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ORIGINAL ARTICLE

Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides

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KEYWORDS

Benzohydrazides;
Antimicrobial activity;
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QSAR

Abstract In the present study, a series of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides was synthesized and evaluated *in vitro* for its antimicrobial and anticancer potentials. The results of antimicrobial and anticancer study indicated that compounds **3**, **15** and **18** ($pMIC_{am}$ = 1.62 μM/ml) were found to be most potent antimicrobial agents and compound **4** (IC_{50} = 1.88 ± 0.03 μM) was found to be the most potent anticancer agent. The results of QSAR analysis indicated the importance of topological parameters, Balaban index (*J*) and valence first and second order molecular connectivity indices ($^1\chi^v$ and $^2\chi^v$) in describing antimicrobial activity of the synthesized benzohydrazides.

1. Introduction

In recent years, a number of life-threatening infections caused by multi-drug resistant Gram-positive and Gram-negative pathogenic bacteria have reached an alarming level in many countries around the world (Berber et al., 2003). A number of clinical reports in the United States and worldwide have independently described the emergence of vancomycin resistance in methicillin-resistance *Staphylococcus aureus* (MRSA) isolates and other human pathogen Gram-negative isolates (Lee and Hecker, 1999). Infections caused by these microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to a search for novel antibacterial agents (Akbas et al., 2005).

Cancer is a disease characterized by a shift in the controlled mechanisms that govern cell proliferation and differentiation. Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by outside exposure of the body to chemicals, radiation, or even infectious agents.

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Ideal anticancer drugs would eradicate cancer cells without harming normal tissues. Unfortunately, no currently available anticancer agents meet this criterion and clinical use of drugs involves a weighing of benefits against toxicity in a search of favorable therapeutic index (Al-Omary et al., 2012).

Similar molecules with just a slight variation in their structures can have quite different biological activities. This kind of relationship between molecular structure and changes in biological activity is the center of focus for the field of quantitative structure–activity relationships (QSAR), the main objective of which is to investigate these relationships by building mathematical models that explain the relationship in a statistical way. With the success of its applications, QSAR has become one of the well-developed areas in computational chemistry (Jurs and He, 2005).

Keeping this observation in mind and in continuation of our study on exploring the biological profile of Schiff bases (Kumar et al., 2010a,b, 2012; Judge et al., 2012a,b; Narang et al., 2012a,b), we hereby report the synthesis, antimicrobial, anticancer evaluation and QSAR studies of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides (**1**–**22**).

2. Materials and methods

Starting materials were obtained from commercial sources 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 and ^{13}C nuclear magnetic resonance (^{1}H NMR and ^{13}C) spectra were determined by a Brucker DRX-300 FTNMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on an Agilent Resolutions Pro FTIR spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer. Mass spectra were taken on Waters Micromass Q-ToF Micro instrument.

2.1. General procedure for the synthesis of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides (*1*–*22*)

The mixture of 2/3 benzoic acid (0.08 mol) and ethanol (0.74 mol) was refluxed in the presence of sulfuric acid till the completion of reaction. Once the reaction has been completed, the reaction mixture was added to 200 mL ice cold water and the ester formed was extracted with ether (50 mL). The ether layer was separated and this on evaporation yielded the crude ester which was then recrystallized from alcohol. Hydrazine-hydrate (99%) (0.015 mol) was added to ethanolic solution of ester (0.01 mol) and refluxed for 5 h. The reaction mixture was then cooled and the precipitates were filtered off, washed with water, dried and recrystallized from ethanol.

A solution of different aldehydes (0.05 mol) in ethanol was added to a solution of 2/3-bromobenzohydrazide (synthesized above, 0.05 mol) in 50 mL ethanol. The mixture was refluxed for 5–7 h. Then the reaction mixture was allowed to cool at room temperature and the precipitated title compounds were filtered, dried and recrystallized from ethanol.

2.2. Evaluation of antimicrobial activity

The antimicrobial activity of the synthesized benzohydrazides was carried out against Gram-positive bacteria: *S. aureus*, *Bacillus. subtilis*, Gram-negative bacterium: *Escherichia coli* and fungal strains: *Candida albicans* and *Aspergillus niger* were determined using the tube dilution method (Cappucino and Sherman, 1999). Dilutions (50–1.56 μg/ml) of test and standard (norfloxacin and fluconazole for antibacterial and antifungal activities respectively) 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 ± 1 °C for 24 h (bacteria), at 25 ± 1 °C for 7d (*A. niger*) and at 37 ± 1 °C for 48 h (*C. albicans*), and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

2.3. Determination of MBC/MFC

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

2.4. Evaluation of anticancer activity

The anticancer activity of the synthesized compounds (**1**–**22**) was determined against human colon (HCT116) cancer cell line. The cell line was cultured in RPMI 1640 (Sigma) supplemented with 10% heat inactivated 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 the synthesized compounds at various concentrations was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann, but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 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. Anticancer activity was expressed as the mean IC_{50} of three independent experiments (Mosmann, 1983).

