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2nd Cancer Update

Synthesis, *in vitro* antimicrobial, anticancer evaluation and QSAR studies of *N*′-(substituted)-4-(butan-2-lideneamino)benzohydrazides

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Abstract A series of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (**1–21**) was synthesized and characterized by physicochemical as well as spectral means. The synthesized compounds were screened for their *in vitro* antimicrobial and anticancer potentials. The synthesized compounds displayed higher antifungal potential as compared to antibacterial potential. Besides having good antifungal potential, the synthesized compounds were having appreciable anticancer potential and a number of compounds displayed higher anticancer potential than the standard drug, carboplatin. The results of QSAR studies demonstrated the importance of steric parameter, molar refractivity (MR), topological parameters, third order molecular connectivity index ($^3\chi$), Kier's first order shape index (κ_1) in describing the antimicrobial activity of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

1. Introduction

One of the key objectives of organic and medicinal chemistry is to design and synthesize molecules that possess potent therapeutic values. The rapid development of resistance to existing antimicrobial drugs generates a serious challenge to the scientific community. Consequently, there is a vital need for the

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development of new antimicrobial agents with potent activity against drug resistant microorganisms (Malhotra et al., 2011).

Cancer incidences continue to rise despite the enormous amount of research and rapid developments during the past decade. According to the recent statistics, cancer accounts for about 23% of the total deaths in the USA and is the second most common cause of death after heart disease (Hou et al., 2011).

QSAR has been a very useful tool in designing libraries of various ligands targeted toward particular receptors and to ensure increase in the probability of synthesizing therapeutically active drugs. Therefore, to understand the influence of physiochemical and structural properties, QSAR studies have been carried out (Kashaw et al., 2011).

Literature reports reveal that there are very few reports on the biological activity of ethyl methyl ketone derivatives *viz*. anticonvulsant activity (Nobuyoshi et al., 2010) and analgesic and anti-inflammatory activity (Sondhi et al., 2009).

Schiff bases are considered to be among the most important group of compounds in medicinal chemistry due to their preparative accessibility, structural variety and wide biological profile (Rosu et al., 2010). In continuation of our research focused on synthesis of medicinally potent new chemical entities and their biological screening (Judge et al., 2012a,b; Sharma et al., 2012; Sigroha et al., 2012; Narang et al., 2012), the present study is aimed to carry out synthesis and *in vitro* antimicrobial, anticancer evaluation and QSAR studies of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

2. Materials and methods

2.1. Instrumentation

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 nuclear magnetic resonance (^{1}H NMR) spectra were determined by a BrukerAvance II 400 NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard) NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on an Agilent Resolutions Pro FTIR spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer. Mass spectra were recorded using Waters Micromass Q-Tof micro instrument.

2.1.1. General procedure for the synthesis of N′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (1–21)

A solution of ethyl methyl ketone (0.01 mol) was added to the solution of *p*-amino benzoic acid (0.01 mol) in ethanol and the mixture was refluxed for 3–4 h. The resulting solution was poured on crushed ice and the precipitated solid was dried and recrystallized from ethanol. 4-(Butan-2-ylideneamino) benzoic acid (0.02 mol) synthesized as above was added to ethanol (0.5 mol) and the mixture was refluxed for 1 h, in the presence of concentrated sulfuric acid (2.7 ml), cooled and poured into ice-cold water. The solid ester separated out was filtered, dried and recrystallized from ethanol (Bhat and Al-omar, 2011). 4-(Butan-2-ylideneamino) benzoate (0.01 mol) and hydrazine hydrate 99% (0.02 mol) were refluxed in 50 ml ethanol. The mixture was then cooled and poured into ice-cold water. The solid product separated out was filtered, dried and recrystallized from ethanol (Bhat and Al-omar, 2011). Equimolar quantities of 4-(Butan-2-ylideneamino)benzohydrazide (0.01 mol) and substituted aldehydes (0.01 mol) in ethanol were refluxed in the presence of a few drops of glacial acetic acid. Then the mixture was cooled and poured into ice-cold water. The solid product separated out was filtered, dried and recrystallized from ethanol (Ramachandran and Maheswari, 2011).

2.2. Evaluation of antimicrobial activity

The antimicrobial activity of synthesized benzohydrazides 1-21 acid derivatives against Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis,* Gram-negative bacterium: *Escherichia coli* and fungal strains: *Candida albicans* and *Aspergillus niger* was determined using the 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 ± 1 °C for 24 h (bacteria), at 25 ± 1 °C for 7 d (*A. niger*) and at 37 ± 1 °C for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

2.3. Evaluation of anticancer activity

The anticancer activity of the synthesized compounds (**1–21**) 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). Cultures were 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 spectrophotometer at 520 nm. Data generated were used to plot a dose–response curve from which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined (Mosmann, 1983).

2.4. QSAR studies

The structures of synthesized benzohydrazide derivatives 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 are further refined by means of the semiempirical method PM3 (Parametric Method-3). We chose 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 (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

The synthesis of target compounds, *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (**1–21**) was carried out as outlined in Scheme 1. All the compounds were obtained in appreciable yield and their physicochemical characteristics are presented in Table 1. The structures of the synthesized compounds (**1–21**) were ascertained on the basis of their consistent IR, NMR and Mass spectral characteristics in addition to elemental analysis (C, H, N) which were in full agreement with the assigned molecular structures and the data are given in Table 2.

3.2. Antimicrobial activity

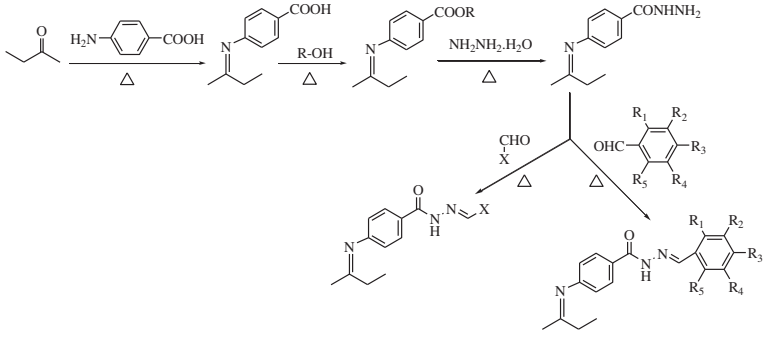
The synthesized compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus, B. subtilis, E. coli* and antifungal activity against *C. albicans* and *A. niger* by tube dilution method using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activity, respectively and the results are presented in Table 3.

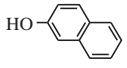
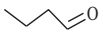
Among the synthesized compounds, *N*′-(3,4-dimethoxybenzylidene)-4-(butan-2-ylideneamino)benzohydrazides (**2**) was found to be an effective antibacterial agent against *S. aureus* with pMIC values of 1.75 μM/ml. In the case of *B. subtilis, E. coli* and *C. albicans*, *N*′-(4-bromobenzylidene)-4-(butan-2-ylideneamino)benzohydrazide **(6)** was found to be the most effective with pMIC values of 1.77, 1.47 and 2.07 μM/ml, respectively (Table 3). *N*′-(4-hydroxybenzylidene)-4-(butan-2-ylideneamino)benzohydrazide (**12**) was found to be most effective against *A. niger* with pMIC value of 1.76 μM/ml.

Results of antimicrobial screening indicated that the synthesized compounds were found to have more potent antifungal than antibacterial activity and compound **6** was found to be the most active antifungal agent against *C. albicans* ($pMIC_{ca}$ = 2.07 μM) which may be taken as lead molecule for the development of novel antifungal agents.

