of PABA (17-27)

A mi.xture of *p*-amino benzoic acid (0.08 mol) and corresponding alcohol (0.74 mol) was heated under reflux in presence of sulphuric acid for 3-4 h.

Then the reaction mixture was added to 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 PABA.

Compound 1: Yield – 77.47%; m.p. – 100-102; IR (KBr pellets, cm-1): 1533 (C=C str., phenyl nucleus), 3001 (C-H str., phenyl nucleus), 1665 (C=N str. –N=CH), 673 (C-Cl str., C_6H_5-Cl), 3406 (N-H str., sec-amide), 1072 (N-N str., -NHN=CH) ; ^{1}H NMR (DMSO): 7.316-8.013 (m, 13H, ArH), 8.650 (s, 1H, -NH), 8.680 (s, 1H, N=CH); Anal. Calculated for $C_{21}H_{16}ClN_3O$: C, 69.71; H, 4.46; N, 11.61; Found: C, 69.80; H, 4.51; N, 11.64

Compound 11: Yield – 70.58%; m.p. – 110-112; IR (KBr pellets, cm-1) 1530 (C=C str., phenyl nucleus), 3001 (C-H str., phenyl nucleus), 1650 (C=N str. –N=CH), 698 (C-Br str., C_6H_5-Br), 1680 (C=O str., sec-amide), 1066 (N-N str., -NHN=CH); ^{1}H NMR (DMSO) 7.460-7.986 (m, 13H, ArH), 8.015 (s, 1H, -NH), 8.707 (s, 1H, N=CH-C_6H_5); Anal. Calculated for $C_{21}H_{16}BrN_3O$ C, 62.08; H, 3.97; N, 10.34; Found: C, 61.97; H, 4.00; N, 10.38

Compound 13: Yield – 61.06 %; m.p. – 155-157; Yield – 77.47%; m.p. – 100-102; IR (KBr pellets, cm-1) 3039 (C-H str., phenyl nucleus), 1716 (C=N str. –N=CH), 3415 (N-H str., sec-amide), 956 (N-N str., -NHN=CH), 2951 (C-H str., alkene); ^{1}H NMR (DMSO) 7.116-7.835 (m, 14H, ArH), 8.383 (s, 1H, N=CH), 6.765 (d, 1H, CH=CH); Anal. Calculated for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89; Found: C, 78.12; H, 5.49; N, 11.84

Compound 20: Yield – 87.90%; m.p. – 110-112; IR (KBr pellets, cm-1) 1546 (C=C str., phenyl nucleus), 2933 (C-H str., phenyl nucleus), 1604 (N-H in plane bending, 1^0 amine), 1820 (C=O str., ester), 2933 (CH_3 asym. str., alkane); ^{1}H NMR (DMSO) 6.394-7.588 (m, 4H, ArH), 1.130 (t, 3H, CO -CH_2-), 5.016 (m, 2H, -CH_3); Anal. Calculated for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48; Found; C, 65.39; H, 6.76; N, 8.43

2.4 Evaluation of antimicrobial activity (Determination of MIC)

The antimicrobial activity was performed against Gram-positive bacteria: *Staphylcococcus aureus* MTCC 2901, *Bacillus sublitis* MTCC 2063, Gram-negative bacterium: *Escherichia coli* MTCC 1652 and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 8189 using tube dilution method (Cappucino and Sherman, 1999). Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) (Pharmacopoeia of India, 2007). The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. niger*)

and at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of MIC.

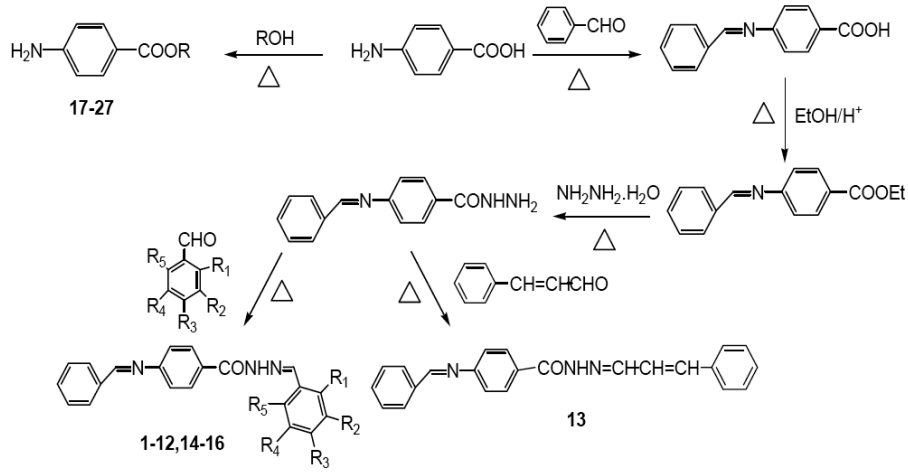
2.5 QSAR studies

The structures of PABA derivatives were first pre-optimized with the Molecular Mechanics Force Field (MM^+) procedure included in Hyperchem 6.03 (1993) and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). We chose a gradient norm limit of 0.01 kcal/A˚ for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows (2000). Further, the regression analysis was performed using the SPSS software package (1999).

