Acta Poloniae Pharmaceutica – Drug Research, Vol. 73 No. 1 pp. 65–78, 2016 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

2-AZETIDINONE DERIVATIVES: SYNTHESIS, ANTIMICROBIAL, ANTICANCER EVALUATION AND QSAR STUDIES

AAKASH DEEP[1]*, PRADEEP KUMAR[1], BALASUBRAMANIAN NARASIMHAN[1]*, SIONG MENG LIM[2,4], KALAVATHY RAMASAMY[2,4], RAKESH KUMAR MISHRA[2,3] and VASUDEVAN MANI[2,3]

[1] Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001, India
[2] Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia
[3] Brain Degeneration and Therapeutics Group, [4] Collaborative Drug Discovery Research (CDDR) Group, Brain and Neuroscience Communities of Research, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

Abstract: A series of 2-azetidinone derivatives was synthesized from hippuric acid and evaluated for its *in vitro* antimicrobial and anticancer activities. Antimicrobial properties of the title compounds were investigated against Gram positive and Gram negative bacterial as well as fungal strains. Anticancer activity was performed against breast cancer (MCF7) cell lines. Antimicrobial activity results revealed that *N*-{2-[3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (**4**) was found to be the most potent antimicrobial agent. Results of anticancer study indicated that the synthesized compounds exhibited average anticancer potential and *N*-[2-(3-chloro-2-oxo-4-styrylazetidin-1-ylamino)-2-oxoethyl]benzamide (**17**) was found to be most potent anticancer agent against breast cancer (MCF7) cell lines. QSAR models indicated that the antibacterial, antifungal and the overall antimicrobial activities of the synthesized compounds were governed by topological parameters, Balaban index (J) and valence zero and first order molecular connectivity indices ($^0\chi^v$ and $^1\chi^v$).

Keywords: 2-azetidinone, antimicrobial, anticancer, QSAR

Infectious diseases are answerable for a significant proportion of deaths worldwide and according to the World Health Organization, antimicrobial agents are considered to be "miracle drugs" that are the leading armaments in the treatment of infectious diseases. Unfortunately, however, a number of the current clinically efficacious antimicrobial agents are becoming less effective because of the spreading out of antimicrobial resistance and there is evidence for the rapid global spread of resistant clinical isolates and the appearance of drug-resistant strains among community acquired infections. So, there is an urgent need for the discovery of novel antimicrobial agents that are active against these resistant strains (1).

Cancer is a serious disease that can affect almost every tissue lineage in the human body and poses great challenges to medical science. Hence, there is necessity for the discovery and development of novel antitumor drug molecules which could effectively inhibit proliferative pathways (2).

The 2-azetidinones are of immense attention in diverse areas of medicinal chemistry because of their different pharmacological activities like antimicrobial (3, 4) anticancer (5, 6), antimycobacterial (7), anti-inflammatory and analgesic (8) and antiviral activities (9).

QSAR models are highly effective in describing the structural basis of biological activity. The success of QSAR approach can be explained by the insight offered into the structural determination of chemical properties and the possibility to estimate the properties of new chemical compounds without the need to synthesize and test them (10).

In the light of above facts and in continuation of our efforts in the development of novel antimi-

* Corresponding authors: e-mail: aakashdeep82@gmail.com; naru2000us@yahoo.com; phone: +91-1262-272535; fax: +91-1262-274133

crobial and anticancer agents (11-15), in the present study, we have planned to carry out synthesis, antimicrobial, anticancer evaluation and QSAR studies of 2-azetidinone derivatives.

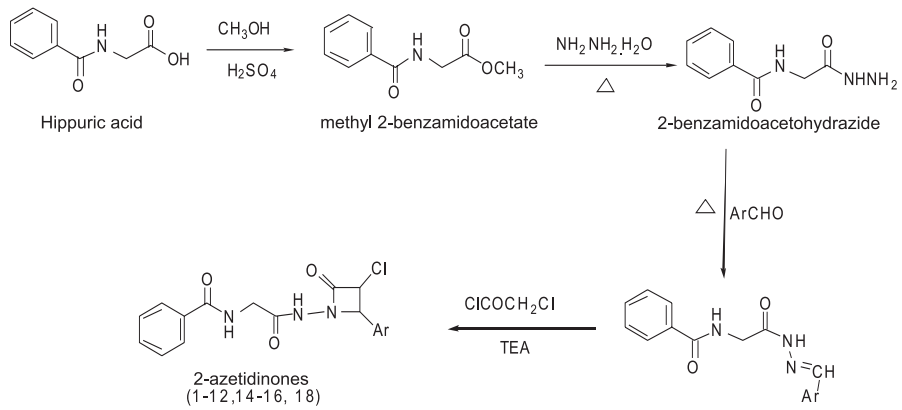
RESULTS AND DISCUSSION

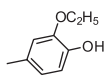
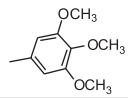
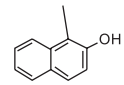
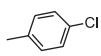
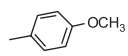
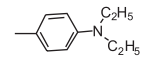
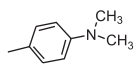
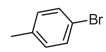
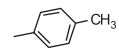
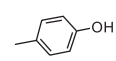
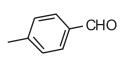
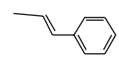
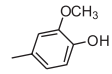
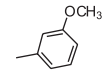
Chemistry

2-Azetidinone derivatives (**1**-**18**) were synthesized according to synthetic procedure described in Scheme 1. The structure of synthesized compounds was confirmed by NMR and IR spectra which were in full agreement with assigned molecular structures. The physicochemical properties of synthesized compounds are given in Table 1.

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was carried out using the tube dilution method (16) and results indicated that none of the synthesized compounds was found to be better antimicrobial agent than standard drugs, norfloxacin and fluconazole (Table 2). The antimicrobial activity results of the synthesized compounds indicated that compound **7** was found to be the most potent antimicrobial agent against *B. subtilis* (pMIC = 1.86 µM/mL). In case of *S. aureus* and fungal strain *C. albicans,* compound **9** (pMIC = 1.84 µM/mL) was found to be the most potent antimicrobial agent. Against Gram negative bacterium *E. coli* and fungal strain *A. niger*, compound **14** (pMIC = 1.84 µM/mL) was found to be the most potent antimicrobial agent.



Comp.	Ar	Comp.	Ar	Comp.	Ar
1		**7**		**13**	
2		**8**		**14**	
3		**9**		**15**	
4		**10**		**16**	
5		**11**		**17**	
6		**12**		**18**	

Scheme 1. Scheme for the synthesis of 2-azetidinone derivatives (**1-18**).

Table 1. Physicochemical properties and anticancer activity of the 2-azetidinone derivatives.

