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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimycobacterial, antiviral, antimicrobial activities, and QSAR studies of nicotinic acid benzylidene hydrazide derivatives

Rakesh Narang · Balasubramanian Narasimhan · Sunil Sharma · Dharmarajan Sriram · Perumal Yogeeswari · Erik De Clercq · Christophe Pannecouque · Jan Balzarini

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Abstract A series of nicotinic acid benzylidene hydrazide derivatives (**1–18**) was synthesized and tested in vitro for biological evaluations. The antimycobacterial activity results indicated that the presence of electron-withdrawing halogen groups at *para* position of the phenyl ring improved their activity. The results of antiviral evaluation depicted that none of the synthesized derivatives inhibited the replication of viruses at subtoxic concentration. Further, the antimicrobial screening results indicated that compounds having OCH_3 and NO_2 substituents were the most active ones. QSAR investigations revealed that multi-target QSAR models were effective in describing the antimicrobial activity.

Keywords Hydrazides · Antimycobacterial · Antiviral · Antimicrobial · QSAR

R. Narang · S. Sharma
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India

D. Sriram · P. Yogeeswari
Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Hyderabad 500078, India

E. De Clercq · C. Pannecouque · J. Balzarini
Laboratory of Virology & Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium

B. Narasimhan (✉)
Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124001, India
e-mail: naru2000us@yahoo.com

Introduction

Tuberculosis (TB) is presently regarded as the most dangerous infective disease world-wide and one of the major AIDS-associated infections. At present, according to statistics, TB kills four people every minute somewhere in the world and accounts for about two million deaths per year. It is estimated that one-third of the world's population is currently infected with the TB bacillus and 30 million people will die in the next 10 years (Lourenco *et al*., 2008). The simultaneous presence of HIV infection, the spread of drug-resistant strains of *Mycobacterium tuberculosis* (MTB) and the poor compliance with the lengthy complex therapies often complicate the treatment of tuberculosis (Bedia *et al*., 2006).

Acquired immunodeficiency syndrome (AIDS) is a leading cause of death worldwide. An estimated 39.5 million people are living with human immunodeficiency virus (HIV). Between them about 5,30,000 children less than 15 years of age were infected mainly through mother-to-children transmission. In 2006, 2.9 million people died of AIDS-related illness (Pirhadi and Ghasem, 2010). HIV-1 is the causative organism for AIDS belongs to the *lenti-viridae* family of pathogenic retroviruses. A number of HIV-1 protease inhibitors (indinavir, ritonavir, nelfinavir, etc.) are marketed as anti-HIV drugs. Recently, these were combined with nucleoside and/or non-nucleoside reverse transcriptase inhibitors for highly active antiretroviral therapy (HAART). However, none of the available drugs or treatment is completely devoid of untoward effects. Phenotypic resistance and cross resistance also restricted their uses (Halder and Jha, 2010). Hence, search for new more active as well as less toxic HIV inhibitors always remain an important and challenging task for medicinal chemists.

During the past decades, the human population affected with life-threatening infectious diseases caused by

multidrug-resistant Gram-positive and Gram-negative pathogenic bacteria increased at an alarming level around the world (Bayrak *et al*., 2009). Hence, the development of newer antimicrobial agents is essential to overcome the rapidly developing drug resistance and side effects.

Quantitative structure–activity relationships (QSAR) attempt to find relationships between the molecular properties of molecules and the biological responses they elicit when applied to a biological system. The advancements in computer hardware and software now allow the molecular properties of molecules to be easily estimated without the need to synthesize the molecules in question. Thus, the use of predictive computational (in silico) QSAR models allows the biological properties of virtual structures to be predicted, and a more informed choice of target to be selected for synthesis (Painea *et al*., 2010). The use of computational approaches for the estimation of the activity of various molecules as drug candidates prior to their synthesis can save the resources and accelerate the drug discovery procedure (Zhoua *et al*., 2007).

Hydrazide–hydrazone derivatives have been claimed to possess antimicrobial (Bayrak *et al*., 2009), antimycobacterial (Nayyar *et al*., 2007), antitumour (Lembege *et al*., 2008), anti-inflammatory (Bhandari *et al*., 2008), trypanocidal (Leite *et al*., 2006), antiviral (Osama *et al*., 2009), and antimalarial activities (Gemma *et al*., 2006). Further, careful literature survey for functional groups which could be considered as pharmacophores for the antitubercular activities revealed that the hydrazone moiety is common among most of the antitubercular agents (Joshi *et al*., 2008; Sriram *et al*., 2006). Therefore, the target compounds were rationalized so as to comprise the hydrazone pharmacophores that are believed to be responsible for the biological significance of some relevant chemotherapeutic agents. The substitution pattern of such hydrazone derivatives was carefully selected so as to confer a different electronic environment to the molecules. We described previously the antimicrobial profile of hydrazide–hydrazone derivatives of cinnamic acid, benzoic acid, and their derivatives, some of which have exhibited a remarkable antimicrobial activity (Kumar *et al*., 2008, 2009, 2010a, b). Motivated by the aforementioned findings and with the aim of obtaining new antimicrobial compounds we hereby report the synthesis, antimycobacterial, antiviral, antibacterial, antifungal, and QSAR studies of nicotinic acid benzylidene hydrazide derivatives.

Experimental

Melting points were determined in open glass capillaries on a sonar melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel-G) and purity of compounds was ascertained by single spot TLC. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer (400 MHz) at 298 K, in appropriate deuterated solvents. Chemical shifts were reported as δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded as KBr pellet on Perkin Elmer FTIR spectrometer. The wave number is given in cm^{-1}.

General procedure for synthesis of nicotinic acid benzylidene hydrazides

The mixture of nicotinic acid (0.08 mol) and ethanol (0.80 mol) was refluxed in the presence of a few drops of concentrated sulfuric acid till the completion of reaction. Then the reaction mixture was added to 200 ml ice cold water and the residual acid was removed by treatment with sodium bicarbonate. The ester formed was extracted with ether. The ether layer was separated which on evaporation yielded the crude ester, which was recrystallized from alcohol. Hydrazine–hydrate (99%) (0.015 mol) was added in ethanolic solution of ester (0.01 mol) synthesized above and refluxed for 8 h. The reaction mixture was then cooled and the precipitates of acid hydrazide were filtered off, washed with water, dried, and recrystallized from ethanol. Then the solution of acid hydrazide (0.01 mol) and appropriate aldehyde (0.01 mol) in ethanol was refluxed for 5–6 h. The precipitated title compounds were filtered off, washed with water, and recrystallized from ethanol.

*Nicotinic acid benzylidene-hydrazide (**1**)*

Mp (°C) 93–96; Yield—74.20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 11.91 (s, 1H, NH), 7.35–9.15 (m, 10H, ArH); IR (KBr pellets): cm^{-1} 3247.78 (N–H str.), 3064.53 (C–H str., aromatic), 2980.00 (C–H str., aliphatic), 1655.27 (C=O str.), 1446.90 (C=C str., aromatic), 706.50 (ring bending, 3-substituted pyridine).

*Nicotinic acid (3-chloro-benzylidene)-hydrazide (**3**)*

Mp (°C) 141–144; Yield—65.70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 12.01 (s, 1H, NH), 7.33–9.16 (m, 9H, ArH); IR (KBr pellets): cm^{-1} 3181.00 (N–H str.), 3022.00 (C–H str., aromatic), 2975.58 (C–H str., aliphatic), 1669.53 (C=O str.), 1471.17 (C=C str., aromatic), 798.83 (C–H out of plane bending, 3-substituted pyridine), 722.44 (C–Cl str.), 703.47 (ring bending, 3-substituted pyridine).

*Nicotinic acid (3-bromo-benzylidene)-hydrazide (**6**)*

Mp (°C) 106–109; Yield—68.90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 12.06 (s, 1H, NH), 7.31–9.14 (m, 9H,

ArH); IR (KBr pellets): cm^{-1} 3178.90 (N–H str.), 3020.00 (C–H str., aromatic), 2910.10 (C–H str., aliphatic), 1667.23 (C=O str.), 1472.88 (C=C str., aromatic), 795.83 (C–H out of plane bending, 3-substituted pyridine), 705.25 (ring bending, 3-substituted pyridine), 638.05 (C–Br str.).