3. RESULTS AND DISSCUSSION

3.1 Chemistry

Schiff bases (**1-16**) and esters (**17-27**) of PABA were synthesized using the synthetic procedures outlined in Scheme 1. Esters were synthesized by reaction of PABA with different alcohols in the presence of sulphuric acid. In order to synthesize Schiff bases of PABA, firstly *p*-amino benzoic acid was coupled with benzaldehyde in order to block amino group of PABA. The later was reacted with ethanol to synthesize ethyl 4-(benzylideneamino)benzoate. 4-(benzylideneamino)



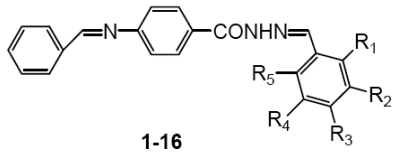
Scheme1: Synthetic scheme for the synthesis of Schiff bases (1-16) and esters of PABA (17-27).

benzohydrazide was synthesized by reaction of ethyl 4-(benzylideneamino) benzoate with hydrazine hydrate which on reaction with corresponding aldehydes yielded Schiff bases of *p*-amino benzoic acid. The synthesized compounds were characterised by physicochemical as well as spectral means. The elemental analysis results were within ± 0.4% of the theoretical values. Physicochemical properties of synthesized compounds are given in Table 1.

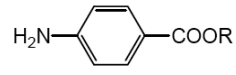
3.2 Antimicrobial activity

The results of antimicrobial activity of synthesized compounds are presented in Table 2 which indicated that synthesized compounds displayed more antibacterial

Table 1: The physicochemical properties of PABA derivatives **(1-27).**



1-16



17-27

S. NO.	R_1	R_2	R_3	R_4	R_5	Mol. Formula	M. Wt.	M.P. (°C)	Rf Value (Benzene)	% Yield
1.	H	Cl	H	H	H	$C_{21}H_{16}ClN_3O$	361.82	100-102	0.75	77.47
2.	H	OCH_3	OCH_3	OCH_3	H	$C_{24}H_{23}N_3O_4$	417.46	180-182	0.46	67.62
3.	H	H	CHO	H	H	$C_{22}H_{17}N_3O_2$	355.39	220-222	0.67	68.54
4.	Cl	H	H	H	H	$C_{21}H_{16}ClN_3O$	361.82	103-105	0.50	63.95
5.	H	H	Br	H	H	$C_{21}H_{16}BrN_3O$	406.28	102-104	0.78	92.00
6.	H	H	$N(CH_3)_2$	H	H	$C_{23}H_{22}N_4O$	370.45	80-82	0.30*	57.96
7.	H	H	CH_3	H	H	$C_{22}H_{19}N_3O$	341.41	143-145	0.48	78.84
8.	H	OCH_3	OCH_3	H	H	$C_{23}H_{21}N_3O_3$	387.43	155-157	0.74*	91.75
9.	H	H	OCH_3	H	H	$C_{22}H_{19}N_3O_2$	357.41	140-142	0.69	87.64
10.	H	OC_2H_5	OH	H	H	$C_{23}H_{21}N_3O_3$	387.43	135-137	0.62*	76.28
11.	H	Br	H	H	H	$C_{21}H_{16}BrN_3O$	406.28	110-112	0.54	70.58
12.	H	NO_2	H	H	H	$C_{21}H_{16}N_4O_3$	372.38	95-97	0.56	93.02
13.	-	-	-	-	-	$C_{23}H_{19}N_3O$	353.42	115-117	0.67	61.06
14.	H	OCH_3	OH	H	H	$C_{22}H_{19}N_3O_3$	373.40	175-177	0.78	57.92
15.	OCH_3	H	H	H	H	$C_{22}H_{19}N_3O_2$	357.41	92-94	0.83	73.33
16.	H	H	Cl	H	H	$C_{21}H_{16}ClN_3O$	361.82	180-182	0.71	78.43