Comp.	Molecular formula	M. P. (°C)	M. W.	R_f value*	% yield	IC_{50} (µM) MCF-7)
1	$C_{20}H_{20}ClN_3O_5$	102-104	417.84	0.67	71	38.29
2	$C_{18}H_{15}Cl_2N_3O_3$	113-115	392.24	0.63	66	45.89
3	$C_{20}H_{21}ClN_4O_3$	125-127	400.86	0.56	79	107.27
4	$C_{18}H_{15}Cl_2N_3O_3$	109-111	392.24	0.69	82	> 254.95
5	$C_{19}H_{16}ClN_3O_4$	157-159	385.8	0.58	76	64.80
6	$C_{19}H_{18}ClN_3O_4$	134-136	387.82	0.62	77	232.07
7	$C_{21}H_{22}ClN_3O_6$	142-144	447.87	0.53	73	> 223.28
8	$C_{19}H_{18}ClN_3O_4$	130-132	387.82	0.65	69	> 257.85
9	$C_{18}H_{15}BrClN_3O_3$	118-120	436.69	0.74	70	57.25
10	$C_{18}H_{15}ClN_4O_5$	160-162	402.79	0.73	74	> 248.27
11	$C_{18}H_{16}ClN_3O_3$	195-197	357.79	0.67	71	111.80
12	$C_{19}H_{18}ClN_3O_5$	149-151	403.82	0.59	88	> 247.64
13	$C_{22}H_{18}ClN_3O_4$	171-173	423.85	0.71	79	61.34
14	$C_{22}H_{25}ClN_4O_3$	180-182	428.91	0.65	67	100.25
15	$C_{19}H_{18}ClN_3O_3$	189-191	371.82	0.75	80	75.31
16	$C_{18}H_{16}ClN_3O_4$	121-123	373.79	0.55	59	133.76
17	$C_{20}H_{18}ClN_3O_3$	144-146	383.83	0.64	65	28.66
18	$C_{19}H_{18}ClN_3O_4$	201-203	387.82	0.67	68	> 257.85
5-FU**						6.00
Carboplatin						> 100

*TLC mobile phase: benzene; ** 5-fluorouracil

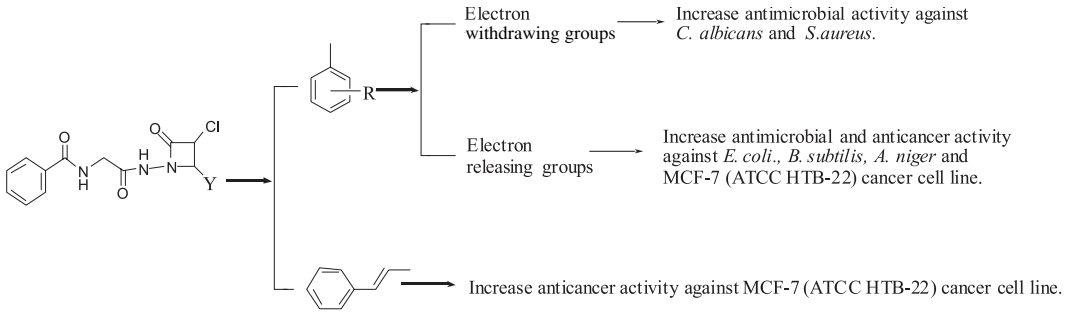


Figure 1. Structural requirements for the antimicrobial and anticancer activities of 2-azetidinone derivatives (**1-18**)

Overall antimicrobial activity results indicated that compound **4** (pMIC = 1.68 µM/mL) was found to be most potent antimicrobial agent.

Anticancer activity

The *in vitro* anticancer activity of the synthesized derivatives was determined against an estrogen receptor positive human breast adenocarcinoma, MCF-7 (ATCC HTB-22) cancer cell line using the sulforhodamine B (SRB) assay (17) and the results are presented in Table 1. In general, the synthesized compounds showed average anticancer activity as none of the synthesized compounds displayed better anticancer potential than standard drug – 5-fluorouracil (5-FU) (IC_{50} = 6.00 µM) and compounds **1**, **2**, **5**, **9**, **13**, **15** and **17** showed better anticancer potential than standard drug carboplatin (IC_{50} = > 100 µM). Compound **17** (IC_{50} = 28.66 µM) was found to be the most potent anticancer agent.

SAR (Structure Activity Relationship) studies

From the anticancer and antimicrobial screening results of synthesized 2-azetidinone derivatives, the following structure activity relationship (SAR) can be derived:

1. The presence of electron withdrawing group (-Br, compound **9**) on benzylidene portion improved the antimicrobial activity of the synthesized compounds against *C. albicans* and *S. aureus*.
2. The presence of electron releasing groups (compounds **1, 7** and **14**) on benzylidene portion improved the antimicrobial activity of synthesized compounds against *A. niger, B. subtilis*, *E. coli* and their anticancer activity against MCF-7 (ATCC HTB-22) cancer cell line.
3. Compound **17** (synthesized using cinnamaldehyde) was found to be active against an estrogen receptor positive human breast adenocarcinoma, MCF-7 (ATCC HTB-22) cancer cell line.
4. From these result we may conclude that different structural requirements are required for a compound to be effective against different targets. This is similar to the results of Sortino et al. (18).

These findings are summarized in Figure 1.

QSAR studies

Quantitative structure activity relationship (QSAR) is a predictive tool for preliminary evaluation of the activity of chemical compounds by using computer-aided models. For QSAR studies, the biological activity data determined as MIC values were first transformed into pMIC (–log MIC) values and used as dependent variables QSAR study (Table 2). The different molecular descriptors selected and the values of selected descriptors are listed in Tables 3 and 4, respectively.

In the present study, we 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 as a common *mt*-QSAR model for describing the antimicrobial (overall antibacterial and antifungal) activity of synthesized compounds by calculating their average antibacterial activity, antifungal activity and

Table 2. Antimicrobial activity (pMIC in µM/mL) of synthesized compounds.

Compound	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{sa}$	$pMIC_{an}$	$pMIC_{ca}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	0.92	1.83	1.22	1.52	1.52	1.32	1.52	1.40
2	1.50	1.80	0.89	1.20	1.50	1.20	1.35	1.38
3	1.81	1.51	1.51	1.51	1.21	1.50	1.36	1.51
4	1.50	1.80	1.80	1.80	1.50	1.80	1.65	1.68
5	0.89	0.89	1.19	1.49	1.79	1.19	1.64	1.25
6	1.19	1.49	1.79	1.79	1.49	1.39	1.64	1.55
7	1.86	1.25	1.55	0.95	1.25	1.33	1.10	1.37
8	1.19	1.49	1.79	1.49	1.49	1.71	1.49	1.49
9	1.24	1.54	1.84	1.54	1.84	1.53	1.69	1.60
10	1.81	1.51	1.21	1.81	1.81	1.32	1.81	1.63
11	1.46	1.46	1.46	1.16	1.16	1.57	1.16	1.34
12	0.91	1.81	0.91	1.51	1.81	1.39	1.66	1.39
13	1.53	0.93	1.53	1.83	1.23	1.12	1.53	1.41
14	1.84	1.84	0.93	1.84	1.54	1.43	1.69	1.60
15	1.47	1.77	1.47	1.47	1.47	1.69	1.47	1.53
16	1.48	0.87	1.78	1.78	1.78	1.37	1.78	1.54
17	1.26	1.57	0.96	0.96	1.57	1.34	1.26	1.26
18	1.19	1.79	1.19	1.49	1.79	1.42	1.64	1.49
S.D.	0.32	0.33	0.34	0.28	0.23	0.18	0.21	0.12
Standard	2.61*	2.61*	2.61*	2.61*	2.64**			

*norfloxacin, **fluconazole

Table 3. QSAR descriptors used in the study.