2.5. QSAR studies

The structures of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides (**1**–**22**) were first pre-optimized with the Molecular Mechanics Force Field (MM^{+}) procedure included in Hyperchem 6.03 (Hyperchem 6.0, 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/Å

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for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows (TSAR 3D Version 3.3, 2000). Further, the regression analysis was performed using the SPSS software package (SPSS for Windows, 1999).

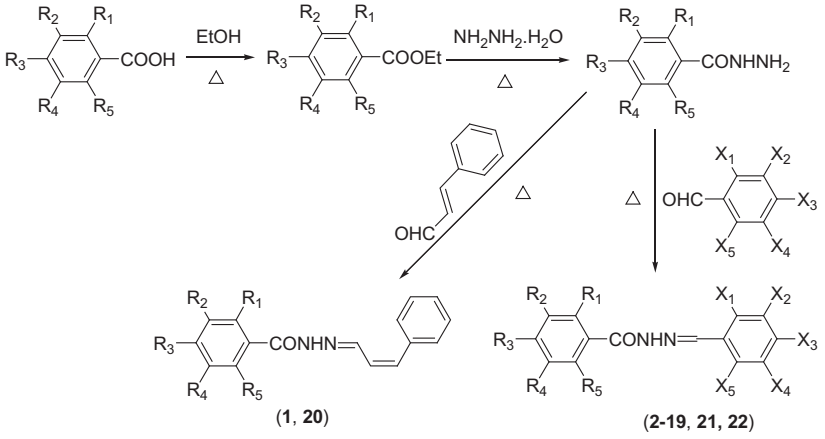
3. Results and discussion

3.1. Chemistry

Synthesis of target compounds (**1**–**22**) was carried out in appreciable yield by adopting the synthetic procedures outlined in Scheme 1. The structures of all the synthesized compounds (**1**–**22**) were ascertained on the basis of their consistent IR, NMR and Mass spectral characteristics in addition to elemental (C, H, N) analysis which were in full agreement with their assigned molecular structures.

3.2. Antimicrobial activity

The synthesized compounds were screened for their *in vitro* antibacterial activity against *S. aureus*, *B. subtilis*, and *E. coli* and antifungal activity against *C. albicans* and *A. niger* by tube dilution method (Cappucino and Sherman, 1999) using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activities, respectively and the results are presented in Table 1 which indicated that none of the synthesized compounds exhibited better antimicrobial activity than standard drugs, norfloxacin and fluconazole. Results of MBC/MFC studies of the synthesized compounds are given in Table 2.



S. No.	R_1	R_2	R_3	R_4	R_5	X_1	X_2	X_3	X_4	X_5
1	H	Br	H	H	H	-	-	-	-	-
2	H	Br	H	H	H	H	H	OCH_3	H	H
3	H	Br	H	H	H	H	OCH_3	OCH_3	OCH_3	H
4	H	Br	H	H	H	OH	H	H	H	H
5	H	Br	H	H	H	H	H	Br	H	H
6	H	Br	H	H	H	H	OCH_3	OCH_3	H	H
7	H	Br	H	H	H	H	OC_2H_5	OH	H	H
8	H	Br	H	H	H	H	H	$N(CH_3)_2$	H	H
9	Br	H	H	H	H	H	H	H	H	H
10	Br	H	H	H	H	H	H	Cl	H	H
11	Br	H	H	H	H	H	Cl	H	H	H
12	Br	H	H	H	H	H	H	Br	H	H
13	Br	H	H	H	H	H	H	NO_2	H	H
14	Br	H	H	H	H	H	OCH_3	OCH_3	H	H
15	Br	H	H	H	H	H	H	$N(CH_3)_2$	H	H
16	Br	H	H	H	H	H	H	CHO	H	H
17	Br	H	H	H	H	H	OC_2H_5	OH	H	H
18	Br	H	H	H	H	H	OCH_3	OCH_3	OCH_3	H
19	Br	H	H	H	H	H	H	OCH_3	H	H
20	Br	H	H	H	H	-	-	-	-	-
21	Br	H	H	H	H	H	H	CH_3	H	H
22	Br	H	H	H	H	H	OCH_3	OH	H	H

Scheme 1 Scheme for the synthesis of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides.