*Nicotinic acid (4-fluoro-benzylidene)-hydrazide (**8**)*

Mp (°C) 183–186; Yield—76.30%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 11.97 (s, 1H, NH), 9.14 (s, 1H, CH of -N=CH-), 7.05–8.75 (m, 8H, ArH); IR (KBr pellets): cm^{-1} 3183.46 (N–H str.), 3010.00 (C–H str., aromatic), 2951.50 (C–H str., aliphatic), 1650.81 (C=O str.), 1509.55 (C=C str., aromatic), 1027.60 (C–F str.), 838.57 (C–H out of plane bending, 3-substituted pyridine), 702.08 (ring bending, 3-substituted pyridine).

*Nicotinic acid (2-nitro-benzylidene)-hydrazide (**9**)*

Mp (°C) 178–181; Yield—73.3%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 12.39 (s, 1H, NH), 9.11 (s, 1H, N–CH–C of nicotinic acid), 8.79–8.88 (d, 2H, central CH of N–CH–CH and C–CH–CH of nicotinyl ring), 7.59–8.31 (m, 6H, ArH); IR (KBr pellets): cm^{-1} 3189.94 (N–H str.), 3050.95 (C–H str., aromatic), 2921.72 (C–H str., aliphatic), 1667.92 (C=O str.), 1518.55 (NO_2 asym. str., aromatic nitro group), 1343.55 (NO_2 sym. str., aromatic nitro group),1476.43 (C=C str., aromatic), 704.92 (ring bending, 3-substituted pyridine).

*Nicotinic acid (3-nitro-benzylidene)-hydrazide (**10**)*

Mp (°C) 169–172; Yield—74.80%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 12.32 (s, 1H, NH), 9.09 (s, 1H, N–CH–C of nicotinic acid), 8.79–8.80 (d, 1H, central CH of N–CH–CH of pyridine ring), 8.56–8.58 (d, 1H, Central CH of C–CH–CH pyridine ring), 7.58–8.30 (m, 6H, ArH); IR (KBr pellets): cm^{-1} 3220.22 (N–H str.), 3061.68 (C–H str., aromatic), 2911.00 (C–H str., aliphatic), 1676.54 (C=O str.), 1531.63 (NO_2 asym. str., aromatic nitro group), 1357.62 (NO_2 sym. str., aromatic nitro group), 1476.29 (C=C str., aromatic), 709.21 (ring bending, 3-substituted pyridine).

*Nicotinic acid (2-hydroxy-benzylidene)-hydrazide (**12**)*

Mp (°C) 168–171; Yield—58.90%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 12.29 (s, 1H, NH), 11.98 (s, 1H, OH), 9.12 (s, 1H, N–CH–C of pyridine ring), 8.79–8.80 (d, 1H, central CH of N–CH of pyridine ring), 8.68 (s, 1H, CH of N=CH), 8.29–8.32 (d, 1H, central CH of C–CH–CH of pyridine ring), 6.81–7.62 (m, 5H, ArH); IR (KBr pellets): cm^{-1} 3449.29 (O–H str.), 3268.71 (N–H str.), 3043.47 (C–H str., aromatic), 2950.00 (C–H str., aliphatic), 1682.94 (C=O str.), 1480.76 (C=C str., aromatic), 703.03 (ring bending, 3-substituted pyridine).

*Nicotinic acid (4-hydroxy-benzylidene)-hydrazide (**14**)*

Mp (°C) above 240; Yield—73.70%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 11.97 (s, 1H, NH), 9.14 (s, 1H, CH of -N=CH-), 7.05–8.75 (m, 8H, ArH); IR (KBr pellets): cm^{-1} 3390.22 (O–H str.), 3180.00 (N–H str.), 3070.52 (C–H str., aromatic), 2950.50 (C–H str., aliphatic), 1654.94 (C=O str.), 1515.29 (C=C str., aromatic), 818.52 (C–H out of plane bending, 3-substituted pyridine), 692.32 (ring bending, 3-substituted pyridine).

*Nicotinic acid (4-methoxy-benzylidene)-hydrazide (**15**)*

Mp (°C) 141–144; Yield—72.50%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 11.90 (s, 1H, NH), 9.06 (s, 1H, N–CH–C of nicotinic acid), 8.75–8.77 (d, 1H, central CH of N–CH–CH of pyridine ring), 8.39 (s, 1H, CH of N=CH), 8.23–8.26 (d, 1H, central CH of C–CH–CH pyridine ring), 6.99–7.77 (m, 5H, ArH), 3.36–3.82 (s, 3H, OCH_3); IR (KBr pellets): cm^{-1} 3255.86 (N–H str.), 3091.47 (C–H str., aromatic), 1681.57 (C=O str.), 1421.20 (C=C str., aromatic), 1295.45 (asym. C–O–C str.), 1128.95 (sym. C–O–C str), 702.14 (ring bending, 3-substituted pyridine).

*Nicotinic acid (3-methoxy-4-hydroxy-benzylidene)-hydrazide (**17**)*

Mp (°C) 195–198; Yield—68.90%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 11.85 (s, 1H, NH), 9.62 (s, 1H, OH), 9.26 (s, 1H, N–CH–C of pyridine ring), 8.75- 8.76 (d, 1H, central CH of N–CH of pyridine ring), 9.05 (s, 1H, CH of N=CH), 8.22–8.25 (d, 1H, central CH of C–CH–CH of pyridine ring), 6.83–7.58 (m, 4H, ArH), 3.37–3.83 (s, 3H, OCH_3); IR (KBr pellets): cm^{-1} 3449.00 (O–H str.), 3248.50 (N–H str.), 3050.53 (C–H str., aromatic), 1663.65 (C=O str.), 1451.43 (C=C str., aromatic), 1276.42 (asym. C–O–C str.), 1125.89 (sym. C–O–C str), 697.06 (ring bending, 3-substituted pyridine).

Evaluation of antimycobacterial activity

All compounds were screened for their in vitro antimycobacterial activity against MTB, in Middlebrook 7H11agar medium supplemented with OADC (contain Oleic acid and Dextrose as Carbon sources) by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards (1995) for the determination of MIC in triplicate. The minimum inhibitory concentration (MIC) is defined as the minimum concentration

of compound required to give complete inhibition of bacterial growth.

Evaluation of antimicrobial activity (determination of minimum inhibitory concentration)

Antibacterial assay

A 24-h fresh culture was obtained by inoculation of respective bacteria in double strength nutrient broth-I.P. followed by incubation at 37 ± 1°C. The stock solution (100 µg/ml) of synthesized substituted benzylidene hydrazide derivatives was serially diluted in tube (Cappucino and Sherman, 1999) containing 1 ml of sterile double strength nutrient broth I.P. (Pharmacopoeia of India, 2007) to get a concentration of 50–3.125 µg/ml and then inoculated with 100 µl of suspension of respective organisms in sterile saline (*Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*). The inoculated tubes were incubated at 37 ± 1°C for 24 h and minimum inhibitory concentrations (MIC) were determined.

Antifungal assay

The antifungal activity of synthesized hydrazide derivatives against the fungal species *Candida albicans* and *Aspergillus niger* was determined by serial dilution method similar to antibacterial assay using Sabouraud dextrose broth-I.P. (Pharmacopoeia of India, 2007). The inoculated tubes were incubated at 37 ± 1°C and 25 ± 1°C for a period of 2 and 7 days in case of *C. albicans* and *A. niger*, respectively.

QSAR studies

Data set is the set of molecules whose biological activity is regressed with its molecular descriptor values. Our data set consisted of 18 hydrazide analogs of nicotinic acid, synthesized and biologically evaluated, for antimicrobial activity by the tube dilution method. Descriptor is any molecular property which is characteristic of a molecule and can be utilized to determine new QSAR. The number of descriptors selected for this study fell into four categories, viz. electronic, steric, lipophilic, and topological (Table 7). The structures of benzylidene hydrazide derivatives are first pre-optimized with the Molecular Mechanics Force Field (MM^{+}) procedure included in Hyperchem 6.03 (1993) and the resulting geometries are further refined by means of the semiempirical method PM3 (parametric Method-3). The lowest energy structure was used for each molecule to calculate the 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).

Cross-validation

The predictive powers of the equations were validated by leave-one-out (LOO) cross-validation method (Schaper, 1999), where a model is built with $N - 1$ compounds and Nth compound is predicted. Each compound is left out of the model derivation and predicted in turn. An indication of the performance is obtained from cross-validated (or predictive q^2) method which is defined as

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

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

Calculation of statistical parameters

The selected models were also validated by the calculation of following statistical parameters (Mandloi *et al*., 2005; Pinheiro *et al*., 2004): probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), standard error of prediction (SEP), quality value (Q), root mean square of errors (RMSE), absolute average error (e).