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 (R)	Topological
12	Balaban topological index (J)	Topological
13	Wiener's topological index (W)	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

antimicrobial activity values which are presented in Table 2.

Our preceding studies in the field of QSAR (11-15) indicated that the multi-target QSAR (*mt*-QSAR) models are better than one-target QSAR (*ot*-QSAR) models in describing the antimicrobial activity.

Different outliers were observed against different microorganisms and QSAR models were developed by removing these outliers (compounds in brackets) i.e., antibacterial (**1, 3, 4, 5, 10** and **12**), antifungal (**2, 3, 9, 10, 11, 14** and **16**) and antimicrobial (**4, 5, 7, 9, 10, 11** and **14**) activities. In multivariate statistics, it is common to define three types of outliers (19).

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.

As there was no difference in the activity (Table 2) as well as the molecular descriptor range (Table 4) of these outliers when compared to other synthesized compounds, these outliers belong to the category of Y outliers (substances for which the reference value of response is invalid). In order to develop mt-QSAR models, initially we calculated the average antibacterial, antifungal and antimicrobial activity values of synthesized compounds which are presented in Table 2. These average antibacterial activity values were correlated with the molecular descriptors of synthesized compounds (Table 5).

In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between topological parameter, valence first order molecular connectivity index ($^{1}\chi^{v}$) and molar refractivity (MR) ($r = 0.989$), and low interrelationship was observed for topological parameter, valence third order molecular connectivity index ($^{3}\chi^{v}$) electronic parameter, energy

Table 4. Values of selected descriptors calculated for synthesized compounds.

Comp. no.	log P	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{3}\chi^{v}$	κ_1	J	LUMO	HOMO	μ
1	1.93	16.41	9.45	0.82	23.66	1.41	-0.39	-8.97	4.95
2	2.64	15.12	8.71	0.89	20.73	1.38	-0.71	-9.90	2.02
3	1.92	16.37	9.23	0.99	22.68	1.39	-0.25	-8.76	7.58
4	2.64	15.12	8.71	0.85	20.73	1.40	-0.73	-9.87	5.91
5	1.80	14.91	8.63	0.79	21.70	1.38	-1.24	-10.04	7.19
6	1.87	15.33	8.73	0.74	21.70	1.43	-0.26	-9.21	3.78
7	1.37	18.00	9.78	0.86	25.62	1.45	-0.27	-9.14	7.65
8	1.87	15.33	8.72	0.76	21.70	1.38	-0.32	-9.13	2.49
9	2.92	15.93	9.11	1.03	20.73	1.38	-0.78	-9.92	2.04
10	2.08	15.19	8.70	0.81	22.68	1.40	-1.22	-9.94	3.93
11	2.13	14.00	8.20	0.69	19.75	1.37	-0.41	-9.85	3.21
12	1.59	15.70	8.86	0.82	22.68	1.40	-0.27	-9.01	4.58
13	2.84	16.53	9.75	0.89	23.17	1.20	-0.93	-9.04	4.97
14	2.60	17.79	10.38	0.88	24.64	1.39	-0.23	-8.66	8.48
15	2.59	14.93	8.61	0.86	20.73	1.38	-0.41	-9.79	3.83
16	1.84	14.37	8.33	0.77	20.73	1.38	-0.44	-9.62	3.27
17	4.34	18.47	10.93	0.89	26.07	1.12	-0.92	-8.89	3.80
18	1.87	15.33	8.72	0.76	21.70	1.39	-0.25	-9.39	4.73

Table 5. Correlation matrix for the antibacterial activity of the synthesized compounds.

	$pMIC_{ab}$	log P	MR	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{3}\chi^{v}$	J	LUMO	HOMO	μ
$pMIC_{ab}$	1.000									
log P	-0.506	1.000								
MR	-0.337	0.614	1.000							
$^{0}\chi^{v}$	-0.116	0.432	0.953	1.000						
$^{1}\chi^{v}$	-0.265	0.612	0.989	0.965	1.000					
$^{3}\chi^{v}$	0.089	0.540	0.424	0.507	0.509	1.000				
J	0.786	-0.835	-0.629	-0.400	-0.580	-0.256	1.000			
LUMO	0.602	-0.779	-0.352	-0.213	-0.353	-0.597	0.794	1.000		
HOMO	-0.190	0.153	0.768	0.747	0.756	-0.029	-0.330	0.122	1.000	
μ	0.238	-0.184	0.536	0.641	0.564	0.042	0.123	0.403	0.685	1.000

of highest occupied molecular orbital (HOMO) (r = -0.029). Corrrelation of molecular descriptors with antibacterial, antifungal and antimicrobial activities of synthesized compounds is shown in Table 6.

From the correlation Table 5, it was observed that Balaban index (J) and topological parameter, was found to be dominating descriptor for antibacterial activity of the synthesized compounds (Eq. 1).

QSAR model for antibacterial activity

$$pMIC_{ab} = 0.809\ J + 0.356 \qquad \text{Eq. 1}$$

n = 12 r = 0.786 q^2 = 0.534s = 0.064 F = 16.14

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 is a positive cor-

relation between Balaban index (J) and antibacterial activity of the synthesized compounds. This is indicated by low antibacterial activity value of compound **13** ($pMIC_{ab}$ = 1.12 μM/mL) having low J value (1.20).

The topological parameters signify the degree of branching, connectivity of atoms and the unsaturation in the molecule, that accounts for variation in activity. The topological parameter, Balaban index J = J(G) of G is defined as

$$J = M/(\mu + 1)\ \Sigma(di.dj)^{-0.5}$$

where M is the number of bonds in G, μ is the cyclomatic number of G, and di (i = 1, 2, 3, N; N is the number of vertices in G) is the distance sum. The cyclomatic number μ = μ(G) of a cyclic graph G is equal to the minimum number of edges necessary to be erased from G in order to transform it into the related acyclic graph. In case of monocyclic graph μ = 1 otherwise it is calculated by means of the following expression:

$$\mu = M - N + 1$$

In order to improve the value of correlation coefficient, we coupled Balaban index (J) with topological parameter, valence third order molecular connectivity index ($^3\chi^v$) which improved r value from 0.786 to 0.841.