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Table 1 Antimicrobial (μM/ml) and anticancer (IC_{50}, μM) activity of the synthesized 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides.

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$	IC_{50} (μM)
1	1.42	1.72	1.72	1.42	1.42	1.62	1.42	1.54	607.90 ± 0.03
2	1.43	1.73	1.43	1.73	1.43	1.53	1.58	1.55	87.09 ± 0.00
3	1.50	1.80	1.50	1.80	1.50	1.60	1.65	1.62	66.16 ± 0.01
4	1.41	1.71	1.41	2.01	1.41	1.51	1.71	1.59	1.88 ± 0.03
5	1.49	1.79	1.49	1.49	1.49	1.59	1.49	1.55	12.49 ± 0.04
6	1.46	1.76	1.46	1.76	1.46	1.56	1.61	1.58	165.29 ± 0.02
7	1.16	1.46	1.16	1.46	1.16	1.26	1.31	1.28	165.29 ± 0.01
8	1.44	1.74	1.44	1.74	1.44	1.54	1.59	1.56	144.51 ± 0.00
9	1.08	1.38	1.38	1.38	1.08	1.28	1.23	1.26	247.52 ± 0.03
10	1.43	1.73	1.43	1.73	1.43	1.53	1.58	1.55	88.76 ± 0.03
11	1.13	1.43	1.13	1.73	0.83	1.23	1.28	1.25	59.17 ± 0.02
12	1.49	1.79	1.49	1.49	1.79	1.59	1.64	1.61	96.86 ± 0.01
13	1.14	1.44	1.14	1.44	1.44	1.24	1.44	1.32	517.24 ± 0.02
14	1.16	1.46	1.16	1.46	1.16	1.26	1.31	1.28	247.93 ± 0.02
15	1.44	1.74	1.44	1.74	1.74	1.54	1.74	1.62	14.45 ± 0.01
16	1.42	1.72	1.42	1.72	1.12	1.52	1.42	1.48	604.23 ± 0.01
17	1.46	1.76	1.46	1.46	1.46	1.56	1.46	1.52	137.74 ± 0.00
18	1.50	1.80	1.50	1.80	1.50	1.60	1.65	1.62	305.34 ± 0.03
19	1.43	1.73	1.43	1.73	1.43	1.53	1.58	1.55	147.15 ± 0.01
20	1.42	1.72	1.72	1.72	1.42	1.62	1.57	1.60	54.71 ± 0.04
21	1.71	1.71	1.40	1.71	1.40	1.60	1.55	1.58	157.73 ± 0.02
22	1.45	1.75	1.45	1.75	1.45	1.55	1.60	1.57	257.88 ± 0.01
SD	0.16	0.14	0.16	0.17	0.21	0.14	0.14	0.13	
Std.	2.61[a]	2.61[a]	2.61[a]	2.64[b]	2.64[b]				
Tetrandrine									1.53 ± 0.01
5FU									4.60 ± 0.01
Carboplatin									> 100.00

SD, standard deviation.
[a] Norfloxacin.
[b] Fluconazole.

Table 2 Minimum bactericidal/fungicidal (μM/ml) activity of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene) benzohydrazides.

Comp.	Minimum Bactericidal/Fungicidal Concentration (μM/ml)				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
2	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
3	> 0.13	> 0.13	> 0.13	> 0.13	> 0.13
4	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
5	> 0.13	> 0.13	> 0.13	> 0.13	> 0.13
6	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
7	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
8	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
9	> 0.17	> 0.17	> 0.17	> 0.17	> 0.17
10	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
11	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
12	> 0.13	> 0.13	> 0.13	> 0.13	> 0.13
13	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
14	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
15	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
16	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
17	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
18	> 0.13	> 0.13	> 0.13	> 0.13	> 0.13
19	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
20	> 0.15	> 0.15	> 0.15	> 0.15	> 0.15
21	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
22	> 0.14	> 0.14	> 0.14	> 0.14	> 0.14
Standard	0.019[a]	0.019[a]	0.019[a]	0.040[b]	0.040[b]

[a] Norfloxacin.
[b] Fluconazole.