3.3. Anticancer activity

The *in vitro* anticancer activity of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides against human colorectal cancer (HCT116) cell line is presented in



Comp.	R_1	R_2	R_3	R_4	R_5	X
1	H	H	CH_3	H	H	-
2	H	OCH_3	OCH_3	H	H	-
3	H	Cl	H	H	H	-
4	H	H	$N(CH_3)_2$	H	H	-
5	H	OCH_3	OCH_3	OCH_3	H	-
6	H	H	Br	H	H	-
7	H	H	OCH_3	H	H	-
8	H	H	Cl	H	H	-
9	H	H	H	H	H	-
10	H	H	OCH_3	OH	H	-
11	H	H	OC_2H_5	OH	H	-
12	H	H	OH	H	H	-
13	H	NO_2	H	H	H	-
14	Cl	H	H	H	H	-
15	H	Br	H	H	H	-
16	OH	H	H	H	H	-
17	-	-	-	-	-	
18	-	-	-	-	-	
19	-	-	CHO	-	-	-
20	-	-	-	-	-	
21	-	-	-	-	-	

Scheme 1 Scheme for the synthesis of *N*′-(substituted)-4-(butan-2-ylideneamino) benzohydrazides.

Table 1 Physicochemical characteristics of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

Comp.	M. formula	M. wt.	*m.p.*	R_f value[a]	% Yield
1	$C_{19}H_{21}N_3O$	307	175–178	0.34	93
2	$C_{20}H_{23}N_3O_3$	353	144–147	0.22	82
3	$C_{18}H_{18}ClN_3O$	327	101–104	0.32	83
4	$C_{20}H_{24}N_4O$	336	61–64	0.27	79
5	$C_{21}H_{25}N_3O_4$	338	167–170	0.26	75
6	$C_{18}H_{18}BrN_3O$	371	189–192	0.23	81
7	$C_{19}H_{21}N_3O_2$	323	117–120	0.29	87
8	$C_{18}H_{18}ClN_3O$	327	112–115	0.48	84
9	$C_{18}H_{19}N_3O$	293	95–98	0.34	78
10	$C_{19}H_{21}N_3O_3$	339	127–130	0.28	79
11	$C_{20}H_{23}N_3O_3$	353	177–180	0.19	80
12	$C_{18}H_{19}N_3O_2$	309	250–253	0.21	79
13	$C_{18}H_{18}N_4O_3$	338	173–176	0.15	73
14	$C_{18}H_{18}ClN_3O$	327	87–90	0.06	80
15	$C_{18}H_{18}BrN_3O$	372	104–107	0.21[b]	90
16	$C_{18}H_{19}N_3O_2$	309	225–228	0.74	94
17	$C_{20}H_{21}N_3O$	319	89–92	0.38	88
18	$C_{22}H_{21}N_3O_2$	359	235–238	0.26	81
19	$C_{19}H_{19}N_3O_2$	321	268–271	0.227	80
20	$C_{16}H_{21}N_3O_2$	287	96–99	0.41	76
21	$C_{16}H_{17}N_3O_2$	283	107–110	0.14	85

[a] Chloroform: toluene = 7:3.
[b] Chloroform: toluene = 1:1.

Table 4. The synthesized compounds exhibited good anticancer potential. Compounds **1**, **2**, **6**, **12**, **13**,**14**, **15**, **19** and **20** were found to be more potent than the standard drug carboplatin (IC_{50} > 100 μM) and compound **14** (IC_{50} = 37.71 μM) was found to be the most potent against human colorectal cancer (HCT116) cell line and may be taken as new lead for the development of novel anticancer agents. The synthesized compounds however did not show good activity when compared to the drugs available in the market which include tetrandrine, doxorubicin and camptothecin having IC_{50} values of 1.530, 0.702 and 0.147 μM, respectively.

3.4. Structure activity relationship studies

The SAR for the antimicrobial and anticancer activity of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides can be summarized as follows.

1. Results of the antimicrobial evaluation indicated that electron releasing groups (compounds **2** and **12** having 3,4-dimethoxy and *p*-hydroxy) on benzylidene portion improved the antimicrobial activity of the synthesized compounds against *S. aureus* and *A. niger*, respectively. This is in accordance with the results obtained by Nandagokula et al. (2013).
2. Presence of electron withdrawing (*p*-Br) substituent on benzylidene portion improved the antimicrobial activity of compound **6** against *B. subtilis*, *E. coli* and *C. albicans*. The role of electron withdrawing groups in improving antibacterial and antifungal activities is supported by the studies of Mostafa et al. (2008).
3. Replacement of phenyl nucleus of benzylidene moiety with heterocyclic (furan, **21**) and alkyl groups (**20**) resulted in less active antimicrobial agents, which shows that phenyl nucleus is essential for antimicrobial activity of the synthesized compounds.
4. Anticancer activity results indicated that presence of *o*-Cl substituent on benzylidene portion (**14**) improved the anticancer activity of the synthesized compounds against human colorectal cancer (HCT116) cell line.
5. From these results, 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. (2007).

The aforementioned findings are summarized in Fig. 1.

3.5. QSAR studies

In order to identify the substituent effect on the antimicrobial activity, quantitative structure activity relationship (QSAR) studies were undertaken, using the linear free energy relationship model (LFER) described by Hansch and Fujita (1964).

In the present study, a dataset of 21 *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (**1–21**) was used for QSAR model development. 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 5.

Our earlier studies (Sigroha et al., 2012; Judge et al., 2012a,b; Narang et al., 2012) indicated that the multi-target QSAR (*mt*-QSAR) models are better than one-target QSAR (*ot*-QSAR) models in describing the antimicrobial activity. Therefore in the present study we developed muti-target QSAR models to describe the antimicrobial activity of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

According to the *ot*-QSAR models one should use five different equations with different errors to predict the activity of a new compound against the five microbial species. The *ot*-QSAR models, which are almost in the whole literature, become impractical to use when we have to predict each compound result for more than one target. In these cases we have to develop one *ot*-QSAR for each target. However, very recently the interest has increased in the development of multi-target QSAR (*mt*-QSAR) models.

As opposed to *ot*-QSAR, the *mt*-QSAR model is a single equation that considers the nature of molecular descriptors, which are common and essential in describing the antibacterial and antifungal activity (Gonzalez-Diaz et al., 2008, 2007; Cruz-Monteagudo et al., 2007; 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 activity of the synthesized compounds against *S. aureus, B. subtilis* and *E. coli*, *mt*-QSAR model to describe antifungal activity of the synthesized compounds against *C. albicans* and *A. niger* as well as a common *mt*-QSAR model to describe the antimicrobial (overall antibacterial and antifungal) activity of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazide derivatives against all the above mentioned microorganisms.

During the regression analysis studies it was observed that the response values of compounds **1, 2, 3, 5, 6, 8** and **19** were outside the limits of response values of other synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides. Thus, these compounds were designated as outliers and were not involved in the data set for QSAR model generation. In

Table 2 Spectral studies of *N′*-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