MLR-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.330\ ^3\chi^v + 0.891\ J - 0.030 \qquad \text{Eq. 2}$$

$$n = 12\ r = 0.841\ q^2 = 0.603\ s = 0.059\ F = 10.89$$

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

Descriptors	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
Cos E	0.350	-0.611	-0.327
log P	-0.506	-0.239	-0.695
MR	-0.337	-0.715	-0.757
$^0\chi$	-0.268	-0.770	-0.765
$^0\chi^v$	-0.116	-0.834	-0.768
$^1\chi$	-0.362	-0.735	-0.768
$^1\chi^v$	-0.265	-0.709	-0.789
$^2\chi$	-0.447	-0.682	-0.769
$^2\chi^v$	-0.299	-0.659	-0.748
$^3\chi$	-0.211	-0.590	-0.489
$^3\chi^v$	0.089	-0.532	-0.348
κ_1	-0.202	-0.792	-0.758
κ_2	-0.217	-0.771	-0.735
κ_3	-0.284	-0.678	-0.706
$\kappa\alpha_1$	-0.097	-0.807	-0.761
$\kappa\alpha_2$	-0.092	-0.796	-0.737
$\kappa\alpha_3$	-0.145	-0.712	-0.698
R	-0.362	-0.735	-0.768
J	0.786	0.254	0.678
W	-0.396	-0.728	-0.786
Te	0.172	0.724	0.782
Ee	0.058	0.816	0.688
Ne	-0.049	-0.821	-0.676
SA	-0.185	-0.790	-0.741
IP	0.190	0.381	0.304
LUMO	0.602	-0.058	0.687
HOMO	-0.190	-0.381	-0.304
μ	0.238	-0.194	0.104

The developed QSAR model (Eq. 2) was cross validated by q^2 value ($q^2 = 0.603$; $q^2 > 0.5$) obtained by leave one out (LOO) method. As the observed and predicted values are close to each other (Table 7), the *mt*-QSAR model for antifungal activity (Eq. 2) is a valid one (20). The plot of predicted $pMIC_{ab}$ against observed $pMIC_{ab}$ (Fig. 2) also favors the developed model expressed by Eq. 2. The absence of systemic error in the model development was indicated by the plot of observed $pMIC_{ab}$ *vs.* residual $pMIC_{ab}$ (Fig. 3) (21).

In case of antifungal activity, topological parameter, valence zero order molecular connectivity index ($^0\chi^v$, Table 6) were found most dominant in expressing antifungal activity of the synthesized compounds.

QSAR model for antifungal activity

$$pMIC_{af} = -0.123\ ^0\chi^v + 3.473 \qquad \text{Eq. 3}$$

$n = 11$ $r = 0.834$ $q^2 = 0.481$ $s = 0.105$ $F = 20.52$

In contrast to antibacterial activity, antifungal activity of the synthesized 2-azetidinone compounds is negatively correlated with their $^0\chi^v$ values, which means that antifungal activity of the synthesized compounds will increase with decrease in their $^0\chi^v$ values and vice versa (Tables 2 and 4).

The validity and predictability of the QSAR model for antifungal activity i.e., Eq. 3 was cross validated by q^2 value ($q^2 = 0.481$) obtained by leave one out (LOO) method. As the observed and predicted values are close to each other (Table 7), the *mt*-QSAR model for antifungal activity Eq. (3) is a valid one (20).

Topological parameter valence first order molecular connectivity index ($^1\chi^v$) was found to be most effective in describing antimicrobial activity of the synthesized compounds (Eq. 4, Table 6).

QSAR model for antimicrobial activity

$$pMIC_{am} = -0.096\ ^1\chi^v + 2.325 \qquad \text{Eq. 4}$$

$n = 11$ $r = 0.789$ $q^2 = 0.500$ $s = 0.058$ $F = 14.87$

Antimicrobial activity of the synthesized 2-azetidinone compounds is negatively correlated with valence first order molecular connectivity index ($^1\chi^v$), which means that antimicrobial activity of the synthesized compounds will decrease with an increase in their $^1\chi^v$ values (Tables 2 and 4). Further, developed model is favored by plot of predicted $pMIC_{am}$ against observed $pMIC_{am}$ (Fig. 4; Eq. 4) and there is no systemic error (Fig. 5; plot of observed $pMIC_{am}$ *vs.* residual $pMIC_{am}$).

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.	Pred.	Res.	Obs.	Pred.	Res.	Obs.	Pred.	Res.
1	1.32	1.49	-0.17	1.52	1.45	0.07	1.40	1.42	-0.02
2	1.40	1.49	-0.09	1.35	1.61	-0.26	1.38	1.49	-0.11
3	1.61	1.53	0.08	1.36	1.46	-0.10	1.51	1.44	0.07
4	1.70	1.50	0.20	1.65	1.61	0.04	1.68	1.49	0.19
5	0.99	1.46	-0.47	1.64	1.64	0.00	1.25	1.50	-0.25
6	1.49	1.48	0.01	1.64	1.59	0.05	1.55	1.49	0.06
7	1.55	1.55	0.00	1.10	1.26	-0.16	1.37	1.39	-0.02
8	1.49	1.45	0.04	1.49	1.59	-0.10	1.49	1.49	0.00
9	1.54	1.54	0.00	1.69	1.51	0.18	1.60	1.45	0.15
10	1.51	1.48	0.03	1.81	1.60	0.21	1.63	1.49	0.14
11	1.46	1.42	0.04	1.16	1.75	-0.59	1.34	1.54	-0.20
12	1.21	1.49	-0.28	1.66	1.54	0.12	1.39	1.47	-0.08
13	1.33	1.33	0.00	1.53	1.44	0.09	1.41	1.39	0.02
14	1.54	1.50	0.04	1.69	1.29	0.40	1.60	1.33	0.27
15	1.57	1.48	0.09	1.47	1.64	-0.17	1.53	1.50	0.03
16	1.38	1.45	-0.07	1.78	1.71	0.07	1.54	1.53	0.01
17	1.26	1.26	0.00	1.26	1.20	0.06	1.26	1.28	-0.02
18	1.39	1.46	-0.07	1.64	1.59	0.05	1.49	1.49	0.00