These parameters were calculated from the following equations.

$$\text{PE} = 2\left(1 - r^2\right)/3\sqrt{n}$$

where r is the correlation coefficient and n is the number of compounds used.

$$\text{LSE} = \sum \left(Y_{\text{obs}} - Y_{\text{calc}}\right)^2$$

where Y_{obs} and Y_{calc} are the observed and calculated values, respectively.

$$\text{LOF} = \text{LSE} \Big/ \{1 - (C + d \cdot p/n)\}^2$$

where LSE is the least square error, C is the number of descriptors $+1$, p is the number of independent parameters, n is the number of compounds used, d is the smoothing parameter which controls the bias in the scoring factor between equations with different number of terms and was kept 1.0.

$$\text{SEP} = \sqrt{\text{LSE}/n}$$

The quality value, Q is given by

$$Q = r/\text{SE}$$

where Q is the quality value, r is the correlation coefficient, and SE is the standard error.

Variation inflation factor (Kumar *et al*., 2006a, b) is employed to determine the multicolinearity between the physicochemical parameters. The VIF value is calculated as

$$\mathrm{VIF} = 1/1 - r^2$$

where r^2 is the squared multiple correlation coefficient of one parameter effect on the remaining parameter. VIF values greater than 5 indicate the presence of unacceptably large multicolinearity between parameters in the correlation.

The root mean square of errors RMSE is calculated from below equation.

$$\mathrm{RMSE} = \sqrt{\left\{\sum (Y_{\mathrm{cal}} - Y_{\mathrm{obs}})^2 \Big/ n\right\}}$$

To illustrate the predictive accuracy more explicitly, the e (absolute average error) is defined by following equation.

$$e = \sum |Y_{\mathrm{cal}} - Y_{\mathrm{obs}}| \Big/ n$$

Evaluation of anti-HIV activity

The anti-HIV activity and cytotoxicity were evaluated against wild-type HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels *et al*., 1988). Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/ml and infected with HIV at a multiplicity of infection of 0.02. Immediately after viral infection, 100 µl of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After 4-day incubation at 37°C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for their cytotoxic effects in uninfected MT-4 cell cultures.

Antiviral assays

The antiviral assays (except anti-human immunodeficiency virus (HIV) assays) were based on inhibition of virus-induced cytopathicity in HEL (herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus), Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 ($CCID_{50}$) of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (100, 20, 4 µg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

Results and discussion

Chemistry

The synthesis of the intermediate and target compounds (**1**–**18**) was performed according to reactions outlined in Scheme 1. The nicotinic acid was refluxed with ethanol in the presence of a few drops of sulfuric acid to yield the ethyl ester. The ethyl ester was refluxed with hydrazine hydrate in ethanol to yield the acid hydrazide, which was then condensed with corresponding aromatic aldehydes to yield the target substituted nicotinic acid benzylidene hydrazides. The physicochemical characteristics of synthesized compounds are presented in Table 1.

Compound	R_1	R_2	R_3	R_4
1	H	H	H	H
2	Cl	H	H	H
3	H	Cl	H	H
4	H	H	Cl	H
5	Br	H	H	H
6	H	Br	H	H
7	H	H	Br	H
8	H	H	F	H
9	NO_2	H	H	H
10	H	NO_2	H	H
11	H	H	NO_2	H
12	OH	H	H	H
13	H	OH	H	H
14	H	H	OH	H
15	H	H	OCH_3	H
16	OCH_3	H	H	OCH_3
17	H	OCH_3	OH	H
18	H	OC_2H_5	OH	H

^{1}H NMR and IR spectral data are in agreement with the proposed structures of synthesized compounds (**1**–**18**). The appearance of singlet signal ranging from δ 11.85 to 12.39 ppm in the synthesized compounds confirmed the presence of NH of hydrazide derivatives. The presence of aromatic protons was confirmed by the multiplet signal in the range of δ 6.50–9.50 ppm. In the synthesized derivatives one singlet and one doublet signal were observed at higher δ value, corresponding to C_1 and C_5 protons, respectively, as they are in close proximity of the

Scheme 1 Synthetic route followed to obtain target compounds

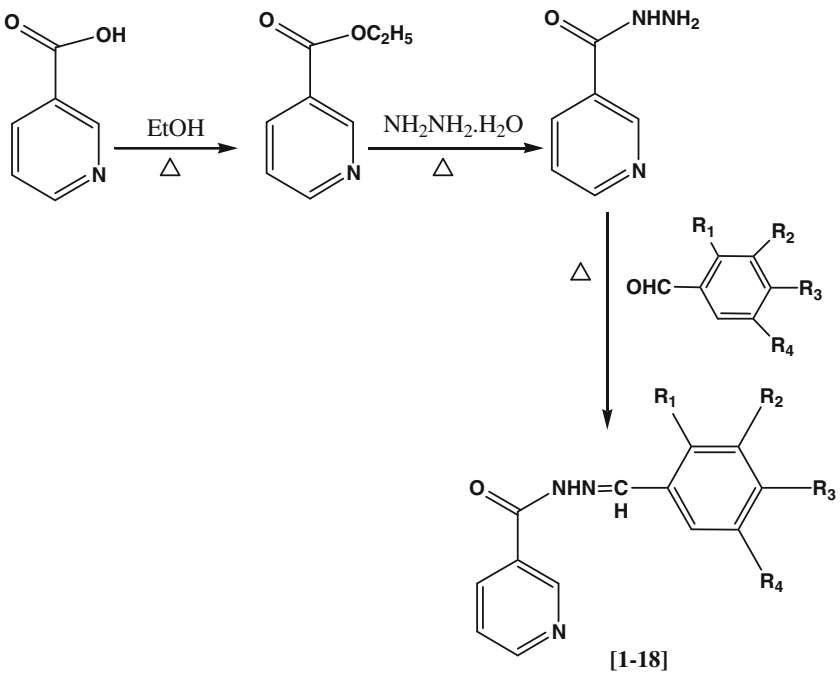

Table 1 Physicochemical characteristics and antimycobacterial activity of synthesized nicotinic acid benzylidene hydrazide derivatives

Compound	M. formula	M. weight	Mp (°C)	R_f^a	% yield	MIC *MTB-H37Rv* (μg/ml)
1	$C_{13}H_{11}N_3O$	225.27	93–96	0.74	74.2	>25
2	$C_{13}H_{10}N_3ClO$	259.71	141–144	0.71	65.7	>25
3	$C_{13}H_{10}N_3ClO$	259.71	108–111	0.68	81.2	>25
4	$C_{13}H_{10}N_3ClO$	259.71	179–182	0.58	84.9	25
5	$C_{13}H_{10}N_3BrO$	304.16	167–170	0.63	74.5	>25
6	$C_{13}H_{10}N_3BrO$	304.16	106–109	0.80	68.9	>25
7	$C_{13}H_{10}N_3BrO$	304.16	207–210	0.75	75.9	25
8	$C_{13}H_{10}N_3FO$	243.26	183–186	0.52	76.3	25
9	$C_{13}H_{10}N_4O_3$	270.27	178–181	0.48	73.3	>25
10	$C_{13}H_{10}N_4O_3$	270.27	169–172	0.67	74.8	>25
11	$C_{13}H_{10}N_4O_3$	270.27	Above 240	0.37	68.8	>25
12	$C_{13}H_{11}N_3O_2$	241.27	168–171	0.65	58.9	>25
13	$C_{13}H_{11}N_3O_2$	241.27	209–212	0.43	65.4	>25
14	$C_{13}H_{11}N_3O_2$	241.27	Above 240	0.56	73.7	>25
15	$C_{14}H_{13}N_3O_2$	255.30	141–144	0.49	72.5	>25
16	$C_{15}H_{15}N_3O_3$	285.33	176–179	0.66	80.5	>25
17	$C_{14}H_{13}N_3O_3$	271.30	195–198	0.50	68.9	>25
18	$C_{15}H_{15}N_3O_3$	285.33	167–170	0.67	64.5	>25
Isoniazid						0.10
Ethambutol						1.56
Ciprofloxacin						3.13

[a] Mobile phase—hexane: ethyl acetate (3:7)

electronegative nitrogen atom, as compared to the remaining two C_3 and C_4 protons of nicotinyl nucleus. The appearance of singlet signal of CH proton at δ 8.68 ppm and δ 8.39 ppm revealed the formation of N=CH bond in compounds **12** and **15**, respectively. In compounds **15** and **17**, singlet signal in the range of δ 3.36–3.83 ppm depicted the presence three protons of OCH_3 group. Further, the appearance singlet signals at δ 11.98 ppm and δ 9.62 ppm

corresponding of OH group confirmed the formation of compounds **12** and **17**. Moreover, high δ value of protons of compounds **9** and **10** as compared to proton of other synthesized derivatives confirmed the presence of electronegative NO_2 group which resulted in deshielding of protons. Furthermore, the absence of singlet signal corresponding to COOH in the NMR spectra of synthesized compounds (**1**–**18**) supports the complete reaction of nicotinic acid.