Comp.	IR (KBr pellets, cm^{-1})	^1H NMR (MEOD)	^{13}C NMR (MEOD)	Analytical data calcd. (found)	MS ES + (ToF): *m/z* $[M^+ + 1]$
1	IR (KBr pellets) cm^{-1} 3066 (C–H str., Ar), 1590 (C═C skeletal str., phenyl), 1624 (C═O str., sec. amide), 1677 (C═N str., N═CH), 2826 (C–H sym. str., Ar–CH_3), 2955 (C–H assym. str., R–CH_3), 933 (N–N str., N–NH)	^1H NMR (MEOD): 7.349–8.356 (m, 8H, Ar–H), 8.706 (s, 1H, N═CH), 2.503 (s, 3H, Ar–CH_3), 1.219 (m, 2H, CH_2 of C_2H_5), 0.871 (s, 3H, CH_3)	23.7, 8.0, 171.9, 22.6, 152.8, 122.2, 128.5, 132.9, 128.8, 122.1, 163.3, 130.6, 129.4, 129.0, 140.5, 129.1, 129.4, 143.3, 24.2	$C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67 (C, 74.19, H, 6.91; N, 13.62)	308
2	IR (KBr pellets) cm^{-1} 3076 (C–H str., Ar), 1597 (C═C skeletal str., phenyl), 1621 (C═O str., sec. amide), 1663 (C═N str., N═CH), 2960 (C–H assym. str., R–CH_3), 957 (N–N str., N–NH), 3120 (C–H str., –OCH_3), 1237 (C–O–C assym. str., Ar–O–CH_3)	^1H NMR (MEOD): 7.096–7.531 (m, 8H, Ar–H), 8.663 (s, 1H, N═CH), 1.241 (m, 2H, CH_2 of C_2H_5), 0.849 (s, 3H, CH_3), 3.874 (s, 6H, OCH_3)	23.5, 8.1, 171.6, 22.4, 152.5, 122.4, 128.0, 132.7, 128.4, 122.5, 163.6, 127.4, 122.8, 115.6, 152.3, 149.5, 114.7, 143.3, 56.5, 56.0	$C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89 (C, 67.99; H, 6.52; N, 11.93)	354
3	IR (KBr pellets) cm^{-1} 3060 (C–H str., Ar), 1596 (C═C skeletal str., phenyl), 1627 (C═O str., sec. amide), 1684 (C═N str., N═CH), 2956 (C–H assym. str., R–CH_3), 955 (N–N str., N–NH), 709 (C–Cl str., C_6H_5Cl)	^1H NMR (MEOD): 7.340–8.018 (m, 8H, Ar–H), 8.64. (s, 1H, N═CH), 1.299 (m, 2H, CH_2 of C_2H_5), 0.896 (s, 3H, CH_3)	23.2, 8.5, 171.2, 22.0, 152.7, 122.2, 128.3, 132.6, 128.1, 122.7, 163.4, 135.5, 127.7, 130.6, 131.0, 134.5, 129.4, 143.1	$C_{18}H_{18}ClN_3O$: C, 65.95; H, 5.53; N, 12.82 (C, 65.91; H, 5.57; N, 12.86)	328
4	IR (KBr pellets) cm^{-1} 3070 (C–H str., Ar), 1582 (C═C skeletal str., phenyl), 1597 (C═O str., sec. amide), 1654 (C═N str., N═CH), 2971 (C–H assym. str., R–CH_3), 938 (N–N str., N–NH), 1346 (C–N str., Aryl 3° amine)	^1H NMR (MEOD): 7.438–7.617 (m, 8H, Ar–H), 8.536 (s, 1H, N═CH), 1.232 (m, 2H, CH_2 of C_2H_5), 0.874 (s, 3H, CH_3), 2.532 (s, 6H, N–$(CH_3)_2$)	23.0, 8.4, 171.7, 22.3, 152.9, 122.2, 128.5, 132.6, 128.4, 122.9, 163.4, 123.7, 130.4, 114.1, 151.5, 114.8, 130.5, 143.3, 40.7, 40.1	$C_{20}H_{24}N_4O$: C,71.40; H, 7.19; N, 16.65 (C,71.37; H, 7.23; N, 16.61)	337
5	IR (KBr pellets) cm^{-1} 3079 (C–H str., Ar), 1577 (C═C skeletal str., phenyl), 1621 (C═O str., sec. amide), 1693 (C═N str., N═CH), 2953 (C–H assym. str., R–CH_3), 946 (N–N str., N–NH), 3011 (C–H str., –O–CH_3), 1230 (C–O–C assym. str., Ar–O–CH_3)	^1H NMR (MEOD): 7.236–7.908 (m, 8H, Ar–H), 8.693 (s, 1H, N═CH), 1.277 (m, 2H, CH_2 of C_2H_5), 0.918 (s, 3H, CH_3), 3.786 (s, 9H, OCH_3)	23.5, 8.7, 171.3, 22.3, 152.6, 122.2, 128.9, 132.8, 128.4, 122.8, 163.9, 128.4, 106.9, 150.5, 141.7, 150.6, 106.5, 143.3, 56.2, 56.5, 56.0	$C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96 (C, 65.80; H, 6.61; N, 10.92)	339
6	IR (KBr pellets) cm^{-1} 3046 (C–H str., Ar), 1582 (C═C skeletal str., phenyl), 1624 (C═O str., sec. amide), 1680 (C═N str., N═CH), 2940 (C–H assym. str., R–CH_3), 931 (N–N str., N–NH), 595 (C–Br str., C_6H_5Br)	^1H NMR (MEOD): 7.259–7.864 (m, 8H, Ar–H), 8.760 (s, 1H, N═CH), 1.232 (m, 2H, CH_2 of C_2H_5), 0.851 (s, 3H, CH_3)	23.3, 8.5, 171.7, 22.5, 152.9, 122.5, 128.7, 132.6, 128.3, 122.5, 163.4, 132.5, 131.7, 131.9, 125.2, 131.6, 131.3, 43.4	$C_{18}H_{18}BrN_3O$: C, 58.08; H, 4.87; N, 11.29 (C, 58.05; H, 4.92; N, 11.33)	372
7	IR (KBr pellets) cm^{-1} 2979 (C–H str., Ar), 1573 (C═C skeletal str., phenyl), 1627 (C═O str., sec. amide), 1680 (C═N str., N═CH), 2937 (C–H assym. str., R–CH_3), 933 (N–N str., N–NH), 1255 (C–O–C assym. str., Ar–O–CH_3)	^1H NMR (DMSO): 6.553–7.987 (m, 8H, Ar–H), 8.564 (s, 1H, N═CH), 1.249 (m, 2H, CH_2 of C_2H_5), 0.873 (t, 3H, CH_3), 3.843 (s, 3H, OCH_3)	23.8, 8.6, 171.4, 22.0, 152.6, 122.4, 128.5, 132.2, 128.6, 122.5, 163.4, 126.5, 130.4, 114.8, 163.3, 114.1, 163.5, 114.7, 130.4, 143.2, 55.6	$C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99 (C, 70.61; H, 6.71; N, 12.93)	324
8	IR (KBr pellets) cm^{-1} 3067 (C–H str., Ar), 1596 (C═C skeletal str., phenyl), 1615 (C═O str., sec. amide), 1677 (C═N str., N═CH), 2957 (C–H assym. str., R–CH_3), 936 (N–N str., N–NH), 744 (C–Cl str., C_6H_5Cl)	^1H NMR (DMSO): 7.351–8.196 (m, 8H, Ar–H), 8.888 (s, 1H, N═CH), 1.353 (m, 2H, CH_2 of C_2H_5), 0.900 (t, 3H, CH_3)	23.2, 8.0, 171.1, 22.0, 152.5, 122.4, 128.5, 132.4, 128.6, 122.2, 163.0, 131.6, 130.2, 129.3, 136.8, 129.3, 130.5, 143.4	$C_{18}H_{18}ClN_3O$: C, 65.95; H, 5.53; N, 12.82 (C, 65.91; H, 5.50; N, 12.85)	328