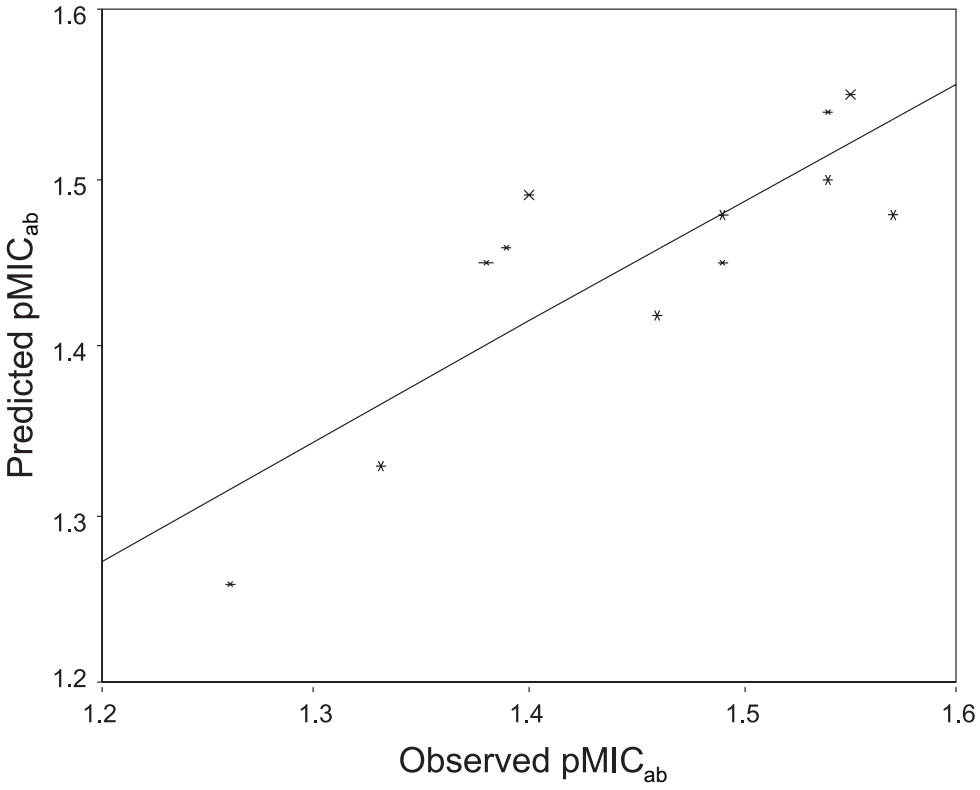


Figure 2. Plot of observed $pMIC_{ab}$ against predicted $pMIC_{ab}$ by Eq. 2

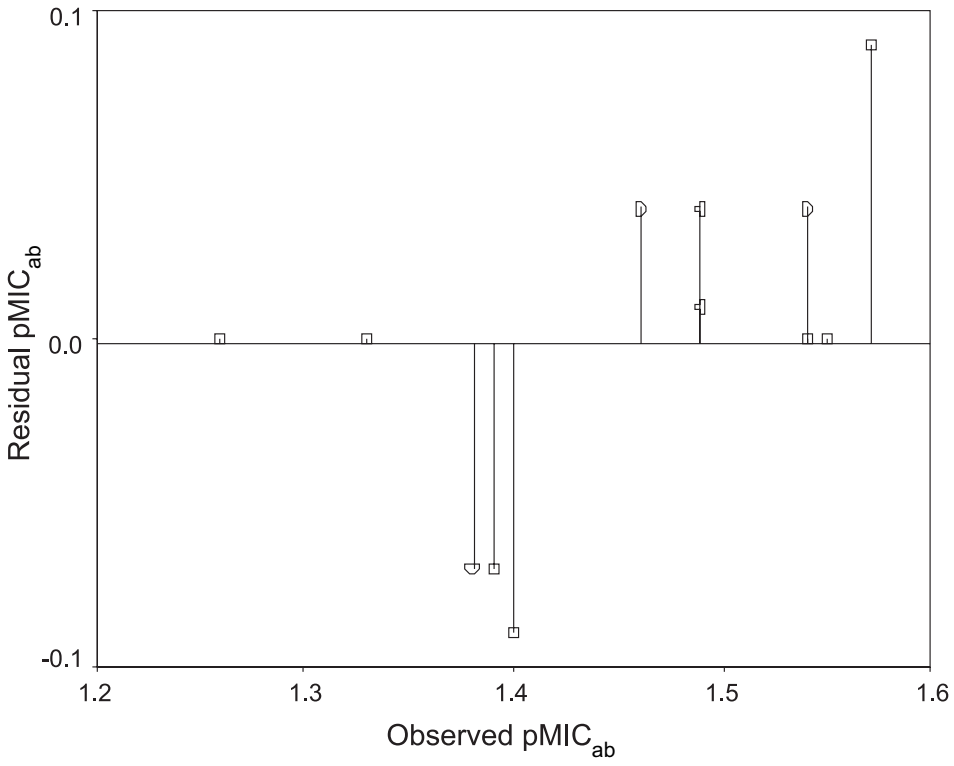


Figure 3. Plot of observed $pMIC_{ab}$ against residual $pMIC_{ab}$ by Eq. 2

EXPERIMENTAL

Chemistry

Reaction progress was observed by thin layer chromatography. 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 Bruker 400 MHz NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). IR spectra were recorded on a Varian Resolutions Pro spectrophotometer in KBr discs.

General procedure for synthesis of 2-azetidinone derivatives

A mixture of hippuric acid (0.25 M) and excess of methanol (250 mL) with 1 mL of sulfuric acid was refluxed for 3–4 h in round bottom flask. The mixture was cooled; the precipitated solid was separated by filtration and recrystallized from methanol to yield methyl 2-benzamidoacetate. A mixture of methyl 2-benzamidoacetate (0.20 M) and excess of hydrazine hydrate (0.30 M) and ethanol (250 mL) was refluxed for about 3 h and cooled. The solid was separated by filtration and recrystallized from ethanol to afford 2-benzamidoacetohydrazide. A mixture of 2-benzamidoacetohydrazide (0.025 M) and required aromatic aldehydes (0.025 M) was refluxed in methanol (50 mL) in the presence of a catalytic amount of glacial acetic acid for about 2 h. The mixture was cooled; the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazones of hippuric acid. A mixture of hydrazone of hippuric acid (0.015 M) and required amount of triethylamine (0.02 M) and chloroacetylchloride (0.02 M) in DMF (40 mL) was refluxed for 3 h on water bath to yield 2-azetidinone derivatives (**1**-**18**). After cooling, the solution was poured on crushed ice to precipitate the product. The product was recrystallized from rectified spirit.

N-{2-[3-chloro-2-(3-ethoxy-4-hydroxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (1)

IR (KBr, cm^{-1}): 3667 (OH), 3398 (NH), 2978 (C-H aromatic), 1770 (C=O of β-lactam), 1702 (C=O), 1629 (C=C aromatic), 1280 (C-N), 1172 (C-O-C str., -OC_2H_5), 769 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.83-7.37 (m, 8H, ArH), 8.09 (s, 1H, NH), 6.83 (d, 1H, CH-Cl), 4.27 (s, 1H, OH), 4.09 (d, 2H, CH_2), 4.05 (m, 2H, CH_2 of -OC_2H_5), 1.36 (t, 3H, CH_3 of -OC_2H_5). Analysis: for $C_{20}H_{20}ClN_3O_5$ calcd.: C, 57.49; H, 4.82; N, 10.06%; found: C, 57.44; H, 4.76; N, 10.09%.