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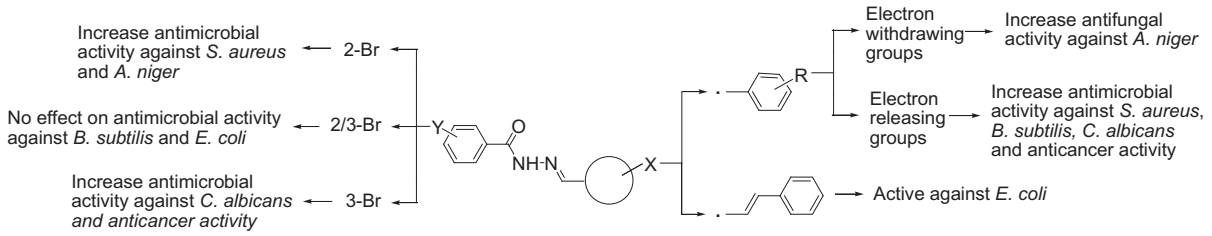

Figure 1 Structural requirements for the antimicrobial and anticancer activities of the synthesized 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides.

Among the synthesized benzohydrazides, compound **21** ($pMIC_{sa}$ = 1.71 μM/ml) was found to be most effective antibacterial agent against *S. aureus*. In case of *B. subtilis,* compounds **3** and **18** ($pMIC_{bs}$ = 1.80 μM/ml) emerged as most effective antibacterial agents. Compounds **1** and **20**, ($pMIC_{ec}$ = 1.72 μM/ml) exhibited most potent antibacterial activity against *E. coli*. In case of antifungal activity against *C. albicans*, compound **4** ($pMIC_{ca}$ = 2.01 μM/ml) was found to be the most potent antifungal agent, whereas, compound **12** ($pMIC_{an}$ = 1.79 μM/ml) exhibited most potent antifungal activity against *A. niger*. Overall antimicrobial activity results (Table 1) indicated that compounds **3**, **15** and **18** ($pMIC_{am}$ = 1.62 μM/ml) were found to be most potent antimicrobial agents.

In general, the results of MBC/MFC studies (Table 2) revealed that the synthesized compounds were bacteriostatic and fungistatic in action as their MFC and MBC values were 3-fold higher than their MIC values (a drug is considered to be bacteriosatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC value).

3.3. Anticancer activity

The *in vitro* anticancer activity of the synthesized hydrazide derivatives was determined against human colorectal (HCT116) cancer cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983) and the results are presented in Table 1. In general, the synthesized compounds showed moderate anticancer activity and compounds **2**–**5**, **10**–**12**, **15** and **20** (IC_{50} = 87.09 ± 0.00, 66.16 ± 0.01, 1.880.03, 12.490.04, 88.76 ± 0.03, 59.17 ± 0.02, 96.86 ± 0.01, 14.45 ± 0.01 and 54.71 ± 0.04 μM respectively) showed better anticancer potential than standard drug carboplatin (IC_{50} = >100.00 μM). None of the synthesized compounds exhibited anticancer potential better than standard drug tetrandrine (IC_{50} = 1.53 ± 0.01 μM) as well as 5-fluorouracil (IC_{50} = 4.60 ± 0.01 μM) except compound **4** (IC_{50} = 1.880.03 μM) which was found to be the more potent anticancer agent than 5-fluorouracil but less potent than tetrandrine. Thus compound **4** was found to be the most potent anticancer agent of the series and may be taken as a lead compound for the development of novel anticancer agents. The structural requirements for the antimicrobial and anticancer activities of the synthesized benzohydrazide derivatives are presented in Fig. 1.

3.4. QSAR studies

In order to identify the substituent effect on the antimicrobial activity, quantitative structure–activity relationship (QSAR)

Table 3 Values of selected descriptors used in the QSAR studies.