9	IR (KBr pellets) cm^{-1} 3067 (C–H str., Ar), 1592 (C═C skeletal str., phenyl), 1623 (C═O str., sec. amide), 1677 (C═N str., N═CH), 2955 (C–H assym. str., R–CH_3), 935 (N–N str., N–NH)	^{1}H NMR (DMSO): 7.337–8.008 (m, 9H, Ar–H), 8.655 (s, 1H, N═CH), 1.298 (m, 2H, CH_2 of C_2H_5), 0.900 (t, 3H, CH_3)	23.6, 8.5, 171.4, 22.4, 152.8, 122.4, 128.7, 132.4, 128.6, 122.5, 163.3, 133.5, 129.6, 128.7, 131.3, 128.5, 129.4, 143.1	$C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.32 (C, 73.65; H, 6.50; N, 14.35)	294
10	IR (KBr pellets) cm^{-1} 3002 (C–H str., Ar), 1600 (C═C skeletal str., phenyl), 1624 (C═O str., sec. amide), 1681 (C═N str., N═CH), 2957 (C–H assym. str., R–CH_3), 968 (N–N str., N–NH) 1238 (C–O–C assym. str., Ar–O–CH_3), 1315 (O–H in plane bending, phenol), 1223 (C–O str., phenol)	^{1}H NMR (DMSO): 6.870–7.897 (m, 7H, Ar–H), 8.324 (s, 1H, N═CH), 1.243 (m, 2H, CH_2 of C_2H_5), 0.885 (t, 3H, CH_3), 3.981 (s, 3H, OCH_3), 5.829 (s, 1H, OH)	23.9, 8.3, 171.1, 22.4, 152.9, 122.4, 128.7, 132.4, 128.6, 122.5, 163.3, 127.3, 116.1, 145.6, 153.9, 115.8, 122.5, 143.2, 56.5	$C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38 (C, 67.29; H, 6.20; N, 12.40)	340
11	IR (KBr pellets) cm^{-1} 3090 (C–H str., Ar), 1581 (C═C skeletal str., phenyl), 1598 (C═O str., sec. amide), 1629 (C═N str., N═CH), 2969 (C–H assym. str., R–CH_3), 930 (N–N str., N–NH), 3121 (C–H str., Ar–O–CH_3), 1245 (C–O–C assym. str., Ar–O–CH_3), 1345 (O–H in plane bending, phenol), 1198 (C–O str., phenol)	^{1}H NMR (DMSO): 6.878–7.700 (m, 7H, Ar–H), 8.552 (s, 1H, N═CH), 1.243 (m, 2H, CH_2 of C_2H_5), 0.905 (t, 3H, CH_3), 1.365 (t, 3H, CH_3 of OC_2H_5), 3.993 (m, 2H, CH_2 of OC_2H_5)	23.4, 8.7, 171.6, 22.3, 152.4, 122.3, 128.5, 132.4, 128.2, 122.0, 163.3, 126.5, 115.8, 145.7, 150.2, 115.6, 122.3, 143.2, 65.0, 14.5	$C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89 (C, 67.93; H, 6.52; N, 11.94)	354
12	IR (KBr pellets) cm^{-1} 3027 (C–H str., Ar), 1596 (C═C skeletal str., phenyl), 1622 (C═O str., sec. amide), 1660 (C═N str., N═CH), 2951 (C–H assym. str., R–CH_3), 963 (N–N str., N–NH), 1303 (O–H in plane bending, phenol), 1222 (C–O str., phenol)	^{1}H NMR (DMSO): 6.696–7.941 (m, 8H, Ar–H), 8.394 (s, 1H, N═CH), 1.240 (m, 2H, CH_2 of C_2H_5), 0.900 (t, 3H, CH_3), 5.832 (s, 1H, OH)	23.5, 8.5, 171.9, 22.3, 152.0, 122.3, 128.7, 132.4, 128.7, 122.0, 163.5, 126.7, 130.9, 116.3, 160.5, 116.1, 130.4, 143.2	$C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58 (C, 69.83; H, 6.22; N, 13.61)	310
13	IR (KBr pellets) cm^{-1} 2958 (C–H str., Ar), 1574 (C═C skeletal str., phenyl), 1625 (C═O str., sec. amide), 1671 (C═N str., N═CH), 2928 (C–H assym. str., R–CH_3), 922 (N–N str., N–NH), 842 (C–N str., Ar–NO_2), 1520 (NO_2 str., Ar–NO_2)	^{1}H NMR (DMSO): 7.608–8.774 (m, 8H, Ar–H), 8.851 (s, 1H, N═CH), 1.289 (m, 2H, CH_2 of C_2H_5), 0.900 (t, 3H, CH_3)	23.4, 8.1, 171.6, 22.2, 152.0, 122.6, 128.7, 132.9, 128.2, 122.0, 163.9, 134.9, 135.5, 129.6, 123.2, 148.5, 124.4, 143.3	$C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56 (C, 63.92; H, 5.40; N, 16.52)	339

(*continued on next page*)

Table 2 (*Continued*)

Comp.	IR (KBr pellets, cm^{-1})	^1H NMR (MEOD)	^{13}C NMR (MEOD)	Analytical data calcd. (found)	MS ES + (ToF): m/z $[M^+ + 1]$
14	IR (KBr pellets) cm^{-1} 3068 (C–H str., Ar), 1588 (C=C skeletal str., phenyl), 1614 (C=O str., sec. amide), 1678 (C=N str., N=CH), 2952 (C–H assym. str., R–CH_3), 936 (N–N str., N–NH), 709 (C–Cl str. C_6H_5Cl)	^1H NMR (DMSO): 7.243–7.991 (m, 8H, Ar–H), 8.886 (s, 1H, N=CH), 1.294 (m, 2H, CH_2 of C_2H_5), 0.882 (t, 3H, CH_3)	23.3, 8.5, 171.3, 22.2, 152.0, 122.5, 128.7, 132.4, 128.2, 122.0, 163.3, 133.5, 130.0, 127.3, 132.7, 129.5, 134.4, 143.1	$C_{18}H_{18}ClN_3O$: C, 65.95; H, 5.53; N, 12.82 (C, 65.91; H, 5.50; N, 12.87)	328
15	IR (KBr pellets) cm^{-1} 2996 (C–H str., Ar), 1593 (C=C skeletal str., phenyl), 1624 (C=O str., sec. amide), 1682 (C=N str., N=CH), 2959 (C–H assym. str., R–CH_3), 940 (N–N str., N–NH), 550 (C–Br str. C_6H_5Br)	^1H NMR (DMSO): 7.464–7.949 (m, 8H, Ar–H), 8.732 (s, 1H, N=CH), 1.292 (m, 2H, CH_2 of C_2H_5), 0.903 (t, 3H, CH_3)	23.7, 8.2, 171.8, 22.5, 152.4, 122.7, 128.9, 132.7, 128.2, 122.0, 163.6, 136.4, 128.1, 131.3, 134.6, 123.5, 132.9, 143.2	$C_{18}H_{18}BrN_3O$: C, 58.08; H, 4.87; N, 11.29 (C, 58.05; H, 4.83; N, 11.33)	373
16	IR (KBr pellets) cm^{-1} 2959 (C–H str., Ar), 1598 (C=C skeletal str., phenyl), 1620 (C=O str., sec. amide), 1678 (C=N str., N=CH), 2817 (C–H sym. str., R–CH_3), 941 (N–N str., N–NH), 1318 (O–H in plane bending, phenol), 1206 (C–O str., phenol)	^1H NMR (DMSO): 6.737–7.716 (m, 8H, Ar–H), 8.035 (s, 1H, N=CH), 1.249 (m, 2H, CH_2 of C_2H_5), 0.898 (t, 3H, CH_3), 5.436 (s, 1H, OH)	23.9, 8.0, 171.5, 22.5, 152.5, 122.3, 128.4, 132.7, 128.2, 122.3, 163.1, 118.7, 130.9, 121.3, 132.5, 116.2, 161.4, 143.1	$C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58 (C, 69.84; H, 6.22; N, 13.60)	310
17	IR (KBr pellets) cm^{-1} 2967 (C–H str., Ar), 1599 (C=C skeletal str., phenyl), 1629 (C=O str., sec. amide), 1668 (C=N str., N=CH), 2923 (C–H assym. str., R–CH_3), 974 (N–N str., N–NH), 1599 (C=C str., CH=CH), 1312 (C–H in plane bending CH=CH)	^1H NMR (DMSO): 6.926–8.158 (m, 9H, Ar–H), 7.575 (d, 1H, N=CH), 1.282 (m, 2H, CH_2 of C_2H_5), 0.883 (t, 3H, CH_3)	23.6, 8.2, 171.3, 22.2, 152.7, 122.4, 128.6, 132.9, 128.2, 122.3, 163.7, 137.6, 126.5, 139.3, 135.0, 126.1, 128.9, 128.3, 128.9, 126.6	$C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.16 (C, 75.25; H, 6.66; N, 13.11)	320
18	IR (KBr pellets) cm^{-1} 3058 (C–H str., Ar), 1578 (C=C skeletal str., phenyl), 1621 (C=O str., sec. amide), 1677 (C=N str., N=CH), 2958 (C–H assym. str., R–CH_3), 932 (N–N str., N–NH), 1306 (O–H in plane bending, phenol), 1210 (C–O str., phenol).	^1H NMR (DMSO): 7.291–7.965 (m, 10H, Ar–H), 8.051 (s, 1H, N=CH), 1.289 (m, 2H, CH_2 of C_2H_5), 0.904 (t, 3H, CH_3), 5.832 (s, 1H, OH)	23.4, 8.5, 171.0, 22.5, 152.9, 122.1, 128.4, 132.6, 128.6, 122.1, 163.5, 143.3, 111.6, 161.5, 126.2, 137.4, 121.3, 126.1, 121.5, 126.8, 126.5, 127.6	$C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69 (C, 73.47; H, 5.91; N, 11.73)	360