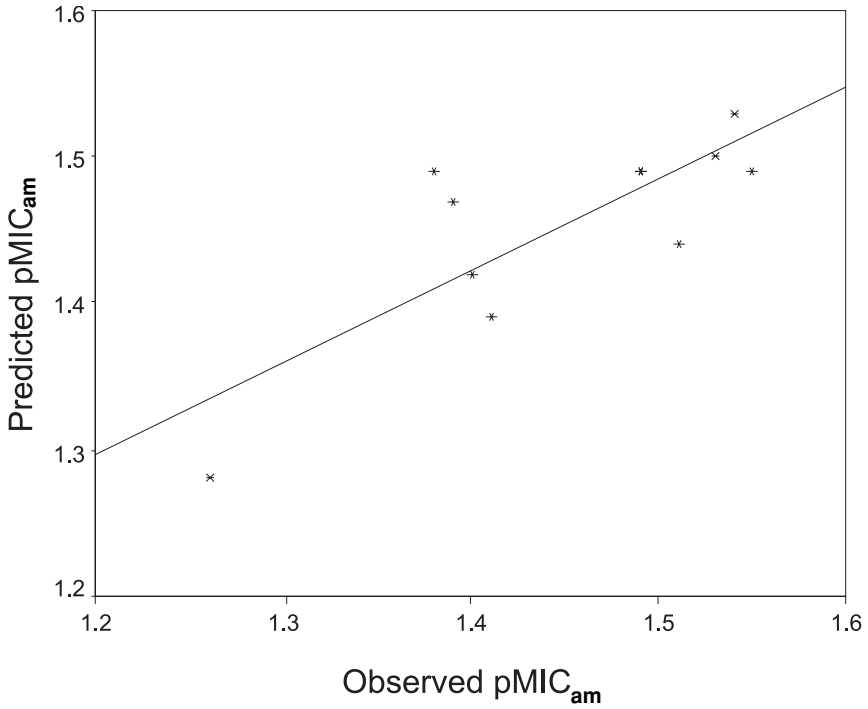


Figure 4. Plot of observed pMIC$_{am}$ against predicted pMIC$_{am}$ by Eq. 4

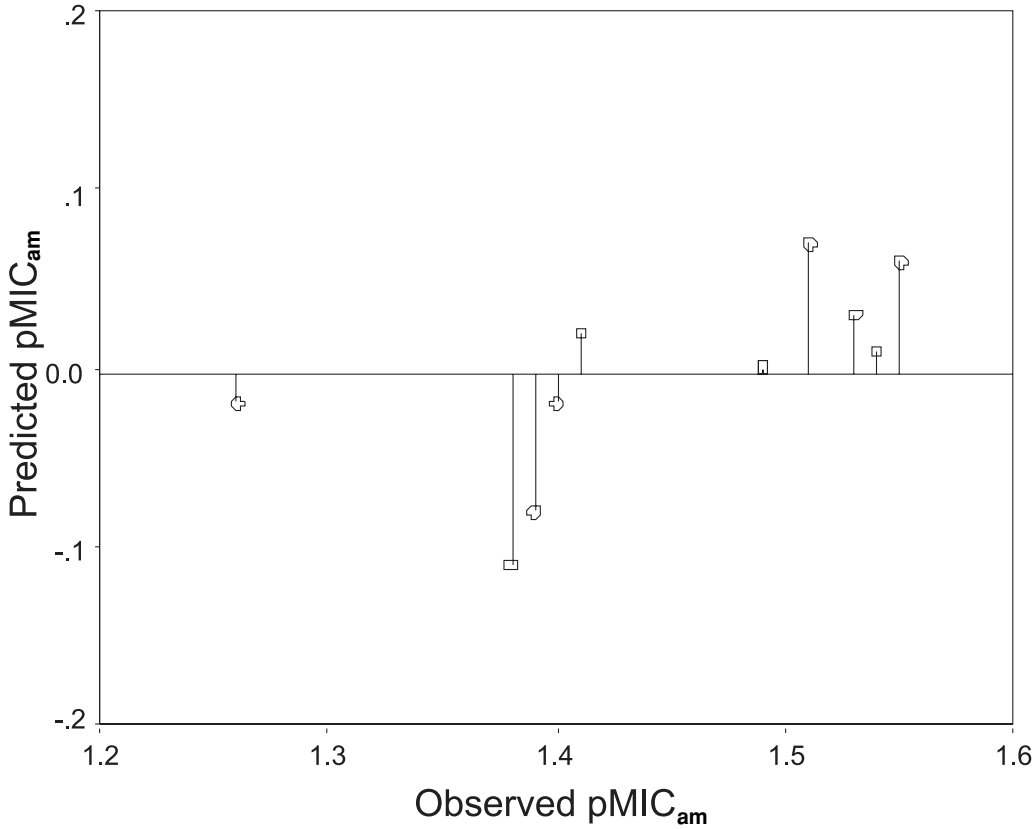


Figure 5. Plot of observed $pMIC_{am}$ against residual $pMIC_{am}$ by Eq. 4

***N*-{2-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (2)**

IR (KBr, cm^{-1}): 3306 (NH), 3046 (C-H aromatic), 1752 (C=O of β-lactam), 1692 (C=O), 1626 (C=C aromatic), 1209 (C-N), 706 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.38-7.58 (m, 9H, ArH), 8.01 (s, 1H, NH), 7.50 (d, 1H, CH-Cl), 4.69 (s, 1H, OH), 4.19 (d, 2H, CH_2). Analysis: for $C_{18}H_{15}Cl_2N_3O_3$ calcd.: C, 55.12; H, 3.85; N, 10.71%; found: C, 55.18; H, 3.91; N, 10.77%.

***N*-{2-[3-chloro-2-(4-dimethylamino)phenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (3)**

IR (KBr, cm^{-1}): 3413 (NH), 3052 (C-H aromatic), 2805 (C-H str., CH_3), 1734 (C=O of β-lactam), 1719 (C=O), 1635 (C=C aromatic), 1368 (C-N str., aryl tertiary amine), 1224 (C-N), 741 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.57-7.47 (m, 9H, ArH), 8.09 (s, 1H, NH), 6.73 (d, 1H, CH-Cl), 4.38 (d, 2H, CH_2), 2.97 (s, 6H, $N(CH_3)_2$). Analysis: for $C_{20}H_{21}ClN_4O_3$ calcd.: C, 59.92; H, 8.84; N, 13.98%; found: C, 59.97; H, 8.89; N, 13.94%.

***N*-{2-[3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (4)**

IR (KBr, cm^{-1}): 3410 (NH), 2997 (C-H aromatic), 1762 (C=O of *β*-lactam), 1714 (C=O), 1636 (C=C aromatic), 1240 (C-N), 714 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.27-7.55 (m, 9H, ArH), 8.04 (s, 1H, NH), 7.48 (d, 1H, CH-Cl), 4.25 (d, 2H, CH_2). Analysis: for $C_{18}H_{15}Cl_2N_3O_3$ calcd.: C, 55.12; H, 18.08; N, 10.71%; found: C, 55.16; H, 18.04; N, 10.75%.

***N*-{2-[3-chloro-2-(4-formylphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (5)**

IR (KBr, cm^{-1}): 3431 (NH), 3009 (C-H aromatic), 2953 (C-H str., CHO), 1739 (C=O of *β*-lactam), 1701 (C=O), 1616 (C=C aromatic), 1273 (C-N), 751 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.98 (s, 1H, CHO), 8.00 (s, 1H, NH), 7.95-7.58 (m, 9H, ArH), 7.42 (d, 1H, CH-Cl), 4.14 (d, 2H, CH_2); Analysis: for $C_{19}H_{16}ClN_3O_4$ calcd.: C, 59.15; H, 9.19; N, 10.89%; found: C, 59.11; H, 9.23; N, 10.85%.

***N*-{2-[3-chloro-2-(2-methoxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (6)**

IR (KBr, cm^{-1}): 3421 (NH), 3063 (C-H aromatic), 1728 (C=O of β-lactam), 1692 (C=O), 1598 (C=C aromatic), 1225 (C-N), 1175 (C-O-C str., $-OCH_3$), 714 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.05 (s, 1H, NH), 7.88-7.49 (m, 9H, ArH), 6.78 (d, 1H, CH-Cl), 4.12 (d, 2H, CH_2), 3.94 (s, 3H, OCH_3). Analysis: for $C_{19}H_{18}ClN_3O_4$ calcd.: C, 58.84; H, 4.68; N, 10.84%; found: C, 58.89; H, 4.64; N, 10.88%.