Comp.	Log *P*	MR	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{2}\chi^{v}$	κ_1	$\kappa\alpha_1$	Te	LUMO	HOMO	μ
1	4.63	85.61	12.28	6.87	4.94	16.37	14.76	−3312.57	−0.69	−8.79	3.69
2	3.96	81.83	12.46	6.73	4.92	16.37	14.98	−3505.27	−0.48	−8.76	3.75
3	3.46	94.76	15.12	7.79	5.60	20.31	18.83	−4456.71	−0.62	−8.85	1.17
4	3.93	77.06	11.50	6.35	4.70	15.39	14.00	−3349.95	−0.63	−8.95	3.35
5	5.01	82.99	13.05	7.12	5.63	15.39	14.51	−3369.00	−0.75	−9.17	2.32
6	3.71	88.29	13.79	7.26	5.26	18.34	16.90	−3980.97	−0.50	−8.58	2.64
7	4.02	88.27	13.54	7.46	5.31	18.34	16.90	−3981.63	−0.51	−8.66	3.74
8	4.01	89.08	13.50	7.24	5.63	17.36	15.96	−3561.08	−0.38	−8.24	5.45
9	4.22	75.37	11.13	6.21	4.46	14.41	13.06	−3029.30	−0.31	−8.98	3.09
10	4.74	80.17	12.25	6.72	5.08	15.39	14.32	−3389.40	−0.56	−9.02	1.76
11	4.74	80.17	12.25	6.72	5.08	15.39	14.32	−3389.39	−0.51	−9.13	2.46
12	5.01	82.99	13.05	7.12	5.54	15.39	14.51	−3368.90	−0.63	−9.07	1.73
13	4.17	82.69	12.32	6.71	4.90	17.36	15.57	−3860.15	−1.41	−9.60	3.67
14	3.71	88.29	13.79	7.26	5.17	18.34	16.90	−3980.86	−0.31	−8.49	2.32
15	4.01	89.08	13.50	7.24	5.54	17.36	15.96	−3560.97	−0.15	−8.16	4.97
16	3.89	81.96	12.04	6.65	4.84	16.37	14.69	−3477.53	−0.91	−9.23	1.70
17	4.02	88.27	13.54	7.46	5.21	18.34	16.90	−3981.53	−0.33	−8.56	2.46
18	3.46	94.76	15.12	7.79	5.51	20.31	18.83	−4456.61	−0.44	−8.78	1.98
19	3.96	81.83	12.46	6.74	4.83	16.37	14.98	−3505.17	−0.27	−8.66	3.48
20	4.63	85.61	12.28	6.88	4.84	16.37	14.76	−3312.46	−0.59	−8.68	2.95
21	4.68	80.41	12.05	6.62	4.96	15.39	14.04	−3185.17	−0.31	−8.83	3.68
22	3.68	83.52	12.83	6.88	4.98	17.36	15.92	−3825.74	−0.35	−8.60	2.04

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Table 4 Correlation matrix for antibacterial activity of the synthesized benzohydrazides.

	$pMIC_{ab}$	Log *P*	MR	$^0\chi^v$	$^1\chi^v$	$^2\chi^v$	κ_1	$\kappa\alpha_1$	LUMO	HOMO	μ
$pMIC_{ab}$	1.000										
Log *P*	0.067	1.000									
MR	0.667	−0.510	1.000								
$^0\chi^v$	0.806	−0.403	0.971	1.000							
$^1\chi^v$	0.826	−0.286	0.954	0.978	1.000						
$^2\chi^v$	0.771	0.191	0.704	0.749	0.799	1.000					
κ_1	0.530	−0.755	0.921	0.887	0.834	0.398	1.000				
$\kappa\alpha_1$	0.618	−0.678	0.943	0.932	0.885	0.481	0.993	1.000			
LUMO	−0.088	−0.297	0.310	0.244	0.240	0.105	0.292	0.287	1.000		
HOMO	−0.133	−0.423	0.443	0.316	0.307	0.239	0.385	0.362	0.881	1.000	
μ	−0.469	−0.075	−0.030	−0.164	−0.165	0.051	−0.155	−0.187	0.579	0.737	1.000

study was undertaken, using linear free energy relationship model (LFER) described by Hansch and Fujita (1964). Biological activity data determined as MIC values were first transformed into pMIC values (*i.e.* –log MIC) and used as dependent variable in QSAR study. The values of selected molecular descriptors used in the QSAR study are presented in Table 3.

Our earlier studies (Kumar et al., 2012; Judge et al., 2012a,b; Narang et al., 2012a,b) indicated that the multi-target QSAR (*mt*-QSAR) models are better than one-target QSAR (*ot*-QSAR) models in describing the antimicrobial activity. So, in the present study we have developed multi-target QSAR models to describe the antimicrobial activity of the synthesized 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene) benzohydrazides.

In light of above, we have attempted to develop three different *mt*-QSAR models *viz*. *mt*-QSAR model for describing antibacterial activity of the synthesized compounds against *S. aureus*, *B. subtilis* and *E. coli*, *mt*-QSAR model for describing antifungal activity of the 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 the synthesized compounds against all the above mentioned microorganisms.

In order to develop *mt*-QSAR models, initially we calculated the average antibacterial, antifungal and antimicrobial activities of 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides which are presented in Table 1.

During the regression analysis studies, different outliers were identified in case of antibacterial, antifungal and antimicrobial activities and the QSAR models have been developed after removal of these outliers (compound numbers in bracket) *i.e.* antibacterial (**1**, **7**, **9**, **11**, **13**, **14**, **20** and **21**), antifungal (**4**, **5**, **7**, **9**, **11**, **14**, **15**, **19** and **22**) and antimicrobial activities (**4**, **7**, **9**, **11**, **13**, **14**, **16**, **17** and **20**). In multivariate statistics, it is common to define three types of outliers.