The presence of the C=O functional group was indicated by the appearance of a stretching band around 1650 cm^{-1}, which is the characteristic of an amide linkage. The appearance of IR band around 3200 cm^{-1} showed the presence of NH linkage of amide bond of hydrazide derivatives. The ring bending observed around vibrational frequency of 700 cm^{-1} indicated the presence of 3-substituted pyridine ring in the structure of synthesized compounds. The presence of peaks slightly above and below 3000 cm^{-1} showed the presence of an aromatic and aliphatic portion in the synthesized compounds, respectively. The appearance of C–Cl, C–Br, C–F, and O–H stretching bands at 722.44, 638.05, 1027.60, and 3390.22 cm^{-1} in compounds **3**, **6**, **8** and **14** depicted the presence of chloro, bromo, fluoro, and hydroxy groups in their structures, respectively. In compound **15**, stretching at 1128.95 cm^{-1} (symmetric C–O–C stretching) and 1295.45 cm^{-1} (asymmetric C–O–C stretching) revealed the presence of methoxy group of anisaldehyde. Further, the aromatic nitro stretching around 1350 cm^{-1} (symmetric NO_2 stretching) and 1520 cm^{-1} (asymmetric NO_2 stretching) depicted the presence of nitro functional group in synthesized compounds **9** and **10**.

Antimycobacterial activity

The in vitro antimycobacterial activity of synthesized compounds against MTB was carried out in Middlebrook 7H11agar medium supplemented with OADC by agar dilution method and the results are presented in Table 1. At the commencement of this study in the preliminary screening, nicotinic acid hydrazide and nicotinic acid hydrazone derivatives with an unsubstituted phenyl ring (compound **1**) displayed poor antimycobacterial activity with a MIC of >25 μg/ml. So we have taken compound **1** as a lead compound and planned to improve the antimycobacterial activity by making substitutions on phenyl ring. The first step toward lead optimization was incorporation of electron donating methoxy group (compound **15**) in the phenyl ring. The addition of OCH_3 group did not improve the antitubercular activity and value of MIC still remained >25 μg/ml as in compound **1**. Further, the introduction of hydroxyl group (**12**, **13**, and **14**) in the phenyl ring also did not alter antitubercular activity (MIC > 25 μg/ml). Then, we studied the antimycobacterial evaluation of nicotinic acid hydrazide derivatives having both OH and alkoxy groups (compounds **17** and **18**). All these changes resulted in substantial loss in activity (MIC > 25.0 μg/ml). Based on these results we have dropped the idea of introducing electron-donating groups in the phenyl ring. The next structural modification was introduction of electron-withdrawing groups, viz., NO_2, Cl, and Br in the phenyl ring. First, we planned to introduce electron-withdrawing nitro group (**9**, **10**, and **11**) into the phenyl ring. This change resulted in no improvement of antimycobacterial activity (MIC > 25.0 μg/ml). Then we decided to introduce an electron-withdrawing chloro group. The presence of chloro group at *para* position of phenyl ring improved the antimycobacterial potential (MIC = 25.0 μg/ml) of synthesized hydrazide derivative (compound **4**) in comparison to compounds having *ortho* and *meta* chloro groups (compounds **2** and **3**), evidenced by their MIC values presented in Table 1. Enhancement in activity proves that this modification is a step up toward the synthesis of a pharmacophore for antitubercular activity. On the basis of biological data, improvement in biological activity was observed with an increase in electro-negativity of the molecule due to the halogen group. Hence, we planned to introduce other halogen atoms (viz. fluoro and bromo) on the phenyl ring of the hydrazide derivatives. This modification also produced similar type of results as in case of chloro derivatives, *para*-substituted bromo (compound **7**) and fluoro derivatives (compound **8**) have shown good improvement in the antitubercular activity (MIC = 25.0 μg/ml) in comparison to *ortho*- and *meta*-substituted derivative (MIC > 25.0 μg/ml).

From the aforementioned results we can conclude that the presence of electron-withdrawing halogen groups (viz. chloro, bromo, and fluoro) at *para* position of the phenyl ring improves the antimycobacterial activity (compounds **4**, **7**, and **8**) as compared to the electron-donating groups in the synthesized nicotinic acid hydrazide derivatives. This is similar to the results observed by Mamolo *et al.* (2004), who stated that the presence of bromo and chloro substituents, at the *para* position of the benzene ring attached to imidazole improved the antitubercular activity.

Cytotoxic and antiviral activity

All the synthesized compounds were evaluated for antiviral activity and cytotoxicity. The inhibitory concentration of compounds was expressed as the concentration that caused 50% inhibition of viral cytopathogenicity (EC_{50}). The cytotoxic concentration (CC_{50}) of the compounds was monitored based on the growth of non-infected cells by the MTT method and corresponded to the compound concentration required to cause 50% cell death (Table 2) or on

microscopically visible alteration of cell morphology (Tables 3, 4, 5).

None of the synthesized derivatives inhibited the viral replication at subtoxic concentrations. Although in general most compounds were poorly cytotoxic, several compounds showed pronounced cytotoxicity. In particular, compounds **2**, **5** (for MT-4), **11**, and **12** (for MT-4, HEL, HeLa and Vero) proved highly cytotoxic.

It was interesting to note that the position of the different groups on the phenyl group is of crucial importance for eventual toxicity in the MT-4 cell cultures. The introduction of Cl, Br, and OH groups at *ortho* position (compounds **2**, **5**, and **12**) results in high toxicity (CC_{50}: ≥0.57, 0.22, and 0.61 μg/ml, respectively), whereas in *meta* (compounds **3**, **6**, and **13**) and *para* (compounds **4**, **7**, and **14)** position they cause poor toxicity (CC_{50}: ≥60 μg/ml) pointing to the *ortho* substitution of these groups as a prerequisite for cytotoxic activity. In contrary to above results, compound **11** having NO_2 group at *para* position in phenyl ring showed highest toxicity (CC_{50} = 0.1 μg/ml) among the synthesized derivatives.

Table 2 Cytostatic and anti-HIV activity of nicotinic acid hydrazide derivatives in MT-4 cells

Compound	EC_{50}^{a} (μg/ml)		CC_{50}^{b} (μg/ml)
	HIV-1(IIIB)	HIV-2 (ROD)	
1	>125	>125	>125
2	>0.57	>0.57	≥0.57
3	>62	>62	62 ± 14
4	>76	>76	76 ± 12
5	>0.22	>0.22	≥0.22
6	>66	>66	66 ± 1.0
7	>61	>61	61 ± 11
8	>125	>125	>125
9	>68	>68	68 ± 6.1
10	>54	>54	54 ± 6.8
11	>0.10	>0.10	0.10 ± 0.01
12	>0.61	>0.61	0.61 ± 0.22
13	>98	>98	98 ± 14
14	>104	>104	104 ± 10
15	>99	>99	99 ± 11
16	>13	>13	13 ± 0.8
17	>93	>93	≥93
18	>117	>117	≥117
Nevirapine	0.050 ± 0.011	>4.0	>4.0
Zidovudine	0.002 ± 0.001	0.0009 ± 0.0005	>25

[a] 50% Effective concentration or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cell cultures by 50%

[b] 50% Cytotoxic concentration or compound concentration required to reduce viability of the MT-4 cell cultures by 50%, using the MTT methodology

Antimicrobial activity

The synthesized nicotinic acid benzylidene-hydrazide derivatives were evaluated for their in vitro antibacterial activity against Gram-positive *S. aureus*, *B subtilis* and Gram-negative *E coli* and antifungal activity against *C. albicans* and *A niger* by tube dilution method. Double strength Nutrient broth I.P. and Sabouraud dextrose broth I.P. have been employed as media for growth of bacterial and fungal cells, respectively. The results of antimicrobial activity are presented in Table 6.