19	IR (KBr pellets) cm^{-1} 1591 (C=C skeletal str., phenyl), 1621 (C=O str., sec. amide), 1678 (C=N str., N=CH), 961 (N–N str., N–NH), 1678 (C=O str., Ar–CHO), 858 (C–H out of plane bending, CHO)	^{1}H NMR (DMSO): 7.316–8.272 (m, 8H, Ar–H), 8.659 (s, 1H, N=CH), 1.288 (m, 2H, CH_2 of C_2H_5), 0.900 (t, 3H, CH_3), 10.126 (s, 1H, CHO)	23.4, 8.6, 171.7, 22.5, 152.5, 122.3, 128.4, 132.4, 128.0, 122.3, 163.5, 139.4, 129.9, 130.3, 139.4, 130.2, 129.5, 143.3, 191.2	$C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08 (C, 73.50; H, 5.92; N, 11.71)	322
20	IR (KBr pellets) cm^{-1} 2932 (C–H str., Ar), 1518 (C=C skeletal str., phenyl), 1600 (C=O str., sec. amide), 1673 (C=N str., N=CH), 2866 (C–H sym. str., R–CH_3), 960 (N–N str., N–NH), 2794 (C–H str., R–CHO), 1440 (C–CHO skeletal str., R–CHO)	^{1}H NMR (DMSO): 7.604–7.732 (m, 8H, Ar–H), 6.558 (t, 1H, N=CH), 1.294 (m, 2H, CH_2 of C_2H_5), 0.960 (t, 3H, CH_3), 1.311–2.561 (m, 6H, R–CH_2)	23.2, 8.3, 171.9, 22.4, 152.5, 122.7, 128.1, 132.4, 128.0, 122.6, 163.1, 158.6, 25.8, 18.5, 43.1, 202.4	$C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62 (C, 66.83; H, 7.41; N, 14.56)	288
21	IR (KBr pellets) cm^{-1} 2960 (C–H str., Ar), 1598 (C=C skeletal str., phenyl), 1634 (C=O str., sec. amide), 1678 (C=N str., N=CH), 2928 (C–H assym. str., R–CH_3), 931 (N–N str., N–NH), 1015 (ring breathing, 2-substituted furan ring)	^{1}H NMR (DMSO): 7.59–7.95 (m, 4H, Ar–H), 7.56 (s, 1H, N=CH), 1.29 (m, 2H, CH_2 of C_2H_5), 0.89 (t, 3H, CH_3), 6.43–7.38 (m, 3H, furan)	23.0, 8.4, 171.6, 22.1, 152.2, 122.3, 128.6, 132.9, 128.2, 122.3, 163.5, 134.5, 149.3, 109.8, 109.6, 143.5	$C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83 (C, 67.77; H, 6.01; N, 14.86)	284

multivariate statistics, it is common to define three types of outliers (Furusjo et al., 2006).

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

There was no difference in the activity (Table 3) as well as the molecular descriptor range (Table 5) of the outliers (**1, 2, 3, 5, 6, 8** and **19**) when compared to other N'-(substituted)-4-(butan-2-ylideneamino)benzohydrazides. This indicated the fact that the 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 activity, antifungal activity and antimicrobial activity values of N'-(substituted)-4-(butan-2-ylideneamino)benzohydrazides which are presented in Table 3. These average antifungal activity values correlated with molecular descriptors of the synthesized compounds (Table 6). In general, high colinearity ($r > 0.5$) was observed between different parameters (Table 6). The high interrelationship was observed between Kier's first order shape index (κ_1) and W ($r = 0.975$), and low interrelationship was observed between energy of lowest unoccupied molecular orbital (LUMO) ($r = 0.044$, Table 6).

From the correlation matrix (Table 6), it was observed that the steric parameter, molar refractivity (MR) was found to be most effective in describing the antifungal activity of the synthesized compounds (Eq. (1)).

3.5.1. LR mt-QSAR model for antifungal activity

$$\begin{aligned} &\mathrm{pMIC_{af}} = 0.018\mathrm{MR} - 0.078 \\ &n = 14 \quad r = 0.808 \quad q^2 = 0.533 \quad s = 0.088 \quad F = 22.50 \end{aligned} \tag{1}$$

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

The coefficient of molar refractivity (MR) is positive in QSAR model developed to describe the antifungal activity of the synthesized N'-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (Eq. (1)), which indicated that there is a positive correlation between antifungal activity and molar refractivity (MR) i.e. antifungal potential of the synthesized compounds will increase with increase in their MR values and vice versa. This is evidenced by high antifungal activity of compound **12** ($\mathrm{pMIC_{af}} = 1.91$ μM/ml, Table 3) having high MR value (MR = 107.38, Table 5) and minimum antifungal activity of compound **20** ($\mathrm{pMIC_{af}} = 1.35$ μM/ml, Table 3) having least MR value (MR = 81.63, Table 5).

The developed QSAR model (Eq. (1)) was cross validated by q^2 value ($q^2 = 0.533$) obtained by leave one out (LOO) method. The value of q^2 (more than 0.5) indicated that the developed model is a valid one. Further, the observed and predicted values are close to each other (Table 7), the *mt*-QSAR

Table 3 Antimicrobial activity (pMIC in μM/ml) of the synthesized *N′*-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	1.69	1.69	1.39	1.99	1.39	1.59	1.69	1.63
2	1.75	1.75	1.45	1.75	1.75	1.65	1.75	1.69
3	1.42	1.72	1.42	2.02	1.72	1.52	1.87	1.66
4	1.73	1.43	1.43	2.03	1.43	1.53	1.73	1.61
5	1.43	1.43	1.43	2.03	1.43	1.43	1.73	1.55
6	1.47	1.77	1.47	2.07	1.47	1.57	1.77	1.65
7	1.41	1.71	1.11	1.71	1.41	1.41	1.56	1.47
8	1.42	1.72	1.42	1.72	1.12	1.52	1.42	1.48
9	1.41	1.71	1.11	2.01	1.41	1.41	1.71	1.53
10	1.37	1.67	1.07	1.97	1.37	1.37	1.67	1.49
11	1.43	1.73	1.43	1.73	1.43	1.53	1.58	1.55
12	1.46	1.76	0.86	2.06	1.76	1.36	1.91	1.58
13	1.45	1.75	1.15	2.05	1.75	1.45	1.90	1.63
14	1.39	1.39	1.39	1.69	1.39	1.39	1.54	1.45
15	1.43	1.73	1.43	1.73	1.43	1.53	1.58	1.55
16	1.42	1.42	1.42	1.72	1.42	1.42	1.57	1.48
17	1.47	1.47	1.17	1.77	1.47	1.37	1.62	1.47
18	1.41	1.71	1.11	1.71	1.71	1.41	1.71	1.53
19	1.36	1.66	1.06	1.66	1.36	1.36	1.51	1.42
20	1.35	1.66	1.05	1.35	1.35	1.35	1.35	1.35
21	1.39	1.39	1.39	1.69	1.69	1.39	1.69	1.51
S.D.	0.12	0.14	0.19	0.19	0.17	0.09	0.15	0.09
Std.	2.61[a]	2.61[a]	2.61[a]	2.64[b]	2.64[b]	–	–	–

[a] Norfloxacin.
[b] Fluconazole.