***N*-{2-[3-chloro-2-oxo-4-(3,4,5-trimethoxyphenyl)azetidin-1-ylamino]-2-oxoethyl}benzamide (7)**

IR (KBr, cm^{-1}): 3378 (NH), 2945 (C-H aromatic), 1745 (C=O of β-lactam), 1697 (C=O), 1622 (C=C aromatic), 1295 (C-N), 720 (Cl), 1184 (C-O-C str., $-OCH_3$); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.16-7.22 (m, 7H, ArH), 7.93 (s, 1H, NH), 7.01 (d, 1H, CH-Cl), 4.43 (d, 2H, CH_2), 3.98 (s, 9H, $(OCH_3)_3$). Analysis: for $C_{21}H_{22}ClN_3O_6$ calcd.: C, 56.32; H, 7.92; N, 9.38%; found: C, 56.36; H, 7.91; N, 9.32%.

***N*-{2-[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (8)**

IR (KBr, cm^{-1}): 3337 (NH), 3036 (C-H aromatic), 1717 (C=O of β-lactam), 1701 (C=O), 1634 (C=C aromatic), 1247 (C-N), 1166 (C-O-C str., $-OCH_3$), 712 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.96 (s, 1H, NH), 7.90-7.07 (m, 9H, ArH), 6.99 (d, 1H, CH-Cl), 4.41 (d, 2H, CH_2), 3.98 (s, 3H, (OCH_3). Analysis: for $C_{19}H_{18}ClN_3O_4$ calcd.: C, 58.84; H, 4.68; N, 10.84%; found: C, 58.80; H, 4.73; N, 10.89%.

***N*-{2-[2-(4-bromophenyl)-3-chloro-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (9)**

IR (KBr, cm^{-1}): 3312 (NH), 3005 (C-H aromatic), 1727 (C=O of β-lactam), 1701 (C=O), 1630 (C=C aromatic), 1288 (C-N), 707 (Cl), 659 (C-Br); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.99 (s, 1H, NH), 7.84-7.56 (m, 9H, ArH), 7.46 (d, 1H, CH-Cl), 4.43 (d, 2H, CH_2); Analysis: for $C_{18}H_{15}BrClN_3O_3$ calcd.: C, 49.51; H, 3.46; N, 9.62%; found: C, 49.57; H, 3.41; N, 9.67%.

***N*-{2-[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (10)**

IR (KBr, cm^{-1}): 3432 (NH), 3006 (C-H aromatic), 1753 (C=O of β-lactam), 1688 (C=O), 1630 (C=C aromatic), 1352 (NO_2), 1214 (C-N), 700 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.36-7.50 (m, 9H, ArH), 7.88 (s, 1H, NH), 7.26 (d, 1H, CH-Cl), 4.27 (d, 2H, CH_2). Analysis: for $C_{18}H_{15}ClN_4O_5$ calcd.: C, 53.67; H, 3.75; N, 13.91%; found: C, 53.61; H, 3.79; N, 13.88%.

***N*-[2-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)-2-oxoethyl]benzamide (11)**

IR (KBr, cm^{-1}): 3461 (NH), 3003 (C-H aromatic), 1735 (C=O of β-lactam), 1723 (C=O), 1645 (C=C aromatic), 1264 (C-N), 715 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.96 (s, 1H, NH), 7.87-6.99 (m, 9H, ArH), 6.83 (d, 1H, CH-Cl), 3.17 (d, 2H, CH_2). Analysis: for $C_{18}H_{16}ClN_3O_3$ calcd.: C, 60.42; H, 4.51; N, 11.74%; found: C, 60.46; H, 4.57; N, 11.68%.

***N*-{2-[3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (12)**

IR (KBr, cm^{-1}): 3595 (OH), 3404 (NH), 3005 (C-H aromatic), 1722 (C=O of β-lactam), 1707 (C=O), 1633 (C=C aromatic), 1280 (C-N), 1139 (C-O-C str., $-OCH_3$), 756 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.70-7.46 (m, 8H, ArH), 8.02 (s, 1H, NH), 7.44 (d, 1H, CH-Cl), 4.44 (s, 1H, OH), 4.27 (d, 2H, CH_2), 3.94 (s, 3H, (OCH_3). Analysis: for $C_{19}H_{18}ClN_3O_5$ calcd.: C, 56.51; H, 4.49; N, 10.41%; found: C, 56.57; H, 4.43; N, 10.46%.

***N*-{2-[3-chloro-2-(2-hydroxynaphthalen-1-yl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (13)**

IR (KBr, cm^{-1}): 3612 (OH), 3416 (NH), 3033 (C-H aromatic), 1777 (C=O of β-lactam), 1696 (C=O), 1621 (C=C aromatic), 1282 (C-N), 746 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.04 (s, 1H, NH), 7.63-7.28 (m, 11H, ArH), 7.23 (d, 1H, CH-Cl), 4.28 (d, 2H, CH_2), 4.27 (s, 1H, OH). Analysis: for $C_{22}H_{18}ClN_3O_4$ calcd.: C, 62.34; H, 4.28; N, 9.91%; found: C, 62.39; H, 4.23; N, 9.99%.

***N*-{2-[3-chloro-2-(4-(diethylamino)phenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (14)**

IR (KBr, cm^{-1}): 3399 (NH), 3035 (C-H aromatic), 2966 (C-H str., CH_3), 1751 (C=O of β-lactam), 1702 (C=O), 1599 (C=C aromatic), 1354 (C-N str., aryl tertiary amine), 1267 (C-N), 714 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.19-7.48 (m, 9H, ArH), 8.06 (s, 1H, NH), 7.46 (d, 1H, CH-Cl). Analysis: for $C_{22}H_{25}ClN_4O_3$ calcd.: C, 61.61; H, 5.87; N, 13.06%; found: C, 61.66; H, 5.82; N, 13.01%.

***N*-[2-(3-chloro-2-oxo-4-p-tolylazetidin-1-ylamino)-2-oxoethyl]benzamide (15)**

IR (KBr, cm^{-1}): 3317 (NH), 3060 (C-H aromatic), 2878 (C-CH_3) 1760 (C=O of β-lactam), 1737

(C=O), 1641 (C=C aromatic), 1278 (C-N), 775 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.12-7.47 (m, 9H, ArH), 7.89 (s, 1H, NH), 6.92 (d, 1H, CH-Cl), 4.13 (d, 2H, CH_2), 2.51 (s, 3H, CH_3). Analysis: for $C_{19}H_{18}ClN_3O_3$ calcd.: C, 61.38; H, 4.88; N, 11.30%; found: C, 61.33; H, 4.92; N, 11.34%.

***N*-{2-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (16)**

IR (KBr, cm^{-1}): 3581 (OH), 3415 (NH), 3005 (C-H aromatic), 1747 (C=O of β-lactam), 1717 (C=O), 1632 (C=C aromatic), 1282 (C-N), 717 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.25-7.48 (m, 9H, ArH), 7.91 (s, 1H, NH), 7.43 (d, 1H, CH-Cl), 4.43 (s, 1H, OH), 4.28 (d, 2H, CH_2). Analysis: for $C_{18}H_{16}ClN_3O_4$ calcd.: C, 57.84; H, 4.31; N, 11.24%; found: C, 57.89; H, 4.27; N, 11.28%.