In case of *B. subtilis* nicotinic acid (2-nitro-benzylidene)-hydrazide (**9**) was found to be more active than the other synthesized derivatives with $pMIC_{bs}$ value of 1.94 (Table 6) which is comparable to the reference drug norfloxacin ($pMIC_{bs}$ = 2.61). Against *S. aureus* nicotinic acid (2,5-dimethoxy-benzylidene)-hydrazide (**16**) and nicotinic acid (3-ethoxy-4-hydroxy-benzylidene)-hydrazide (**18**) emerged as most active ones with $pMIC_{sa}$ values of 1.36 in comparison to other synthesized derivatives (Table 6). (3-Nitro-benzylidene)-hydrazide (**10**), (4-nitro-benzylidene)-hydrazide (**11**) and (2,5-dimethoxy-benzylidene)-hydrazide (**16**) derivatives were found to be most potent against the Gram-negative bacteria, *E. coli* having 1.94, 1.94 and 1.96 $pMIC_{ec}$ values, respectively (Table 6) which is comparable to the reference drug norfloxacin ($pMIC_{ec}$ = 2.61). Among the tested bacterial strains, Gram-negative *E. coli* showed relatively high sensitivity towards the tested compounds.

On the other hand, investigation of antifungal activity revealed that the nicotinic acid benzylidene-hydrazide derivatives having bromo group (**5**, **6**, and **7**) were able to produce good inhibitory activity against *C. albicans* having a $pMIC_{ca}$ value 1.39. For antifungal activity against *A. niger* nicotinic acid (3-bromo-benzylidene)-hydrazide (**6**), nicotinic acid (2-nitro-benzylidene)-hydrazide (**9**), nicotinic acid (2, 5-dimethoxy-benzylidene)-hydrazide (**16**), and nicotinic acid (3-ethoxy, 4-hydroxy-benzylidene)-hydrazide (**18**) have shown marked antifungal potential having $pMIC_{an}$ value greater than 1.93 as compared to other synthesized derivatives which is comparable to the reference drug fluconazole ($pMIC_{an}$ = 2.64). It can be seen from Table 6 that synthesized benzylidene hydrazides have higher antifungal potential as compared to antibacterial activity. Further, the results of antimicrobial activity revealed that compounds **9** and **10** displayed maximum inhibitory effect on the growth of tested bacterial and fungal strains of all the synthesized derivatives.

The obtained result revealed that the nature of substituents have a considerable impact on antibacterial and antifungal activities of target hydrazones and following structure activity relationship (SAR) can be deduced:

Table 3 Cytotoxicity and anti-DNA virus activity of synthesized nicotinic acid hydrazide derivatives in HEL cell cultures

Compound	CC_{50}^{a}	Minimum cytotoxic concentration[b] (μg/ml)	EC_{50}^{c} (μg/ml)					
			Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Herpes simplex virus-1 TK^{-} KOS ACV^{r}	VZV (OKA/07-1)	HCMV (AD-169/ Davis)
1	>100	>100	>100	>100	>100	>100	>100	>100
2	12	>100	>100	>100	>100	>100	>100	>100
3	53	>100	>100	>100	>100	>100	>100	>100
4	29	>100	>100	>100	>100	>100	>100	>100
5	2.6	100	>20	>20	>20	>20	51 to >100	>100
6	55	>100	>100	>100	>100	>100	>100	>100
7	48	>100	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100	>100	>100
9	46	>100	>100	>100	>100	>100	>100	>100
10	13	>100	>100	>100	>100	>100	>100	>20
11	<0.16	≥0.8	>0.8	>0.8	>0.8	>0.8	0.33 to >0.16	>0.16
12	0.42	≥4	>4	>4	>4	>4	2.2 to >4	>0.8
13	>100	>100	>100	>100	>100	>100	>100	>100
14	91	>100	>100	>100	>100	>100	54–100	>100
15	>100	>100	>100	>100	>100	>100	>100	>100
16	12	100	>20	>20	>20	>20	>20	>20
17	>100	100	>20	>20	>20	>20	>100	>20
18	63	100	>20	>20	>20	>20	70 to >100	>20

[a] 50% Cytostatic concentration to inhibit cell proliferation by 50%

[b] Required to cause a microscopically detectable alteration of normal cell morphology

[c] Required to reduce virus-induced cytopathicity by 50%

Structure activity relationship

1. The analysis of antimicrobial results indicated that the compounds having alkoxy groups (compounds **15**, **16**, **17**, and **18**) were endowed with high antifungal activity particularly against *A. niger*. The importance of electron-donating groups in enhancing the antifungal activity is supported by similar results observed by Emami *et al*. (2008).
2. The synthesized compounds derived from chlorobenzaldehyde (compounds **2**, **3**, and **4**) displayed greater activity as compared to those derived from unsubstituted benzaldehyde (compound **1**). This observation reveals the fact that presence of a chloro substituent enhances the antibacterial and antifungal potentials of the synthesized hydrazide derivatives. This fact is supported by the observations of Guven *et al*. (2007).
3. The presence of an electron-withdrawing NO_2 group in compounds **9**, **10**, and **11** make them highly potent antibacterial and antifungal agents. The role of an electron-withdrawing group in increasing the antimicrobial potency is similar to the results of Sharma *et al*. (2004).
4. It is noteworthy that introduction of OH group (compounds **12**, **13**, and **14**) in the phenyl residue leads to an increased antibacterial activity as compared to unsubstituted derivative (compound **1**). This observation reveals the fact that the presence of an OH group increases the antimicrobial potential probably by forming hydrogen bond with the target biomacromolecule. This fact is supported by the observations of Vicini *et al*. (2002).
5. The presence of electron-withdrawing bromo (compounds **5**, **6**, and **7**) and fluoro (compound **8**) groups in benzylidene moiety enhance the growth inhibition potency, particularly against *C. albicans*. This is similar to the results observed by Masunari and Tavares (2007) who stated that the presence of bromo and chloro substituents, at the *para* position of the benzene ring showed marked antibacterial activity against *S. aureus*.
6. It is interesting to note that the presence of both hydroxy and ethoxy groups (compound **18**) resulted in an enhanced antibacterial activity. In contrary, the presence of hydroxy and methoxy (compound **17**) groups, make it least active antibacterial of all the synthesized derivatives.

Table 4 Cytotoxicity and anti-RNA virus activity of synthesized nicotinic acid hydrazide derivatives in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}^{b} (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	100	>20	>20	>20
2	100	>20	>20	>20
3	>100	>100	>100	>100
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	≥100	>100	>100	>100
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	>100	>100
10	20	>4	>4	>4
11	0.8	>0.16	>0.16	0.16
12	4	>0.8	>0.8	>0.8
13	>100	>100	>100	>100
14	>100	>100	>100	>100
15	>100	>100	>100	>100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	>100	>100	>100	>100

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathicity by 50%

Table 5 Cytotoxicity and anti-RNA virus activity of synthesized nicotinic acid hydrazide derivatives in Vero cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}^{b} (μg/ml)				
		Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	34	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	≥100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100
10	≥100	>100	>100	>100	>100	>100
11	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8
12	4	>0.8	>0.8	>0.8	>0.8	>0.8
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathicity by 50%

7. The replacement of NH_2 group (compound NAH) with benzylidene ring (compounds **1–18**) led to a noticeable increase in antimicrobial activity of the synthesized compounds. This may be due to the increase in lipophilicity of the molecules which may allow them to easily penetrate the microbial membrane.

Table 6 Antibacterial and antifungal activities of synthesized nicotinic acid derivatives

Compound	$pMIC_{bs}$	$pMIC_{sa}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{b}$	$pMIC_{f}$	$pMIC_{am}$
1	0.95	0.35	1.26	1.26	1.86	0.85	1.56	1.14
2	1.02	1.32	1.02	1.32	1.32	1.12	1.32	1.20
3	1.02	1.32	1.02	1.32	1.92	1.12	1.62	1.32
4	1.02	1.32	1.32	1.32	1.92	1.22	1.62	1.38
5	1.39	1.09	1.39	1.39	1.69	1.29	1.54	1.39
6	1.39	1.09	1.39	1.39	1.99	1.29	1.69	1.45
7	1.39	1.09	1.39	1.39	1.39	1.29	1.39	1.33
8	1.29	1.29	1.29	1.29	1.59	1.29	1.44	1.35
9	1.94	1.33	1.33	1.33	1.94	1.54	1.64	1.58
10	1.64	1.33	1.94	1.33	1.64	1.64	1.49	1.58
11	1.33	1.03	1.94	1.33	1.33	1.44	1.33	1.40
12	1.59	1.29	1.29	1.29	1.29	1.39	1.29	1.35
13	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29
14	1.29	1.29	1.29	1.29	1.59	1.29	1.44	1.35
15	1.31	1.31	1.31	1.31	1.61	1.31	1.46	1.37
16[a]	1.36	1.36	1.96	1.36	1.96	1.56	1.66	1.60
17[a]	0.43	0.43	0.43	1.34	1.64	0.43	1.49	0.85
18[a]	1.36	1.36	1.06	1.36	1.96	1.26	1.66	1.42
SD[a]	0.27	0.25	0.26	0.04	0.26	0.18	0.13	0.12
NAH	1.04	0.74	0.74	1.04	1.04	0.84	1.04	0.92
Std.	2.61[b]	2.61[b]	2.61[b]	2.64[c]	2.64[c]	2.61	2.64	2.62

SD standard deviation, *NAH* nicotinic acid hydrazide

[a] Outliers, [b] Norfloxacin, [c] Fluconazole

8. The aforementioned results indicated the fact that different structural requirements are essential for a compound to be selected as antibacterial or antifungal agent. This is similar to the results obtained by Sortino *et al*. (2007). The SAR results are summarized in Fig. 1.