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 1) as well as the molecular descriptor range (Table 3) of these outliers when compared to the other benzohydrazides, these outliers belong to the category of Y outliers (Substances for which the reference value of response is invalid) (Furusjo et al., 2006).

Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity of the synthesized compounds is presented in Table 4. In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between topological parameters, Kier's first order and alpha first order shape indices (κ_1 and $\kappa\alpha_1$) ($r = 0.993$) and low interrelationship was observed between steric parameter, molar refractivity (MR) and electronic parameter, dipole moment (μ) ($r = -0.030$).

The structural effects on variations in antibacterial activity of the synthesized benzohydrazides in terms of $pMIC_{ab}$ were examined by regression analysis with molecular parameters shown in Table 3. For 14 benzohydrazides, Eq. (1) was derived as that of the best quality using the topological parameter, valence first order molecular connectivity index ($^1\chi^v$, $r = 0.826$, Table 4).

3.4.1. LR *mt*-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.0590\ ^1\chi^v + 1.136 \quad (1)$$

$$n = 14 \quad r = 0.826 \quad q^2 = 0.621 \quad s = 0.0179 \quad F = 25.78$$

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.

Coefficient of $^1\chi^v$ in Eq. (1) is positive which indicates that antibacterial activity of the synthesized compounds is positively correlated to valence first order molecular connectivity index ($^1\chi^v$) *i.e.* antibacterial activity of the synthesized compounds will increase with an increase in value of $^1\chi^v$ and vice versa. This is evidenced by the antibacterial activity data of the synthesized compounds (Table 1) and their $^1\chi^v$ values (Table 3) *i.e.* compounds **3** and **18** having highest $^1\chi^v$ value of 7.79 are having highest antibacterial activity ($pMIC_{ab}$ = 1.60 μM/ml) except compounds **1** and **20** ($pMIC_{ab}$ = 1.62 μM/ml) which were removed as outliers prior to model development for antibacterial activity of the synthesized benzohydrazides.

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Table 5 Observed, predicted and residual antimicrobial activities of the synthesized compounds obtained by developed QSAR models.

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.62	1.54	0.08	1.42	1.50	−0.08	1.54	1.54	0.00
2	1.53	1.53	0.00	1.58	1.50	0.08	1.55	1.56	−0.01
3	1.60	1.61	−0.01	1.65	1.63	0.02	1.62	1.62	0.00
4	1.51	1.51	0.00	1.71	1.46	0.25	1.59	1.56	0.03
5	1.59	1.57	0.02	1.49	1.64	−0.15	1.55	1.57	−0.02
6	1.56	1.56	0.00	1.61	1.57	0.04	1.58	1.59	−0.01
7	1.26	1.57	−0.31	1.31	1.58	−0.27	1.28	1.59	−0.31
8	1.54	1.54	0.00	1.59	1.64	−0.05	1.56	1.58	−0.02
9	1.28	1.50	−0.22	1.23	1.41	−0.18	1.26	1.55	−0.29
10	1.53	1.54	−0.01	1.58	1.53	0.05	1.55	1.57	−0.02
11	1.23	1.54	−0.31	1.28	1.53	−0.25	1.25	1.56	−0.31
12	1.59	1.57	0.02	1.64	1.62	0.02	1.61	1.58	0.03
13	1.24	1.56	−0.32	1.44	1.50	−0.06	1.32	1.57	−0.25
14	1.26	1.55	−0.29	1.31	1.55	−0.24	1.28	1.59	−0.31
15	1.54	1.54	0.00	1.74	1.62	0.12	1.62	1.58	0.04
16	1.52	1.54	−0.02	1.42	1.49	−0.07	1.48	1.56	−0.08
17	1.56	1.57	−0.01	1.46	1.56	−0.10	1.52	1.59	−0.07
18	1.60	1.60	0.00	1.65	1.62	0.03	1.62	1.63	−0.01
19	1.53	1.53	0.00	1.58	1.48	0.10	1.55	1.56	−0.01
20	1.62	1.54	0.08	1.57	1.49	0.08	1.60	1.55	0.05
21	1.60	1.52	0.08	1.55	1.51	0.04	1.58	1.56	0.02
22	1.55	1.53	0.02	1.60	1.51	0.09	1.57	1.58	−0.01