***N*-[2-(3-chloro-2-oxo-4-styrylazetidin-1-ylamino)-2-oxoethyl]benzamide (17)**

IR (KBr, cm^{-1}): 3441 (NH), 2922 (C-H aromatic), 2866 (C-H str., -CH=CH-), 1719 (C=O of β-lactam), 1686 (C=O), 1641 (C=C aromatic), 1285 (C-N), 711 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.67-7.48 (m, 10H, ArH), 7.98 (s, 1H, NH), 7.21 (d, 1H, CH-Cl), 7.19 (d, 1H, CH), 4.41 (d, 2H, CH_2); Analysis: for $C_{20}H_{18}ClN_3O_3$ calcd.: C, 62.58; H, 4.73; N, 10.95%; found: C, 62.63; H, 4.68; N, 10.90%.

***N*-{2-[3-chloro-2-(3-methoxyphenyl)-4-oxoazetidin-1-ylamino]-2-oxoethyl}benzamide (18)**

IR (KBr, cm^{-1}): 3315 (NH), 3053 (C-H aromatic), 1755 (C=O of β-lactam), 1729 (C=O), 1634 (C=C aromatic), 1221 (C-N), 1162 (C-O-C str., -OCH_3), 692 (Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.79-7.49 (m, 9H, ArH), 7.91 (s, 1H, NH), 7.46 (d, 1H, CH-Cl), 4.12 (d, 2H, CH_2), 3.94 (s, 3H, OCH_3). Analysis: for $C_{19}H_{18}ClN_3O_4$ calcd.: C, 58.84; H, 4.68; N, 10.84%; found: C, 58.89; H, 4.63; N, 10.88%.

Antimicrobial assay

The antimicrobial activity of the synthesized compounds was performed against Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, the Gram-negative bacterium *Escherichia coli* and fungal strains: *Candida albicans* and *Aspergillus niger* using the tube dilution method (16). Dilutions of test and standard compounds were prepared in double strength nutrient broth – Indian Pharmacopoeia (bacteria) or Sabouraud dextrose broth – Indian Pharmacopoeia (fungi) (22). The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 7 days (*A. niger*) and at 37°C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC).

Evaluation of anticancer activity

The anticancer activity of synthesized compounds (**1**-**17**) was determined against an estrogen receptor positive human breast adenocarcinoma, MCF-7 (ATCC HTB-22) cancer cell line. The cell line was cultured in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin (PAA Laboratories). Culture was maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2. Anticancer activity of synthesized compounds at various concentrations was assessed using the sulforhodamine B (SRB) assay as previously described by Skehan et al. (17), but with minor modifications, following 72 h of incubation. Assay plates were read using a spectrophotometer at 570 nm. Data generated were used to plot a dose-response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined (17).

Software used for QSAR studies

The structures of synthesized 2-azetidinone derivatives were first pre-optimized with the Molecular Mechanics Force Field (MM^+) procedure included in Hyperchem 6.03 (23) and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). We had chosen a gradient norm limit of 0.01 kcal/Å for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows (24). Further, the regression analysis was performed using the SPSS software package (25).

CONCLUSION

A novel series of 2-azetidinone derivatives was synthesized and evaluated for its *in vitro* anticancer and antimicrobial potentials. Results of newly obtained derivatives displayed average antimicrobial and anticancer potentials and compounds **4** and **17** were found to be most potent antimicrobial and anticancer agents, respectively. Developed QSAR models indicated the importance of topological parameters: Balaban index (J) as well as valence zero and first order molecular connectivity indices ($^0\chi^v$ and $^1\chi^v$) in determining the antimicrobial activity of the synthesized compounds.

REFERENCES

1. Jin X., Zheng C.J., Song M.X., Wu Y., Sun L.P. et al.: Eur. J. Med. Chem. 56, 203 (2012).
2. Murty M.S.R., Rao B.R,, Katiki M.R., Nath L.R., Anto R.J.: Med. Chem. Res. 22, 4980 (2013).
3. Desai N.C., Dodiya A.M., Rajpara K.M., Rupala Y.M.: J. Saudi Chem. Soc. 18, 255 (2014).
4. Trivedi A.R., Desai J.M., Dholariya B.H., Dodiya D.K., Shah V.H.: Med. Chem. Res. 21, 1471 (2012).
5. Maia D.P., Wilke D.V., Mafezoli J., da Silva Júnior J.N., de Moraes M.O. et al.: Chem. Biol. Interact. 180, 220 (2009).
6. Keri R.S., Hosamani K.M., Shingalapur R.V., Reddy H.R.: Eur. J. Med. Chem. 44, 5123 (2009).
7. Pathak R.B., Chovatia P.T., Parekh H.H.: Bioorg. Med. Chem. Lett. 22, 5129 (2012).
8. Kumar A., Rajput C.S., Bhati S.K.: Bioorg. Med. Chem. 15, 3089 (2007).
9. Pandey V.K., Gupta V.D., Upadhyay M., Singh V.K., Tandon M.: Indian J. Chem. 44, 158 (2005).
10. Sawant R.L., Bansode C.A., Wadekar J.B.: Med. Chem. Res 22, 1884 (2012).
11. Kumar P., Narasimhan B., Sharma D., Judge V., Narang R.: Eur. J. Med. Chem. 44, 1853 (2009).
12. Narang R., Narasimhan B., Sharma S., Sriram D., Yogeeswari P. et al.: Med. Chem. Res. 21, 1557 (2012).
13. Narang R., Narasimhan B., Sharma S.: Med. Chem. Res. 21, 2526 (2012).
14. Judge V., Narasimhan B., Ahuja M., Sriram D., Yogeeswari P. et al.: Med. Chem. Res. 21, 1451 (2012).
15. Judge V., Narasimhan B., Ahuja M., Sriram D., Yogeeswari P. et al.: Med. Chem. Res. 21, 1935 (2012).
16. Cappucino J. G., Sherman N., Microbiology – A Laboratory Manual. p. 263, Addison Wesley, California 1999.
17. Skehan P., Storeng R., Scudiero D., Monks A., McMahon J. et al.: J. Natl. Cancer Inst. 82, 1107 (1990).
18. Sortino M., Delgado P., Jaurez S., Quiroga J., Abonia R. et al.: Bioorg. Med. Chem. Lett. 15, 484 (2007).
19. Gonzalez-Diaz H., Gonzalez-Diaz Y., Santana L., Ubeira F. M., Uriarte E.: Proteomics. 4, 750 (2008).
20. Golbraikh A., Tropsha A: J. Mol. Graphics Model. 20, 269 (2002).
21. Kumar A., Narasimhan B., Kumar D.: Bioorg. Med. Chem. 15, 4113 (2007).
22. Pharmacopoeia of India vol. I, p. 37, Controller of Publications, Ministry of Health Department, Govt. of India, New Delhi 2007.
23. Hyperchem version 6.0, Hypercube, Inc., Gainesville, Florida 1993.
24. TSAR 3D Version 3.3, Oxford Molecular Limited, Oxford, UK 2000.
25. SPSS for Windows, version 10.05, SPSS Inc., Bangalore, India 1999.

Received: 17. 10. 2014