QSAR studies

Development of one-target QSAR model (ot-QSAR)

In order to understand the experimental antimicrobial data of 18 substituted benzylidene derivatives on theoretical basis, we established a quantitative structure activity relationship (QSAR) between their in vitro activity and descriptors coding for lipophilic, electronic, steric, and topological properties of the molecules under consideration using the linear free energy relationship model (LFER) described by Hansch and Fujita (1964). Biological activity data determined as MIC values was first transformed into pMIC values and used as a dependent variable in the QSAR study. The different molecular descriptors (independent variables) like log of octanol–water partition coefficient (log *P*), molar refractivity (MR), Kier's molecular connectivity ($^0\chi$, $^0\chi^v$, $^1\chi$, $^1\chi^v$, $^2\chi$, $^2\chi^v$) and shape (κ_1, κ_2, κ_3, $\kappa\alpha_1$, $\kappa\alpha_2$, $\kappa\alpha_3$) topological indices, Randic topological index (*R*), Balaban topological index (*B*), Wiener topological index (*W*), total energy (T_e), energies of highest occupied molecular orbital (HOMO), and lowest unoccupied molecular orbital (LUMO), dipole moment (μ) and electronic energy (Ele.E), calculated for benzylidene hydrazides are presented in Table 7 (Hansch and Fujita, 1964; Hansch *et al*., 1973; Kier and Hall, 1976; Randic, 1975; Balaban, 1982; Wiener, 1947; Randic, 1993). Units of the energies and dipole were electron volts (eV), and atomic units (a.u.), respectively (Dai *et al*., 1999).

In this study, compounds **16**, **17**, and **18** were removed as outliers as their presence resulted in very low correlation (*r*), whereas their removal improved the *r* value significantly ($r = 0.969$, Eq. 1), hence a data set of 15 benzylidene hydrazide derivatives was subjected to linear free energy regression analysis for model generation. The reference drugs norfloxacin and fluconazole were not included in model development as they belong to different structural series.

Depending on the intercorrelation among the total 28 independent descriptors (calculated using Molecular Mechanics Force Field (MM^+) procedure included in

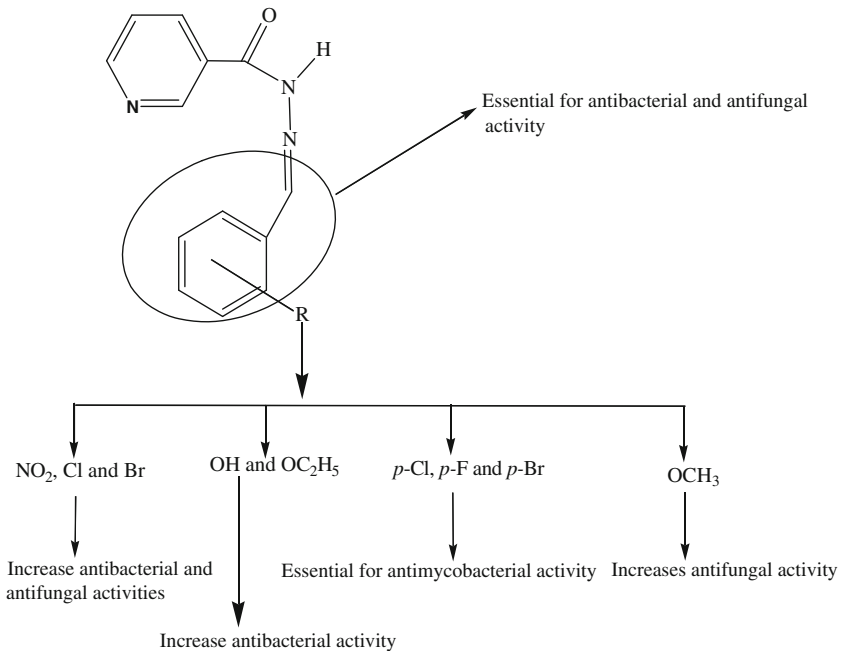

Fig. 1 Structural requirements for the antimycobacterial and antimicrobial activities of nicotinic acid hydrazide derivatives

Table 7 Values of selected descriptors used in the regression analysis

Compound	log *P*	MR	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$	κ_3	$\kappa\alpha_1$	*B*	LUMO	T.D.
1	2.58	65.56	9.08	5.15	6.81	3.35	4.64	11.94	1.93	−0.71	3.28
2	3.10	70.36	10.19	5.66	7.33	3.90	4.57	13.20	1.93	−0.72	3.86
3	3.10	70.36	10.19	5.65	7.44	3.97	4.90	13.20	1.93	−0.78	4.50
4	3.10	70.36	10.19	5.65	7.43	3.96	4.90	13.20	1.93	−0.80	4.08
5	3.37	73.18	11.00	6.06	7.33	4.34	4.57	13.38	1.93	−0.73	3.98
6	3.37	73.18	11.00	6.06	7.44	4.43	4.90	13.38	1.93	−0.79	4.42
7	3.37	73.18	11.00	6.06	7.43	4.43	4.90	13.38	1.93	−0.82	4.11
8	2.72	65.77	9.38	5.25	7.43	3.49	4.90	12.85	1.93	−0.79	4.35
9	2.53	72.88	10.26	5.65	8.26	3.76	5.06	14.44	2.02	−1.22	2.53
10	2.53	72.88	10.26	5.65	8.34	3.79	5.38	14.44	1.96	−1.13	8.87
11	2.53	72.88	10.26	5.65	8.33	3.79	5.38	14.44	1.97	−1.36	8.59
12	2.29	67.25	9.45	5.29	7.33	3.50	4.57	12.88	1.97	−0.79	4.41
13	2.29	67.25	9.45	5.28	7.44	3.54	4.90	12.88	1.94	−0.74	2.18
14	2.29	67.25	9.45	5.28	7.43	3.53	4.90	12.88	1.95	−0.71	2.79
15	2.33	72.02	10.41	5.67	7.60	3.71	5.12	13.85	1.98	−0.69	2.33

Hyperchem 6.03 (1993) and the resulting geometries are further refined by means of the semiempirical method PM3 (parametric Method-3) algorithm) and also their individual correlation with antimicrobial activities, different possible combinations of descriptors were subjected to linear regression (LR) and multiple linear regression (MLR) analysis. Out of hundreds of equations generated, some of the best QSAR equations having significant statistical qualities are selected and the descriptors used in these equations selected for the QSAR study. These equations were generated in stepwise manner by forward selection method starting with best single variable and adding further significant variable according to their contribution to the model that leads to the smallest standard deviation, until there is no other variable outside the equation that satisfies the selection criteria.

Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antifungal activity against *C. albicans* is presented in Table 8. The correlations of different molecular descriptors with antibacterial and antifungal activities are presented in Table 9. In general, both high and low colinearity was

observed between different parameters. A high interrelationship was observed between $^{0}\chi$ and $^{1}\chi$ ($r = 0.995$), $^{0}\chi^{v}$ and $^{1}\chi^{v}$ ($r = 0.995$), and low interrelationship was observed between $^{2}\chi^{v}$ and LUMO ($r = 0.003$). The correlation matrix indicated the predominance of topological parameters in describing the antibacterial and antifungal activity of synthesized compounds.