*Chloroform:Toluene = 7:3

S.NO.	R	Mol. Formula	M. Wt.	M.P. (°C)	R*f* Value**	% Yield
17.	n-butyl	$C_{11}H_{15}NO_3$	193.24	65-67	0.54	65.70
18.	sec-butyl	$C_{11}H_{15}NO_3$	193.24	58-60	0.60	76.02
19.	iso-butyl	$C_{11}H_{15}NO_3$	193.24	80-85	0.76	45.54
20.	Ethyl	$C_9H_{11}NO_2$	165.19	110-112	0.45	87.90
21.	n-propyl	$C_{10}H_{13}NO_2$	179.22	90-92	0.32	67.98
22.	iso-propyl	$C_{10}H_{13}NO_2$	179.22	88-90	0.73	66.20
23.	n-amyl	$C_{12}H_{17}NO_2$	207.27	65-67	0.86	57.34
24.	iso-amyl	$C_{12}H_{17}NO_2$	207.27	120-122	0.66	78.04
25.	Octyl	$C_{15}H_{23}NO_2$	249.35	113-115	0.77	76.22
26.	cyclohexyl	$C_{13}H_{17}NO_2$	219.28	95-97	0.56	54.67
27.	Benzyl	$C_{14}H_{13}NO_2$	227.26	140-142	0.78	87.45

**Hexane:Acetone = 1:1

activity as compared to antifungal activity. In case of antimicrobial activity against *S. aureus,* compound **2**, *N*'-(3,4,5-trimethoxy benzylidene)-4-(benzylidene amino) benzohydrazide with $pMIC_{sa}$ value 1.82 µM/ml was found to be most potent among the synthesized PABA derivatives. The antimicrobial activity against *B. subtilis* suggested that compound **11,** *N*'-(3-bromo benzylidene)-4-(benzylidene amino)benzohydrazide ($pMIC_{bs}$ = 2.11µM/ml) was found most potent among the synthesized compounds. In case of *E. coli*, compound **14,** *N*'-(3-methoxy-4-hydroxy benzylidene)-4-(benzylidene amino)benzohydrazide ($pMIC_{ec}$=1.78 µM/ml) was found better antibacterial agent among the synthesized series. The antifungal activity results showed that compound **5**, *N*'-(4-bromo benzylidene)-4-(benzylidene amino)benzohydrazide was found to be most potent than the other members of the series against both fungal strains tested *i.e. A. niger* as well as *C. albicans* ($pMIC_{an, ca}$=1.81 µM/ml).

3.3 Structure Activity Relationship (SAR) studies

From the antimicrobial activity results of PABA derivatives, the following structure activity relationship may be concluded:

1 In general, Schiff's bases of PABA were found to be more potent than its esters.

2 Electron withdrawing group (bromo) increased antimicrobial activity of the synthesized compounds against *B. subtilis*, *C. albicans* and *A. niger. m*-Bromo derivative (**11**) was found to be active against *B. subtilis* while *p*-bromo derivative (**5**) was found to be active against *C. albicans* and *A. niger.*

Table 2. Antimicrobial activity (pMIC in μM/ml) of PABA derivatives (**1-27**).

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	1.16	1.46	1.46	1.46	1.16	1.36	1.31	1.34
2	1.82	1.52	1.22	1.52	1.22	1.52	1.37	1.46
3	1.45	1.45	1.45	1.45	1.15	1.45	1.30	1.39
4	0.86	1.16	1.46	1.76	1.46	1.16	1.61	1.34
5	1.81	0.91	1.21	1.81	1.81	1.31	1.81	1.51
6	1.47	1.77	1.17	1.77	1.17	1.47	1.47	1.47
7	1.44	1.74	1.14	1.74	1.74	1.44	1.74	1.56
8	1.49	1.79	1.19	1.79	1.49	1.49	1.64	1.55
9	1.46	1.46	1.16	1.76	1.46	1.36	1.61	1.46
10	1.49	1.49	1.49	1.79	1.49	1.49	1.64	1.55
11	1.51	2.11	1.21	1.51	1.51	1.61	1.51	1.57
12	1.47	2.08	1.17	1.47	1.78	1.57	1.62	1.59
13	0.85	1.45	1.15	1.75	1.45	1.15	1.60	1.33
14	1.48	2.08	1.78	1.78	1.78	1.78	1.78	1.78
15	1.46	1.46	1.46	1.76	1.76	1.46	1.76	1.58
16	1.46	1.46	1.76	1.76	1.76	1.56	1.76	1.64
17	1.19	1.19	0.89	1.49	1.49	1.09	1.49	1.25
18	1.19	1.19	1.19	1.49	1.19	1.19	1.34	1.25
19	1.49	1.19	0.89	1.49	1.49	1.19	1.49	1.31
20	1.42	1.12	0.82	1.42	1.42	1.12	1.42	1.24
21	1.46	1.16	0.86	1.16	1.46	1.16	1.31	1.22
22	1.16	1.16	0.86	1.46	1.16	1.06	1.31	1.16
23	1.22	1.22	1.22	1.52	0.92	1.22	1.22	1.22
24	1.22	1.22	1.22	1.22	1.52	1.22	1.37	1.28
25	1.30	1.30	1.60	1.30	1.30	1.40	1.30	1.36
26	1.24	1.24	1.24	1.24	1.55	1.24	1.39	1.30
27	1.26	1.26	1.26	1.56	1.26	1.26	1.41	1.32
SD	0.22	0.32	0.26	0.20	0.24	0.18	0.18	0.16
Std.	2.61*	2.61*	2.61*	2.64**	2.64**			