Table 4 Anticancer activity (IC_{50} in μM) of the synthesized *N′*-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

Compound	IC_{50} (HCT116, μM)
1	96.64
2	74.59
3	243.64
4	163.69
5	209.08
6	79.06
7	216.72
8	152.91
9	178.6
10	141.59
11	173.74
12	89.55
13	60.15
14	37.71
15	88.71
16	184.47
17	156.74
18	158.77
19	87.23
20	95.23
21	181.38
Tetrandrine	1.53
Doxorubicin	0.70
Camptothecin	0.15
Carboplatin	> 100

model for antibacterial activity Eq. (1) is a valid one (Golbraikh and Tropsha, 2002). The plot of predicted $pMIC_{af}$ against observed $pMIC_{af}$ (Fig. 2) also favors the developed model expressed by Eq. (1). The plot of observed $pMIC_{af}$ *vs* residual $pMIC_{af}$ (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).

The topological parameter, third order molecular connectivity index ($^3\chi$) was found to be effective in describing the antibacterial activity of the synthesized compounds (Eq. (2)).

3.5.2. LR mt-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.225\,{}^3\chi + 1.117$$
$$n = 14 \quad r = 0.637 \quad q^2 = 0.156 \quad s = 0.051 \quad F = 8.174 \tag{2}$$

The molecular connectivity index, an adjacency based topological index proposed by Randic is denoted by χ and is defined as sum 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* are 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).

The addition of topological parameter, Kier's third order shape index (κ_3) to the topological parameter, third order molecular connectivity index ($^3\chi$), significantly improved the value of regression coefficient from 0.637 to 0.778 (Eq. (3)).

3.5.3. MLR mt-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.199\,{}^3\chi + 0.053\kappa_3 + 0.787$$
$$n = 14 \quad r = 0.778 \quad q^2 = 0.296 \quad s = 0.043 \quad F = 8.46 \tag{3}$$

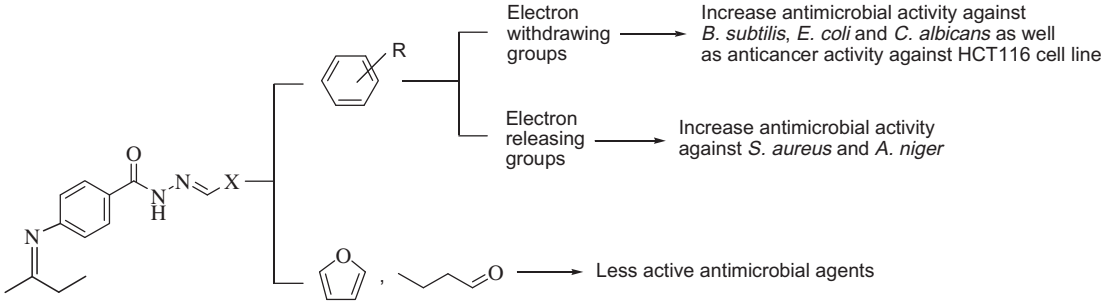


Figure 1 Structural requirements for antimicrobial and anticancer activities of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

The validity and predictability of the QSAR model for antibacterial activity i.e. Eq. (3) were cross validated by q^2 value ($q^2 = 0.296$) 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 their ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other (Table 7), the *mt*-QSAR model for antibacterial activity Eq. (3) is therefore a valid one (Golbraikh and Tropsha, 2002).

A set of very useful topological indices of the second generation is composed of the kappa indices of molecular shape and flexibility (Kier et al., 1999). According to Kier, the shape of a molecule may be partitioned into attributes, each describable by the count of bonds of various path lengths. The basis for devising a relative index of shape is given by the relationship of the number of path of length l in the molecule i, ${}^{l}P_i$, to some reference values based on molecules with a given number of atoms, n, in which the values of ${}^{l}P$ are maximum and minimum, ${}^{l}P_{max}$ and ${}^{l}P_{min}$.

The modified kappa shape indices are given by:

$$\begin{aligned}\kappa\alpha_1 &= (n+\alpha)(n+\alpha-1)^2/({}^1P_i+\alpha)^2\kappa\alpha_2\\ &= (n+\alpha-1)(n+\alpha-2)^2/({}^2P_i+\alpha)^2\kappa\alpha_3\\ &= (n+\alpha-1)(n+\alpha-3)^2/({}^3P_i+\alpha)^2 n\,\text{is odd}\,\kappa\alpha_3\\ &= (n+\alpha-3)(n+\alpha-2)2/({}^3P_i+\alpha)^2 n\,\text{is even}.\end{aligned}$$

The antimicrobial activity of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides is best described by the topological parameter, Kier's first order shape index (κ_1, Eq. (4)).

3.5.4. LR mt-QSAR model for antimicrobial activity

$$\begin{aligned}&\text{pMIC}_{\text{am}} = 0.0463\kappa_1 + 0.5825\\ &n = 14 \quad r = 0.907 \quad q^2 = 0.741 \quad s = 0.0309 \quad F = 55.79\end{aligned} \tag{4}$$

The validity of QSAR model for antimicrobial activity (Eq. (4)) is indicated by their high q^2 value (0.741) as well as the low residual values (Table 7). The high residual values observed in case outliers (**1, 2, 3, 5, 6, 8** and **19**) justify their removal while developing QSAR models.

It was observed from *mt*-QSAR models (Eqs. (1)–(4)) that the antibacterial activity, antifungal activity and overall

Table 5 Values of selected parameters used in the QSAR studies of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

Comp.	log *P*	MR	${}^{2}\chi$	${}^{3}\chi$	κ_1	κ_3	*W*	Te	LUMO	HOMO
1	4.95	94.28	9.56	1.39	19.33	6.79	1504.00	−3661.11	−0.46	−8.66
2	3.98	102.17	10.45	1.43	22.29	7.16	2092.00	−4456.81	−0.47	−8.33
3	5.00	94.04	9.58	1.39	19.33	6.79	1488.00	−3865.33	−0.48	−8.95
4	4.28	102.95	10.46	1.60	21.30	7.26	1924.00	−4036.90	−0.29	−8.02
5	3.73	108.63	11.18	1.57	24.27	7.34	2491.00	−4932.58	−0.53	−8.59
6	5.28	96.86	9.56	1.39	19.33	6.79	1504.00	−3844.85	−0.60	−8.90
7	4.23	95.70	9.73	1.30	20.31	7.04	1713.00	−3981.12	−0.45	−8.50
8	5.00	94.04	9.56	1.39	19.33	6.79	1504.00	−3865.35	−0.56	−8.85
9	4.89	99.48	9.65	1.10	20.31	7.84	1777.00	−3788.40	−0.53	−8.56
10	4.49	89.24	8.94	1.10	18.34	6.57	1318.00	−3505.24	−0.47	−8.80
11	3.95	97.40	10.26	1.50	21.30	6.91	1874.00	−4301.68	−0.49	−8.44
12	5.20	107.38	11.37	1.57	21.70	5.99	2194.00	−4365.36	−0.72	−8.78
13	4.29	102.15	10.67	1.52	22.29	7.51	2051.00	−4457.34	−0.38	−8.59
14	4.20	90.93	9.56	1.39	19.33	6.79	1504.00	−3825.86	−0.47	−8.57
15	4.44	96.56	10.47	1.60	21.30	7.26	1876.00	−4336.33	−1.02	−9.30
16	5.00	94.04	9.46	1.30	19.33	6.43	1472.00	−3865.21	−0.45	−9.07
17	5.28	96.86	9.58	1.39	19.33	6.79	1488.00	−3844.83	−0.55	−8.97
18	4.16	95.83	9.73	1.30	20.31	7.04	1713.00	−3953.48	−0.78	−9.06
19	4.16	95.83	9.73	1.30	20.31	7.04	1713.00	−3953.48	−0.78	−9.06
20	3.43	81.63	8.59	1.10	17.36	5.95	1151.00	−3540.85	−0.51	−8.61
21	4.20	90.93	9.46	1.30	19.33	6.43	1472.00	−3825.82	−0.54	−8.67