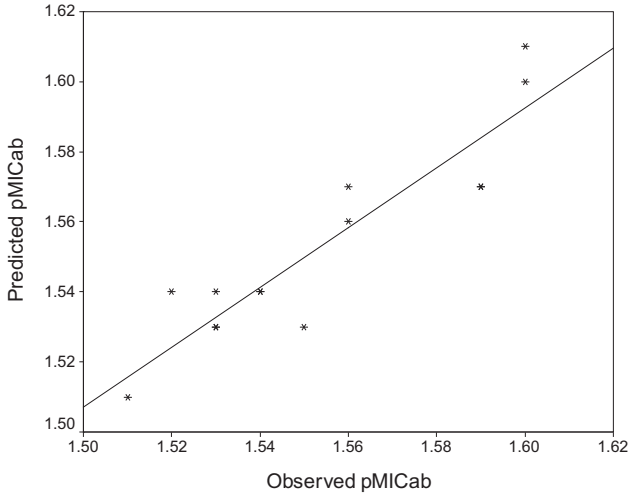

Figure 2 Plot of observed $pMIC_{ab}$ against predicted $pMIC_{ab}$ by Eq. (2).

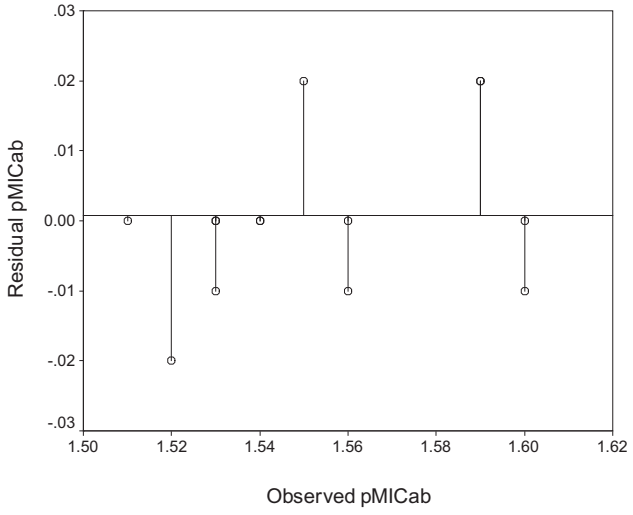

Figure 3 Plot of observed $pMIC_{ab}$ against residual $pMIC_{ab}$ by Eq. (2).

The molecular connectivity index, an adjacency based topological index proposed by Randic is denoted by χ and is defined as sum of over all the edges (ij) as per following

$$\chi = \sum_{i=1}^{n} (V_i V_j)^{-1/2}$$

where V_i and V_j are the degrees of adjacent vertices i and j and n is the number of vertices in a hydrogen suppressed molecular structure (Lather and Madan, 2005). The topological index, χ signifies the degree of branching, connectivity of atoms and unsaturation in the molecule which accounts for variation in activity (Gupta et al., 2003).

In search of a better QSAR model, we coupled valence first order molecular connectivity index ($^1\chi^v$) with electronic parameter, energy of highest occupied molecular orbital (HOMO) which led to best QSAR model for explaining antibacterial activity of the synthesized compounds (Eq. 2) having improved r and q^2 values.

3.4.2. MLR mt-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.0683\ {}^1\chi^v - 0.0406\ HOMO + 0.714 \quad (2)$$
$$n = 14 \quad r = 0.920 \quad q^2 = 0.759 \quad s = 0.0129 \quad F = 30.46$$

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. The extents of these stabilizing interactions are inversely related to the energy gap between the interacting orbitals (Jesudason et al., 2009).

The QSAR model expressed by Eq. (2) was cross validated by its high q^2 value ($q^2 = 0.759$) obtained by leave one out (LOO) method. The value of q^2 greater than 0.5 is the basic requirement for qualifying a QSAR model to be a valid one (Golbraikh and Tropsha, 2002). As the observed and predicted antibacterial activity values are close to each other (Table 5), the *mt*-QSAR model for antibacterial activity (Eq. 2) is a valid one. The plot of predicted $pMIC_{ab}$ against observed $pMIC_{ab}$ (Fig. 2) also favors the developed QSAR model expressed by Eq. (2). 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).

Results of correlation of calculated molecular descriptors with antifungal activity of the synthesized compounds indicated that topological parameter, valence second order molecular connectivity index ($^2\chi^v$) was most dominating descriptor for antifungal activity of the synthesized compounds. So, QSAR model for antifungal activity of the synthesized benzohydrazides was developed by using the topological parameter, valence second order molecular connectivity index ($^2\chi^v$) ($r = 0.685$, Eq. (3)).