*Norfloxacin **Fluconazole

3 Presence of electron donating groups (methoxy and hydroxyl) improved the antimicrobial activity of PABA derivatives against *S. aureus* and *E. coli.* 3,4,5-Trimethoxy derivative (**2**) was found to be active against *S. aureus* and 3-methoxy-4-hydroxyl derivative (**14**) was found to be active against *E. coli.*

4 Presence of an additional conjugation (**13**) decreased antibacterial activity of the synthesized compounds.

5 By analysing the structure of most active compounds, it was evidenced that different structural requirements were necessary for a compound to be active against different microbial species. These results were similar to the findings of Sortino *et al*. (2007).

The findings of antimicrobial study are presented in Figure 1.

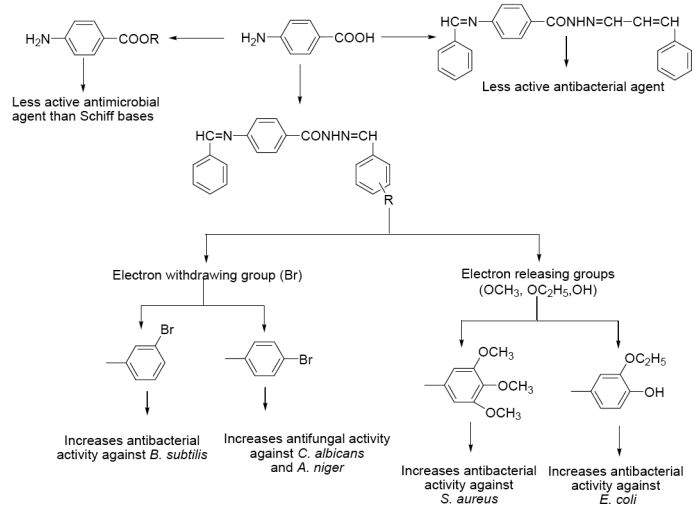


Figure 1: Structural requirements for the antimicrobial activity of Schiff bases and esters of PABA. SAR for antimicrobial activity of PABA derivatives

3.4 QSAR Studies

Quantitative structure activity relationship (QSAR) studies were performed using linear free energy relationship (LFER) model described by Hansch and Fujita (1964). In the present study, a dataset of **27** derivatives of PABA derivatives (**1-27**) was used for linear regression model generation. Standard drugs, norfloxacin and fluconazole were not included in the model generation.

Biological activity *i.e.* pMIC values (*i.e.* –log MIC) were used as dependent variables. Different molecular descriptors (independent variables) selected for the present study are listed in Table 3. The values of selected molecular descriptors used in the QSAR study are presented in Table 4.

mt-QSAR model is a single equation that considered the nature of molecular descriptors which were common and essential for describing the antibacterial and antifungal activity (Gonzalez-Diaz *et al,* 2007, 2008; Gonzalez-Diaz and Prado-Prado, 2008).

In the present study, we attempted to develop three different types of *mt*-QSAR models *viz. mt*-QSAR model to describe antibacterial, antifungal and antimicrobial activity of the synthesized compounds.

Table 3: QSAR descriptors used in the study

S. No.	QSAR descriptor	Type
1	log P	Lipophilic
2	Zero order molecular connectivity index ($^0\chi$)	Topological
3	First order molecular connectivity index ($^1\chi$)	Topological
4	Second order molecular connectivity index ($^2\chi$)	Topological
5	Valence zero order molecular connectivity index ($^0\chi^v$)	Topological
6	Valence first order molecular connectivity index ($^1\chi^v$)	Topological
7	Valence second order molecular connectivity index ($^2\chi^v$)	Topological
8	Kier's alpha first order shape index ($\kappa\alpha_1$)	Topological
9	Kier's alpha second order shape index ($\kappa\alpha_2$)	Topological
10	Kier's first order shape index (κ_1)	Topological
11	Randic topological index	Topological
12	Balaban topological index	Topological
13	Wiener's topological index	Topological
14	Kier's second order shape index (κ_2)	Topological
15	Ionization potential	Electronic
16	Dipole moment (μ)	Electronic
17	Energy of highest occupied molecular orbital (HOMO)	Electronic
18	Energy of lowest unoccupied molecular orbital (LUMO)	Electronic
19	Total energy (Te)	Electronic
20	Nuclear Energy (Nu. E)	Electronic
21	Molar refractivity (MR)	Steric

Table 4: Values of selected descriptors calculated for QSAR studies.