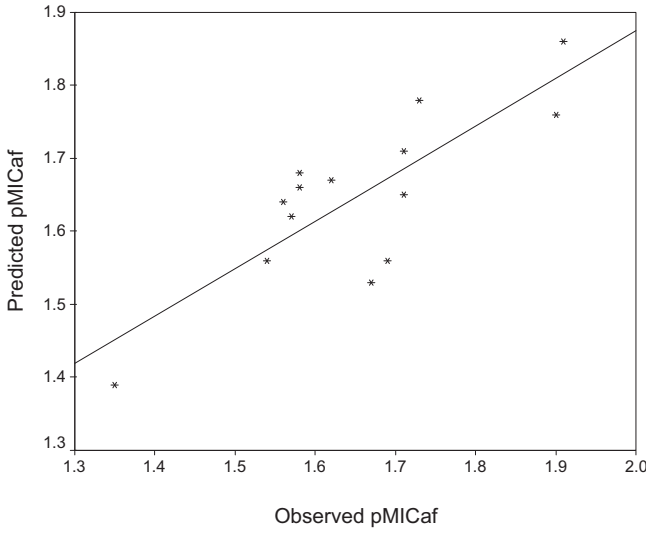


Figure 2 Plot of observed $pMIC_{af}$ against predicted $pMIC_{af}$ for the linear regression model developed by Eq. (1).

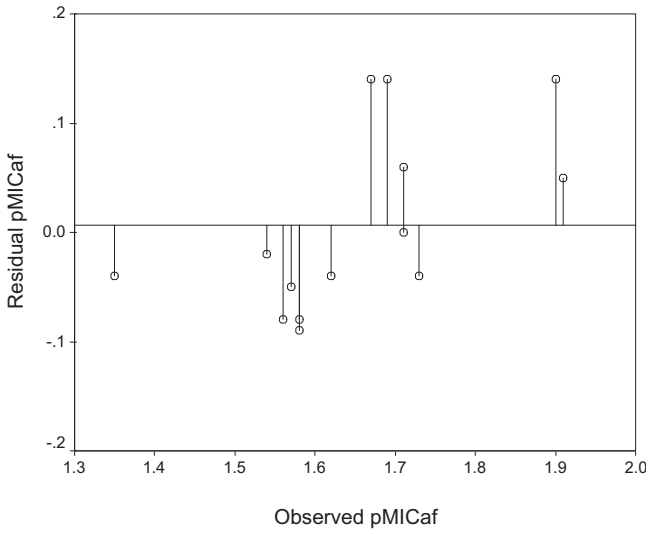


Figure 3 Plot of observed $pMIC_{af}$ against residual $pMIC_{af}$ for the linear regression model developed by Eq. (1).

Table 6 Correlation matrix for antifungal activity of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

	log *P*	MR	$^3\chi$	κ_1	κ_3	*J*	*W*	Te	LUMO	$pMIC_{af}$
log *P*	1.000									
MR	0.561	1.000								
$^3\chi$	0.155	0.683	1.000							
κ_1	0.227	0.893	0.794	1.000						
κ_3	0.063	0.415	0.161	0.526	1.000					
J	−0.491	−0.458	−0.157	−0.207	0.338	1.000				
W	0.290	0.930	0.754	0.975	0.425	−0.409	1.000			
Te	−0.149	−0.761	−0.861	−0.933	−0.303	0.190	−0.899	1.000		
LUMO	−0.147	−0.060	−0.194	−0.151	0.044	0.348	−0.185	0.276	1.000	
$pMIC_{af}$	0.477	0.808	0.428	0.702	0.277	−0.367	0.740	−0.558	−0.001	1.000

Table 7 Comparison of observed and predicted antimicrobial activity obtained by *mt*-QSAR models.

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.59	1.43	0.17	1.69	1.62	0.07	1.63	1.48	0.15
2	1.65	1.45	0.20	1.75	1.76	−0.01	1.69	1.62	0.07
3	1.52	1.43	0.09	1.87	1.62	0.25	1.66	1.48	0.18
4	1.53	1.49	0.04	1.73	1.78	−0.04	1.61	1.57	0.04
5	1.43	1.49	−0.06	1.73	1.88	−0.14	1.55	1.71	−0.16
6	1.57	1.43	0.15	1.77	1.67	0.11	1.65	1.48	0.17
7	1.41	1.42	−0.01	1.56	1.64	−0.08	1.47	1.52	−0.05
8	1.52	1.43	0.09	1.42	1.62	-0.20	1.48	1.48	0.00
9	1.41	1.42	−0.02	1.71	1.71	0.00	1.53	1.52	0.00
10	1.37	1.36	0.01	1.67	1.53	0.14	1.49	1.43	0.06
11	1.53	1.45	0.08	1.58	1.68	-0.09	1.55	1.57	−0.02
12	1.36	1.42	−0.06	1.91	1.86	0.05	1.58	1.59	−0.01
13	1.45	1.49	−0.04	1.90	1.76	0.14	1.63	1.62	0.01
14	1.39	1.43	−0.03	1.54	1.56	−0.02	1.45	1.48	−0.03
15	1.53	1.49	0.04	1.58	1.66	−0.08	1.55	1.57	−0.02
16	1.42	1.39	0.03	1.57	1.62	−0.05	1.48	1.48	0.00
17	1.37	1.43	−0.05	1.62	1.67	−0.04	1.47	1.48	−0.01
18	1.41	1.42	−0.01	1.71	1.65	0.06	1.53	1.52	0.01
19	1.36	1.42	−0.06	1.51	1.65	−0.14	1.42	1.52	−0.10
20	1.35	1.32	0.03	1.35	1.39	−0.04	1.35	1.39	−0.03
21	1.39	1.39	0.00	1.69	1.56	0.14	1.51	1.48	0.03

antimicrobial activity of the synthesized *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides are governed by the steric parameter, molar refractivity (MR) and topological parameters, Kier's first order shape index (κ_1) third order molecular connectivity index ($^3\chi$).

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. (2005) who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range. 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 (Table 3) observed in the antimicrobial activity data justifies its use in QSAR studies.

4. Conclusion

In the present study, a series of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides (**1–21**) was synthesized (Scheme 1) and screened for its *in vitro* antimicrobial and anticancer potentials. The results of antimicrobial studies indicated the synthesized compounds were more potent toward *C. albicans* than other microbial strains tested and compound **6** was found to be the most active antifungal agent ($pMIC_{ca}$ = 2.07 μM/ml). Anticancer activity revealed that compound **14** (IC_{50} = 37.71 μM) was found to be the most potent. The results of QSAR studies demonstrated the importance of steric parameter, molar refractivity (MR), topological parameters, third order molecular connectivity index ($^3\chi$), Kier's first order shape index (κ_1) in describing the antimicrobial activity of *N*′-(substituted)-4-(butan-2-ylideneamino)benzohydrazides.

References

Bajaj, S., Sambi, S.S., Madan, A.K., 2005. Prediction of anti-inflammatory activity of *N*-arylanthranilic acids: computational approach using refined Zagreb indices. Croat. Chem. Acta 2, 165–174.

Bhat, M.A., Al-omar, M.A., 2011. Synthesis, characterization and *in vivo* anticonvulsant and neurotoxicity screening of Schiff bases of phthalimide. Acta Pol. Pharm. 68, 375–380.

Cappucino, J.G., Sherman, N., 1999. Microbiology – A Laboratory Manual. Addison Wesley, California (p. 263).