The antifungal activity of synthesized benzylidene hydrazides against *C. albicans* is explained by the topological parameter, valence first-order molecular connectivity index, $^{1}\chi^{v}$ (Eq. 1).

ot-QSAR model for antifungal activity against C. albicans

$$\mathrm{pMIC_{ca}} = 0.128\,^{1}\chi^{v} + 0.603 \quad (1)$$

$$n = 15, \quad r = 0.969, \quad q^2 = 0.925, \quad s = 0.010,$$
$$F = 205.04, \quad \mathrm{RMSE} = 0.00008, \quad e = 0.006,$$
$$\mathrm{SDEP} = 0.009.$$

Here and thereafter, n is the number of data points, r is the correlation coefficient, q^2 is the cross-validated r^2 obtained by LOO method, s is the standard error of the estimate, F is the Fischer statistics, RMSE is the root mean square of error, e is the absolute average error and SDEP is the standard deviation of error of prediction.

As the coefficient of $^{1}\chi^{v}$ in Eq. 1 is positive, therefore the antifungal activity against *C. albicans* will increase with increase in value of $^{1}\chi^{v}$. This is clearly evident that compounds **5**, **6**, and **7** having highest $^{1}\chi^{v}$ values, 6.06 (Table 7), have highest antifungal activity values, i.e., 1.39 (Table 6). Similarly, compounds **1**, **13**, and **14** having minimum $^{1}\chi^{v}$ values, i.e., 5.15, 5.28, and 5.28 (Table 7), possess minimum $\mathrm{pMIC_{ca}}$, i.e., 1.26, 1.29, and 1.29 (Table 6), respectively. Topological indices (e.g., $^{1}\chi^{v}$) are numerical quantifiers of molecular topology and are sensitive to bonding pattern, symmetry, content of heteroatom as well as degree of complexity of atomic neighborhoods. Since structure of a compound depends on connectivity of its constituent atoms, topological descriptors derived from information based upon connectivity can reveal the role of structural or sub-structural information of a molecule in estimating biological activity, viz., antimicrobial activity. Hence, topological descriptors developed for predicting physicochemical properties and biological activities of chemical substance can be used for drug design (Lather and Madan, 2005).

The QSAR model expressed by Eq. 1 was cross-validated by its high q^2 values ($q^2 = 0.925$) obtained by LOO method. The value of q^2 greater than 0.5 is the basic requirement for qualifying a QSAR model to be valid one (Golbraikh and Tropsha, 2002). The comparison of

Table 8 Correlation matrix of antifungal activity of benzylidene hydrazides against *C. albicans*

	log *P*	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$	$^{3}\chi$	$^{3}\chi^{v}$	$\kappa\alpha_1$	$\kappa\alpha_2$	$\kappa\alpha_3$	LUMO	HOMO	$\mathrm{pMIC_{ca}}$
log *P*	1															
MR	0.474	1														
$^{0}\chi$	−0.295	0.597	1													
$^{0}\chi^{v}$	0.712	0.929	0.293	1												
$^{1}\chi$	−0.329	0.589	0.995	0.279	1											
$^{1}\chi^{v}$	0.769	0.900	0.218	0.995	0.200	1										
$^{2}\chi$	−0.247	0.582	0.985	0.290	0.966	0.222	1									
$^{2}\chi^{v}$	0.870	0.779	0.024	0.941	−0.009	0.967	0.059	1								
$^{3}\chi$	−0.111	0.522	0.864	0.299	0.815	0.247	0.935	0.161	1							
$^{3}\chi^{v}$	0.898	0.658	−0.106	0.861	−0.149	0.899	−0.047	0.981	0.116	1						
$\kappa\alpha_1$	−0.028	0.805	0.951	0.568	0.942	0.500	0.936	0.316	0.838	0.180	1					
$\kappa\alpha_2$	0.129	0.897	0.784	0.729	0.803	0.664	0.728	0.465	0.574	0.306	0.909	1				
$\kappa\alpha_3$	0.328	0.714	0.482	0.675	0.465	0.635	0.536	0.587	0.623	0.550	0.635	0.696	1			
LUMO	0.194	−0.467	−0.869	−0.170	−0.850	−0.128	−0.893	0.003	−0.818	0.094	−0.782	−0.560	−0.360	1		
HOMO	−0.104	−0.529	−0.723	−0.299	−0.693	−0.285	−0.771	−0.199	−0.735	−0.124	−0.693	−0.502	−0.361	0.906	1	
$\mathrm{pMIC_{ca}}$	0.734	0.874	0.273	0.961	0.241	0.970	0.299	0.949	0.356	0.893	0.527	0.616	0.620	−0.228	−0.380	1

Table 9 Correlation of molecular descriptors with antimicrobial (antibacterial and antifungal) activity

Mol. descriptor	$pMIC_{bs}$	$pMIC_{sa}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{b}$	$pMIC_{f}$	$pMIC_{am}$
log *P*	−0.270	−0.087	−0.201	0.734	0.329	−0.266	0.428	−0.054
MR	0.391	0.224	0.426	0.874	0.177	0.495	0.302	0.605
$^{0}\chi$	0.671	0.414	0.696	0.273	−0.058	0.846	−0.015	0.790
$^{0}\chi^{v}$	0.221	0.185	0.209	0.961	0.200	0.291	0.337	0.429
$^{1}\chi^{v}$	0.179	0.136	0.169	0.970	0.215	0.229	0.353	0.379
$^{2}\chi$	0.653	0.436	0.721	0.299	−0.047	0.859	0.000	0.810
$^{2}\chi^{v}$	0.054	0.099	0.057	0.949	0.224	0.099	0.359	0.258
κ_3	0.356	0.243	0.743	0.146	0.050	0.636	0.070	0.632
$\kappa\alpha_1$	0.624	0.458	0.635	0.527	0.009	0.814	0.087	0.808
$\kappa\alpha_2$	0.449	0.286	0.504	0.616	0.133	0.589	0.221	0.656
$\kappa\alpha_3$	0.095	0.268	0.484	0.620	0.204	0.400	0.290	0.510
R	0.491	0.288	0.431	−0.146	−0.196	0.575	−0.211	0.444
B	0.772	0.278	0.285	−0.060	0.001	0.637	−0.008	0.596
W	0.383	0.251	0.384	−0.114	−0.157	0.484	−0.168	0.378
T_e	−0.655	−0.481	−0.669	−0.290	0.057	−0.856	0.011	−0.802
Ele.E	−0.715	−0.430	−0.638	−0.231	0.058	−0.847	0.022	−0.788
LUMO	−0.563	−0.087	−0.744	−0.228	0.019	−0.665	−0.016	−0.634
HOMO	−0.471	−0.006	−0.600	−0.380	−0.178	−0.515	−0.229	−0.590
T.D.	0.148	0.000	0.796	0.258	−0.143	0.449	−0.099	0.377

observed and predicted antibacterial activities is presented in Table 10. The predictability of Eq. 1 is evidenced by the low residual values observed in Table 10 as well by the plot of predicted $pMIC_{ca}$ against observed $pMIC_{ca}$ (Fig. 2). Further, the plot of observed $pMIC_{ca}$ versus residual $pMIC_{ca}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of residuals was observed on both sides of zero (Heravi and Kyani, 2004).

Equations 2 and 3 were developed to predict the antibacterial activity of benzylidene hydrazides against *B. subtilis and E. coli*.

ot-QSAR model for antibacterial activity against B. subtilis

$$pMIC_{bs} = 8.411\,B - 15.082 \quad (2)$$

$$n = 15, \quad r = 0.771, \quad q^2 = 0.500, \quad s = 0.174,$$
$$F = 19.13, \quad \text{RMSE} = 0.026, \quad e = 0.144,$$
$$\text{SDEP} = 0.168$$

A positive correlation of antibacterial activity against *B. subtilis* with Balaban topological index (*B*) indicated that magnitude of antibacterial activity of synthesized derivatives is directly proportional to *B* values (Eq. 2). This was evidenced by the fact that the compounds **9** and **10**, having high *B* values (2.02 and 1.96) compared to other compounds (Table 7), also possess high antibacterial activity values, i.e., 1.94 and 1.64, respectively (Table 6).

ot-QSAR model for antibacterial activity against E. coli

$$pMIC_{ec} = 0.104\ \text{T.D.} - 0.914 \quad (3)$$

$$n = 15, \quad r = 0.795, \quad q^2 = 0.514, \quad s = 0.162,$$
$$F = 22.47, \quad \text{RMSE} = 0.023, \quad e = 0.114,$$
$$\text{SDEP} = 0.157$$

For antibacterial activity against *E. coli*, the developed ot-QSAR model (Eq. 3) describes the importance of total dipole (T.D.). In this case, the positive correlation was observed between total dipole (Table 7) and antibacterial activity against *E. coli* (Table 6). The positive correlation of molecular descriptor with antibacterial activity reveals that increase in value of T.D. (Table 7) will lead to increase in antibacterial activity against *E. coli.* The low residual values presented in Table 10 are in agreement with the models expressed by Eqs. 2 and 3. Further, the low values of RMSE, SDEP, and *e* described the statistical significance of the developed models (Eqs. 1–3).