Comp.	log P	MR	$^{0}\chi$	$^{0}\chi^{v}$	κ_1	κ_2	J	Te	LUMO	HOMO	μ
1	5.72	105.87	18.19	14.66	20.73	11.11	1.16	-4220.83	-0.80	-9.06	2.86
2	4.44	120.46	22.05	17.53	25.62	13.65	1.22	-5288.06	-0.87	-8.75	2.89
3	4.88	107.66	18.90	14.45	21.70	11.87	1.17	-4308.97	-0.96	-9.12	1.84
4	5.72	105.87	18.19	14.66	20.73	11.11	1.19	-4220.73	-0.74	-9.04	3.65
5	5.99	108.69	18.19	15.46	20.73	11.11	1.17	-4200.34	-0.85	-9.05	2.45
6	4.99	114.78	19.77	15.91	22.68	12.00	1.17	-4392.42	-0.72	-8.16	5.59
7	5.67	106.11	18.19	14.46	20.73	11.11	1.17	-4016.61	-0.77	-8.82	3.99
8	4.70	113.99	20.48	16.20	23.66	12.76	1.18	-4812.31	-0.79	-8.49	3.11
9	4.95	107.53	18.90	14.87	21.70	11.87	1.17	-4336.62	-0.77	-8.66	3.89
10	5.01	113.97	20.48	15.95	23.66	12.76	1.17	-4812.97	-0.76	-8.56	2.85
11	5.99	108.69	18.19	15.46	20.73	11.11	1.16	-4200.33	-0.81	-9.06	2.87
12	5.15	108.39	19.77	14.73	22.68	12.00	1.16	-4691.56	-1.15	-9.39	8.60
13	5.61	111.31	18.74	14.70	21.70	12.54	1.13	-4143.91	-0.79	-8.70	3.55
14	4.66	109.22	19.77	15.24	22.68	12.00	1.17	-4657.19	-0.76	-8.57	0.42
15	4.95	107.53	18.90	14.87	21.70	11.87	1.20	-4336.46	-0.71	-8.96	4.79
16	5.72	105.87	18.19	14.66	20.73	11.11	1.17	-4220.85	-0.83	-9.00	2.43
17	2.20	56.16	10.39	8.32	12.07	6.48	2.00	-2463.60	-0.02	-8.65	4.21
18	2.22	55.98	10.55	8.49	12.07	5.78	2.12	-2463.47	-0.13	-8.82	3.40
19	2.21	56.03	10.55	8.49	12.07	5.78	2.06	-2463.48	-0.16	-8.84	3.86
20	1.34	47.03	8.97	6.91	10.08	4.89	2.05	-2151.94	-0.02	-8.65	4.24
21	1.80	51.56	9.68	7.62	11.08	5.67	2.03	-2307.77	-0.02	-8.65	4.14
22	1.75	51.45	9.84	7.78	11.08	5.02	2.10	-2307.66	-0.13	-8.82	3.46
23	2.60	60.76	11.10	9.03	13.07	7.30	1.97	-2619.43	-0.02	-8.65	4.13
24	2.53	60.71	11.26	9.19	13.07	6.55	2.02	-2619.34	-0.15	-8.83	3.60
25	3.79	74.56	13.22	11.15	16.06	9.87	1.88	-3086.94	-0.02	-8.65	4.19
26	2.58	63.30	11.38	9.32	12.46	6.07	1.56	-2747.58	-0.13	-8.82	3.41
27	2.77	66.90	12.09	9.30	13.43	6.81	1.51	-2818.88	-0.16	-8.84	3.67

Compounds **1, 2, 3, 6** and **23** were removed as outliers and were not involved in the data set for QSAR model generation according to recommendations of Furusjo *et al* (Furusjo *et al,* 2006).

The average antibacterial activity values were correlated with the molecular descriptors of synthesized compounds (Table 5). In general, high

Table 5. Correlation matrix for the antibacterial activity of the synthesized PABA derivatives.

	$pMIC_{ab}$	log P	MR	$^0\chi$	$\kappa 1$	J	W	Te	LUMO	HOMO	μ
$pMIC_{ab}$	1.000										
log P	0.644	1.000									
MR	0.711	0.962	1.000								
$^0\chi$	0.738	0.934	0.995	1.000							
κ_1	0.738	0.931	0.992	0.998	1.000						
J	-0.675	-0.927	-0.959	-0.947	-0.933	1.000					
W	0.716	0.908	0.985	0.995	0.993	-0.935	1.000				
Te	-0.758	-0.916	-0.986	-0.997	-0.995	0.940	-0.992	1.000			
LUMO	-0.677	-0.915	-0.949	-0.950	-0.940	0.920	-0.950	0.946	1.000		
HOMO	-0.142	-0.344	-0.205	-0.181	-0.161	0.252	-0.137	0.182	0.401	1.000	
μ	-0.165	-0.098	-0.108	-0.085	-0.079	0.102	-0.080	0.080	-0.043	-0.490	1.000

colinearity (r > 0.5) was observed between different parameters. The high interrelationship was observed between topological parameters, zero order molecular connectivity index ($^0\chi$) and Kier's first order shape index (κ_1) (r = 0.998), and low interrelationship was observed for electronic parameters, dipole moment (μ) and energy of lowest unoccupied molecular orbital LUMO (r = -0.043). The correlation of antibacterial, antifungal and antimicrobial activities of the synthesized compounds with molecular descriptors is presented in Table 6.