Cruz-Monteagudo, M., Gonzalez-Diaz, H., Aguero-Chapin, G., Santana, L., Borges, F., Dominguez, E.R., Podda, G., Uriarte, E., 2007. Computational chemistry development of a unified free energy Markov model for the distribution of 1300 chemicals to 38 different environmental or biological systems. J. Comput. Chem. 11, 1909–1923.

Furusjo, E., Svenson, A., Rahmberg, M., Andersson, M., 2006. The importance of outlier detection and training set selection for reliable environmental QSAR predictions. Chemosphere 63, 99–108.

Golbraikh, A., Tropsha, A., 2002. Beware of q^2! J. Mol. Graphics Modell. 20, 269–276.

Gonzalez-Diaz, H., Prado-Prado, F.J., 2008. Unified QSAR and network-based computational chemistry approach to antimicrobials, part 1: multispecies activity models for antifungals. J. Comput. Chem. 4, 656–667.

Gonzalez-Diaz, H., Vilar, S., Santana, L., Uriarte, E., 2007. Medicinal chemistry and bioinformatics – current trends in drugs discovery with networks topological indices. Curr. Top. Med. Chem. 10, 1015–1029.

Gonzalez-Diaz, H., Gonzalez-Diaz, Y., Santana, L., Ubeira, F.M., Uriarte, E., 2008. Networks and connectivity indices. Proteomics 4, 750–778.

Gupta, S.P., Kumar, A.N., Nagazappa, A.N., Kumar, D., Kumaran, S., 2003. A quantitative structure-activity relationship study on a novel class of calcium-entry blockers: 1-[(4-(aminoalkoxy)phenyl)sulphonyl]indolizines. J. Med. Chem. 38, 867–873.

Hansch, C., Fujita, T., 1964. A method for the correlation of biological activity and chemical structure. J. Am. Chem. Soc. 86, 1616–1626.

Hou, Y.P., Sun, J., Pang, Z.H., Lv, P.C., Li, D.D., Yan, L., Zheng, H.J., Zheng, E.X., Zhao, J., Zhu, H.L., 2011. Synthesis and antitumor activity of 1,2,4-triazoles having 1,4-benzodioxan fragment as a novel class of potent methionine aminopeptidase type II inhibitors. Bioorg. Med. Chem. 19, 5948–5954.

Hyperchem 6.0, 1993. Hypercube, Inc., Florida.

Judge, V., Narasimhan, B., Ahuja, M., Sriram, D., Yogeeswari, P., Clercq, E.D., Pannecouque, C., Balzarini, J., 2012a. Synthesis, antimycobacterial, antiviral, antimicrobial activity and QSAR studies of isonicotinic acid-1-(substituted phenyl)-ethylidene/cycloheptylidene hydrazides. Med. Chem. Res. 21 (8), 1935–1952.

Judge, V., Narasimhan, B., Ahuja, M., Sriram, D., Yogeeswari, P., Clercq, E.D., Pannecouque, C., Balzarini, J., 2012b. Isonicotinic acid hydrazide derivatives: synthesis, antimicrobial activity and QSAR studies. Med. Chem. Res. 21 (7), 1451–1470.

Kashaw, S.K., Kumar, A., Mishra, V., 2011. QSAR of 1,3,5-trisubstituted pyrazoline derivative as monoamine oxidase inhibitors. Asian J. Chem. 23, 4377–4379.

Kier, L.B., Hall, L.H., Devillers, J., Balaban, A.T. (Eds.), 1999. In Topological Indices and Related Descriptors in QSAR and QSPR. Gordon and Breach Sci., Amsterdam, pp. 455–489.

Kumar, A., Narasimhan, B., Kumar, D., 2007. Synthesis, antimicrobial, and QSAR studies of substituted benzamides. Bioorg. Med. Chem. 15, 4113–4124.

Lather, V., Madan, A.K., 2005. Topological models for the prediction of anti-HIV activity of dihydro(alkylthio)(naphthylmethyl)oxopyrimidines. Bioorg. Med. Chem. 13, 1599–1604.

Malhotra, M., Sanduja, M., Samad, A., Deep, A., 2011. New oxadiazole derivatives of isonicotinohydrazide in the search for antimicrobial agents: synthesis and *in vitro* evaluation. J. Serb. Chem. Soc. 76, 1–15.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Mostafa, Y.A.H., Mostafa, A.H., Radwan, A.A., Kfafy, A.E.H.N., 2008. Synthesis and antimicrobial activity of certain new 1,2,4-Triazolo[1,5-a]Pyrimidine derivatives. Arch. Pharmacal Res. 31, 279–293.

Nandagokula, C., Shenoy, S., Poojary, B., Shetty, P., Vittal, S., Tangavelu, A., 2013. Synthesis, characterization, and biological evaluation of some *N*-aryl hydrazones and their 2,3-disubstituted-4thiazolidinone derivatives. Med. Chem. Res. 22, 253–266.

Narang, R., Narasimhan, B., Sharma, S., Sriram, D., Yogeeswari, P., Clercq, E.D., Pannecouque, C., Balzarini, J., 2012. Synthesis, antimycobacterial, antiviral, antimicrobial activity and QSAR studies of nicotinic acid benzylidene hydrazide derivatives. Med. Chem. Res. 21, 1557–1576.

Narasimhan, B., Judge, V., Narang, R., Ohlan, S., Ohlan, R., 2007. Quantitative structure–activity relationship studies for prediction of antimicrobial activity of synthesized 2,4-hexadienoic acid derivatives. Bioorg. Med. Chem. Lett. 17, 5836–5845.

Nobuyoshi, H., Kohji, A., Eriko, S., Rie, H., Osamu, I., 2010. Anticonvulsant effects of methyl ethyl ketone and diethyl ketone in several types of mouse seizure models. Eur. J. Pharmacol. 642, 66–71.

Pharmacopoeia of India, 2007. Controller of Publications, Ministry of Health Department, Govt. of India, New Delhi, 1, 37.

Ramachandran, S., Maheswari, V.U., 2011. Synthesis of analgesic and ulcerogenic evaluation of some novel Schiff and Mannich bases of isatin derivatives. Int. J. Pharm. Biol. Sci. 2 (1), 251–260.

Rosu, T., Negoiu, M., Pasculescu, S., Pahontu, E., Poirier, D., Gulea, A., 2010. Metal-based biologically active agents: synthesis, characterization, antibacterial and antileukemia activity evaluation of Cu(II), V(IV) and Ni(II) complexes with antipyrine-derived compounds. Eur. J. Med. Chem. 45, 774–789.

Sharma, S.K., Kumar, P., Narasimhan, B., Ramasamy, K., Mani, V., Mishra, R.K., Majeed, A.B.A., 2012. Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1H-indol-3-yl]-2-oxo/thi-oxo1,2,3,4-tetrahydro pyrimidine-5-carboxylicacid ethyl esters. Eur. J. Med. Chem. 48, 16–25.

Sigroha, S., Narasimhan, B., Kumar, P., Khatkar, A., Ramasamy, K., Mani, V., Mishra, R.K., Majeed, A.B.A., 2012. Design, synthesis, antimicrobial, anticancer evaluation, and QSAR studies of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones. Med. Chem. Res. 21, 3863–3875.

Sondhi, S.M., Dinodia, M., Jain, S., Kumar, A., 2009. Synthesis of biologically active novel *bis* Schiff base, *bis* guanidine derivatives. Indian J. Chem. 48, 1128–1136.

Sortino, M., Delgado, P., Jaurez, S., Quiroga, J., Abonia, R., Insuasey, B., Nogueras, M., Rodero, L., Garibotto, F.M., Enriz, R.D., Zacchino, S.A., 2007. Synthesis and antifungal activity of (Z)-5-arylidenerhodanines. Bioorg. Med. Chem. 15, 484–494.

SPSS for Windows, 1999. Version 10.05, SPSS Inc., Bangalore, India.

TSAR 3D Version 3.3, 2000. Oxford Molecular Limited.