3.4.3. LR mt-QSAR model for antifungal activity

$$pMIC_{af} = 0.193^2\chi^v + 0.552 \quad (3)$$
$$n = 13 \quad r = 0.685 \quad q^2 = 0.282 \quad s = 0.0657 \quad F = 9.71$$

As in case of antibacterial activity, coefficient of $^2\chi^v$ in Eq. (3) is positive which indicates that antifungal activity of the synthesized compounds is positively correlated to valence second order molecular connectivity index ($^2\chi^v$) *i.e.* antifungal activity of the synthesized compounds will increase with an increase in value of $^2\chi^v$ and vice versa (Tables 2 and 4).

The validity and predictability of the QSAR model for antifungal activity *i.e.* Eq. (3) were cross validated by q^2 value ($q^2 = 0.282$) obtained by leave one out (LOO) method. The value of q^2 less than 0.5 indicated that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha (2002) who reported that the only way to estimate the true predictive power of a model is to test its ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other (Table 5), the *mt*-QSAR model for antifungal activity (Eq. 3) is therefore a valid one.

The mt-QSAR model of antimicrobial activity (Eq. 4) depicted the importance of topological parameter, Balaban index (J) in describing antimicrobial activity of the synthesized compounds.

3.4.4. LR mt-QSAR model for antimicrobial activity

$$pMIC_{am} = 0.424\,J + 0.937 \quad (4)$$
$$n = 13 \quad r = 0.757 \quad q^2 = 0.471 \quad s = 0.021 \quad F = 14.78$$

As in case of antibacterial and antifungal activities, coefficient of J in Eq. (4) is positive which indicates that antimicrobial activity of the synthesized compounds is positively correlated to Balaban index (J) *i.e.* antimicrobial activity of the synthesized compounds will increase with an increase in value of J. This is evidenced by the antimicrobial activity data of the synthesized compounds (Table 1) and their J values (Table 3).

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

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

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 $\mu = \mu(G)$ 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 $\mu = 1$ otherwise it is calculated by means of the following expression (Balaban, 1982).

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

In order to improve the value of correlation coefficient (r), we coupled Balaban index (J) with valence first order molecular connectivity index ($^1\chi^v$) which improved the value of r from 0.757 to 0.808 and q^2 from 0.471 to 0.515 (Eq. 5).

3.4.5. MLR mt-QSAR model for antimicrobial activity

$$pMIC_{am} = 0.0314^1\chi^v + 0.272J + 0.944 \quad (5)$$
$$n = 13 \quad r = 0.808 \quad q^2 = 0.515 \quad s = 0.0198 \quad F = 9.43$$

The QSAR models expressed by Eq. (5) were cross validated by its high q^2 values (q^2 = 0. 515) obtained by leave one out (LOO) method. The value of q^2 greater than 0.5 is the basic requirement for qualifying a QSAR model to be a valid one (Golbraikh and Tropsha, 2002). As the observed and predicted antimicrobial activity values are close to each other (Table 5), the *mt*-QSAR model for antimicrobial activity (Eq. 5) is a valid one. Further, high residual values (Table 5) observed in case of outliers also justified their removal before the development of QSAR models.

It was observed from mt-QSAR models (Eqs. (1)–(5)) that the antibacterial, antifungal and overall antimicrobial activities of the synthesized 2/3-bromo-*N*′-(substituted benzylidene/3-phenylallylidene)benzohydrazides was governed by the topological parameters, Balaban index (J) and valence first and second order molecular connectivity indices ($^1\chi^v$ and $^2\chi^v$).

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of antimicrobial activities of the synthesized compounds is within one order of magnitude. This is in accordance with results suggested by Bajaj et al. who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range (Bajaj et al., 2005). When biological activity data lie in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies (Narasimhan et al., 2007). The minimum standard deviation

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(Table 1) observed in the antimicrobial activity data justifies its use in QSAR studies.

4. Conclusion

In conclusion, a series of substituted benzohydrazide derivatives has been synthesized and its *in vitro* antimicrobial and anticancer activities were evaluated against five representative microorganisms and human colon (HCT116) cancer cell line. The results of antimicrobial and anticancer study indicated that compounds **3**, **15** and **18** ($pMIC_{am}$ = 1.62 μM/ml) were found to be most potent antimicrobial agents and compound **4** (IC_{50} = 1.88 ± 0.03 μM) was found to be the most potent anticancer agent. QSAR studies indicated the importance of the topological parameters, Balaban index (*J*) and valence first and second order molecular connectivity indices ($^1\chi^v$ and $^2\chi^v$) in describing the antimicrobial activity of the synthesized benzohydrazides.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2014.05.010.

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