Electronic parameter, total energy (Te) was found to be dominating descriptor for antibacterial activity of the synthesized compounds (Eq. 1).

LR-*mt*-QSAR model for antibacterial activity

$$pMIC_{ab} = -0.000152\ Te + 0.793 \quad (1)$$

$$n = 22 \quad r = 0.758 \quad q^2 = 0.500 \quad s = 0.131 \quad F = 27.05$$

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

The developed QSAR model for antibacterial activity (Eq. 1) indicated that there was a negative correlation between Te and antibacterial activity of the synthesized compounds. This was evidenced by least antibacterial activity compound **22** ($pMIC_{ab}$ = 1.06 μM/ml) having highest Te value (-2307.66).

The developed QSAR model (Eq. 1) was cross validated by q^2 value (q^2 = 0.500) obtained by leave one out (LOO) method which indicated that the model developed was a valid one. As the observed and predicted values were close to each other (Table 7), the *mt*-QSAR model for antibacterial activity (Eq. 1) was a valid one (Golbraikh and Tropsha 2002). The plot of predicted $pMIC_{ab}$ against observed $pMIC_{ab}$ (Fig. 2) also favoured the developed model expressed by Eq. 1. Further, the plot of observed $pMIC_{ab}$ *vs* residual $pMIC_{ab}$ (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).

Electronic parameter, energy of lowest unoccupied molecular orbital (LUMO) was found most important in expressing antifungal activity of the synthesized compounds (Table 6). So, QSAR model for antifungal activity (Eq. 2) was developed using LUMO.

LR-*mt*-QSAR model for antifungal activity

$$pMIC_{af} = -0.169\ LUMO + 1.40 \qquad (2)$$

$$n = 22 \quad r = 0.663 \quad q^2 = 0.333 \quad s = 0.074 \quad F = 15.69$$

As in case of antibacterial activity, antifungal activity of the synthesized compounds was also negatively correlated with their LUMO values (Tables 2 and 4).

According to the FMO concept, the HOMO and LUMO of a molecule play important roles in intermolecular interactions. Extending the concept to binding in drug-receptor systems, the major contribution to binding involves the interaction between the HOMO of the drug with the LUMO of the receptor and that between LUMO of the drug with the HOMO of the receptor. (Narasimhan *et al,* 2011).

The validity and predictability of the QSAR model for antifungal activity *i.e.* Eq. 2 was cross validated by q^2 value (q^2 = 0.333) obtained by leave one out (LOO) method. In case of Eq. 2, the value of q^2 less than 0.5 indicated that the developed model was an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha, who reported that the only way to estimate the true predictive power of a model was to test their ability to predict accurately the biological activities of compounds. As the observed and predicted values were close to each other (Table 7), the *mt*-QSAR model for antifungal activity Eq. (3) was found to be a valid one (Golbraikh and Tropsha, 2002).

Electronic parameter total energy (Te) was found to be most effective in describing antimicrobial activity of the synthesized compounds (Eq. 3).

Table 6: Correlation of antibacterial, antifungal and antimicrobial activities of synthesized compounds with their molecular descriptors

Descriptor	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
Cos E	0.690	0.627	0.731
log P	0.644	0.605	0.685
MR	0.711	0.607	0.742
$^{0}\chi$	0.738	0.606	0.764
$^{0}\chi^{v}$	0.730	0.603	0.757
$^{1}\chi$	0.725	0.608	0.755
$^{1}\chi^{v}$	0.724	0.602	0.752
$^{2}\chi$	0.732	0.622	0.764
$^{2}\chi^{v}$	0.720	0.624	0.754
$^{3}\chi$	0.691	0.565	0.715
$^{3}\chi^{v}$	0.441	0.482	0.487
κ_1	0.738	0.594	0.761
κ_2	0.712	0.563	0.731
κ_3	0.694	0.533	0.706
$\kappa\alpha_1$	0.743	0.593	0.765
$\kappa\alpha_2$	0.715	0.557	0.731
$\kappa\alpha_3$	0.689	0.517	0.698
R	0.725	0.608	0.755
J	-0.675	-0.621	-0.718
W	0.716	0.597	0.744
Te	-0.758	-0.610	-0.783
Ee	-0.750	-0.604	-0.774
Ne	0.748	0.604	0.773
SA	0.732	0.586	0.754
IP	0.142	0.359	0.211
LUMO	-0.677	-0.663	-0.729
HOMO	-0.142	-0.359	-0.211
μ	-0.165	0.015	-0.128

Table 7: Observed, predicted and residual antimicrobial activities of the synthesized compounds obtained by mt-QSAR models.

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	**Pre.**	**Res.**	**Obs.**	**Pre.**	**Res.**	**Obs.**	**Pre.**	**Res.**
1	1.36	1.43	-.0.07	1.31	1.54	-0.23	1.34	1.47	-0.13
2	1.52	1.60	-0.08	1.34	1.55	-0.21	1.45	1.59	-0.14
3	1.45	1.45	0.00	1.31	1.56	-0.25	1.39	1.48	-0.09
4	1.16	1.43	-0.27	1.46	1.52	-0.06	1.28	1.47	-0.19
5	1.31	1.43	-0.12	1.64	1.54	0.10	1.44	1.47	-0.03
6	1.37	1.46	-0.09	1.32	1.52	-0.20	1.35	1.49	-0.14
7	1.44	1.40	0.04	1.60	1.53	0.07	1.50	1.45	0.05
8	1.49	1.52	-0.03	1.48	1.53	-0.05	1.49	1.54	-0.05
9	1.36	1.45	-0.09	1.46	1.53	-0.07	1.40	1.48	-0.08
10	1.49	1.52	-0.03	1.48	1.53	-0.05	1.49	1.54	-0.05
11	1.61	1.43	0.18	1.49	1.54	-0.05	1.56	1.47	0.09
12	1.57	1.51	0.06	1.62	1.60	0.02	1.59	1.52	0.07
13	1.15	1.42	-0.27	1.46	1.53	-0.07	1.27	1.46	-0.19
14	1.78	1.50	0.28	1.62	1.53	0.09	1.71	1.52	0.19
15	1.46	1.45	0.01	1.61	1.52	0.09	1.52	1.48	0.04
16	1.56	1.43	0.13	1.61	1.54	0.07	1.58	1.47	0.11
17	1.09	1.17	-0.08	1.48	1.40	0.08	1.24	1.27	-0.03
18	1.19	1.17	0.02	1.32	1.42	-0.10	1.24	1.27	-0.03
19	1.19	1.17	0.02	1.48	1.43	0.05	1.30	1.27	0.03
20	1.12	1.12	0.00	1.44	1.40	0.04	1.25	1.23	0.02
21	1.16	1.14	0.02	1.46	1.40	0.06	1.28	1.25	0.03
22	1.06	1.14	-0.08	1.31	1.42	-0.11	1.16	1.25	-0.09
23	1.22	1.19	0.03	1.19	1.40	-0.21	1.21	1.29	-0.08
24	1.22	1.19	0.03	1.49	1.43	0.06	1.33	1.29	0.04
25	1.40	1.26	0.14	1.38	1.40	-0.02	1.39	1.34	0.05
26	1.24	1.21	0.03	1.50	1.42	0.08	1.35	1.30	0.05
27	1.26	1.22	0.04	1.36	1.43	-0.07	1.30	1.31	-0.01

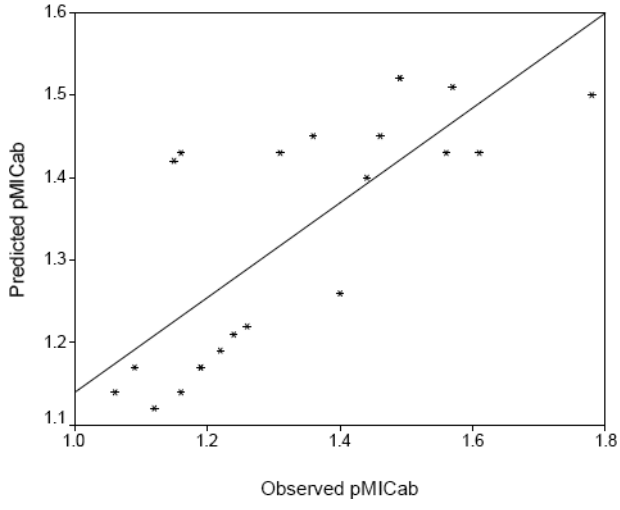


Fig. 2. Plot of observed $pMIC_{ab}$ against predicted $pMIC_{ab}$ by Eq 1.

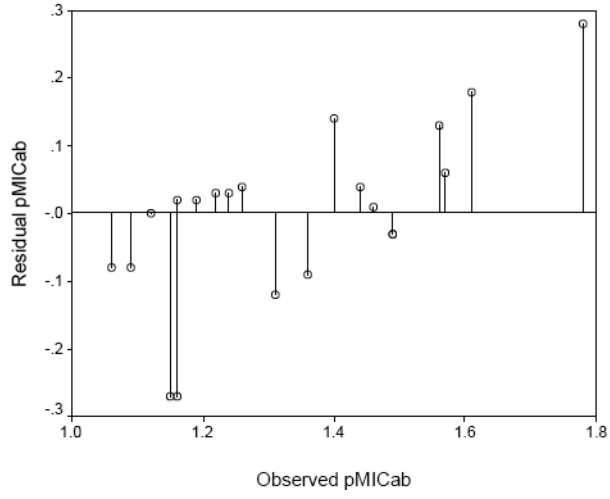


Figure 3: Plot of observed $pMIC_{ab}$ against residual $pMIC_{ab}$ by Eq 1

LR-*mt*-QSAR model for antimicrobial activity

$$pMIC_{am} = -0.000115\ Te + 0.985 \qquad (3)$$

$n = 22 \quad r = 0.783 \quad q^2 = 0.540 \quad s = 0.092 \quad F = 31.60$

Antimicrobial activity of the synthesized compounds was negatively correlated with total energy (Te) which means that antimicrobial activity of the synthesized compounds would increase with decrease in their Te values (Tables 2 and 4).

The total energy (Te) calculated by semi empirical methods can be used as a measure of non-specific interactions of a drug with its target site i.e. the total energies of the protonated and neutral forms of the molecule, can be considered as a good measure of the strength of hydrogen bonds (the higher the energy, the stronger the bond) and can be used to determine the correct localization of the most favorable hydrogen bond acceptor site (Narasimhan *et al,* 2009).

The validity of QSAR model for antimicrobial activity (Eq. 3) was indicated by its high q^2 value (0.540) as well as the low residual values (Table 7). Further, plot of predicted $pMIC_{am}$ against observed $pMIC_{am}$ (Fig. 4) also favoured the developed model expressed by Eq. 3. The plot of observed $pMIC_{am}$ *vs* residual $pMIC_{am}$ (Fig. 5) 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). The high residual values observed in case of outliers (**1, 2, 3, 6** and **23,** Table 7) justified their removal while developing QSAR models.

It was observed from *mt*-QSAR models [Eq. 1-3] that the antibacterial, antifungal and the overall antimicrobial activities of the synthesized PABA derivatives were governed by electronic parameters, total energy (Te) and energy of lowest unoccupied molecular orbital (LUMO).

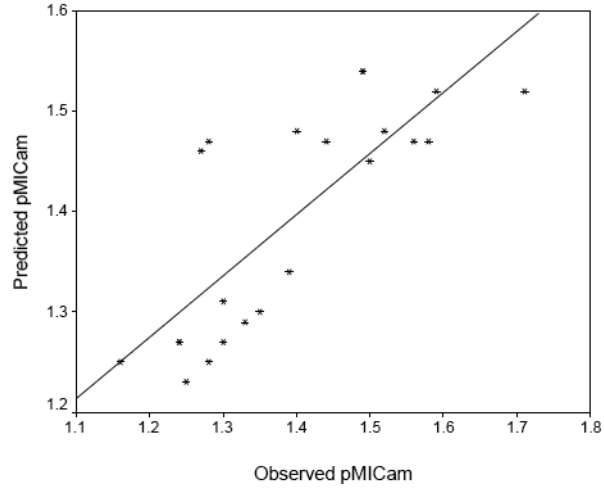


Figure 4: Plot of observed $pMIC_{am}$ against predicted $pMIC_{am}$ by Eq 3.

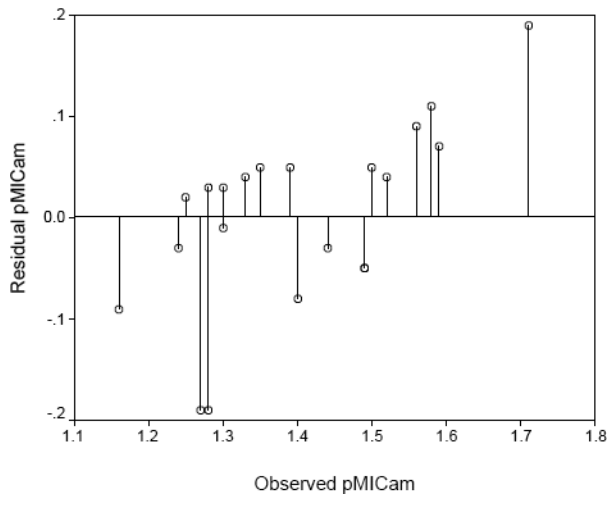


Figure 5: Plot of observed $pMIC_{am}$ against residual $pMIC_{am}$ by Eq 3.

4. CONCLUSION

A series of Schiff bases (**1-16**) and ester (**17-27**) derivatives of *p*-amino benzoic acid (PABA) was synthesized and evaluated for its *in vitro* antimicrobial potential by using tube dilution method. In general, Schiff's bases of PABA were found to be more potent than its esters.

Compound **11** was found to be most promising antibacterial agent. Further, QSAR studies were carried out to find correlation between antimicrobial activity and molecular descriptors of synthesized PABA derivatives which indicated the importance of electronic parameters total energy (Te) and energy of lowest unoccupied molecular orbital (LUMO) in describing the antimicrobial activity of synthesized compounds.

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