The cross-validation of the developed ot-QSAR models was also done by LOO technique. As in case of Eq. 1, the high q^2 values supported the validity of developed QSAR model described by Eq. 3 ($q^2 = 0.514$); however, in Eq. 2 the q^2 value is equal to 0.5, which shows that the developed model (Eq. 4) is an invalid one. One should not forget the recommendations of Golbraikh and Tropsha (2002) who have recently reported that the only way to estimate the true predictive power of a model is to test their ability to

Table 10 Comparison of observed and predicted antibacterial and antifungal activity obtained by ot- and mt-QSAR models

Compound	$pMIC_{ca}$ (Eq. 1)			$pMIC_{bs}$ (Eq. 2)			$pMIC_{ec}$ (Eq. 3)			$pMIC_{b}$ (Eq. 4)			$pMIC_{am}$ (Eq. 5)		
	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res
1	1.25	1.26	−0.01	0.95	1.18	−0.23	1.26	1.26	0.00	0.85	1.01	−0.16	1.14	1.17	−0.04
2	1.32	1.33	−0.01	1.02	1.18	−0.17	1.02	1.32	−0.30	1.12	1.20	−0.09	1.20	1.34	−0.14
3	1.32	1.33	−0.01	1.02	1.18	−0.17	1.02	1.38	−0.37	1.12	1.25	−0.13	1.32	1.34	−0.02
4	1.32	1.33	−0.01	1.02	1.18	−0.17	1.32	1.34	−0.02	1.22	1.24	−0.02	1.38	1.34	0.04
5	1.39	1.38	0.00	1.39	1.18	0.20	1.39	1.33	0.06	1.29	1.20	0.08	1.39	1.37	0.02
6	1.38	1.38	0.01	1.39	1.18	0.20	1.39	1.38	0.01	1.29	1.25	0.04	1.45	1.37	0.08
7	1.39	1.38	0.01	1.39	1.18	0.20	1.39	1.34	0.04	1.29	1.24	0.04	1.33	1.37	−0.04
8	1.29	1.28	0.01	1.29	1.18	0.10	1.29	1.37	−0.08	1.29	1.24	0.05	1.35	1.29	0.06
9	1.33	1.33	0.01	1.94	1.88	0.05	1.33	1.18	0.16	1.54	1.55	−0.01	1.58	1.51	0.07
10	1.33	1.33	0.00	1.64	1.41	0.22	1.94	1.84	0.10	1.64	1.58	0.06	1.58	1.51	0.07
11	1.33	1.33	0.00	1.33	1.46	−0.12	1.94	1.81	0.12	1.44	1.57	−0.14	1.40	1.51	−0.11
12	1.28	1.28	0.00	1.59	1.51	0.07	1.29	1.38	−0.09	1.39	1.20	0.18	1.35	1.30	0.05
13	1.28	1.28	0.00	1.29	1.26	0.03	1.29	1.14	0.14	1.29	1.25	0.04	1.29	1.30	−0.01
14	1.28	1.28	0.00	1.29	1.29	0.00	1.29	1.21	0.08	1.29	1.24	0.04	1.35	1.30	0.05
15	1.31	1.33	−0.02	1.31	1.54	−0.23	1.31	1.16	0.15	1.31	1.30	0.01	1.37	1.43	−0.06

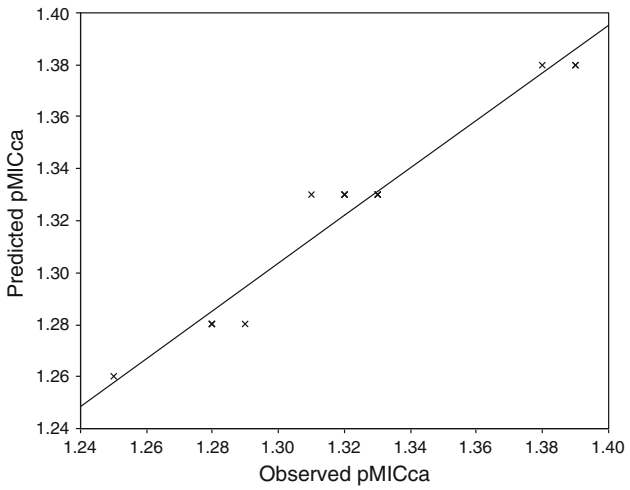

Fig. 2 Plot of predicted $pMIC_{ca}$ values against observed $pMIC_{ca}$ values for the linear regression developed model developed by Eq. 1

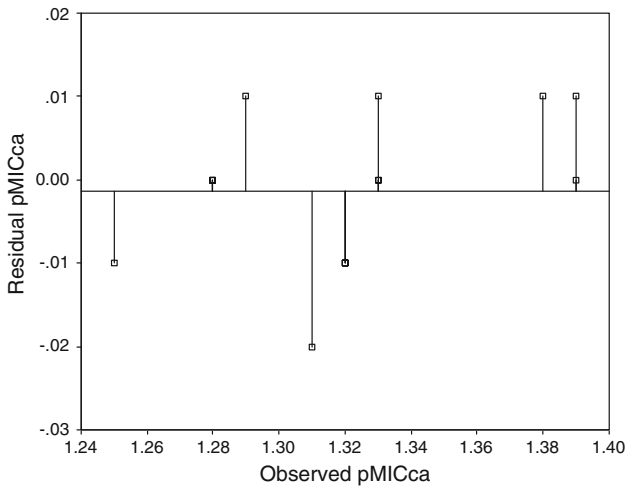

Fig. 3 Plot of residual $pMIC_{ca}$ values against observed $pMIC_{ca}$ values for the linear regression developed model developed by Eq. 1

predict accurately the biological activities of compounds. As the observed and predicted values (Table 10) are close to each other, the QSAR model for *B. subtilis* (Eq. 2) is a valid one.

Even though the sample size and the "rule of thumb" allowed us to go for development of triparametric model in multiple linear regression analysis, the high colinearity among the parameters restricted us to go for a monoparametric model only. The "rule of thumb" gives information about the number of parameters to be selected for regression analysis in QSAR based on the number of compounds. According to this rule for QSAR model development one should select one parameter for a five-compound data set (Narasimhan *et al*., 2006).

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in this study the range of antibacterial and antifungal activities of the synthesized compounds is within one order of magnitude. But it is important to note that the predictability of the QSAR models developed in this study is high, as is evident from their low residual values. This is in accordance with results suggested by the 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.

Table 11 Regression analysis and quality of correlation for modeling antibacterial and antifungal activities of synthesized benzylidene hydrazides

S. no.	QSAR model (pMIC=)	n	r	q^2	s	F
B. subtilis						
1.	−0.00012 Ele. E. − 0.869	15	0.715	0.309	0.191	13.61
2.	0.00014 Nu. E. − 0.798	15	0.721	0.324	0.189	14.09
3.	0.217 $^{1}\chi^{v}$ + 8.651 B − 16.76	15	0.810	0.421	0.167	11.49
E. coli						
4.	0.926 LUMO + 0.572	15	0.744	0.286	0.170	16.16
5.	0.745 κ_3 − 2.293	15	0.742	0.332	0.180	16.09
C. albicans						
6.	0.061 $^{0}\chi^{v}$ + 0.966	15	0.961	0.903	0.011	158.19
7.	0.110 $^{2}\chi^{v}$ + 0.899	15	0.948	0.878	0.013	117.45
8.	0.343 $^{3}\chi^{v}$ + 1.193	15	0.893	0.748	0.018	51.25
9.	0.012 MR + 0.475	15	0.874	0.683	0.020	42.18
10.	0.070 log P + 1.128	15	0.734	0.414	0.028	15.21
Antibacterial						
11.	0.00068 T_e − 0.868	15	0.856	0.609	0.097	35.67
12.	0.213 $^{0}\chi$ − 1.500	15	0.845	0.581	0.100	32.68
13.	1.204 $^{3}\chi$ + 0.217	15	0.835	0.571	0.103	30.15
14.	−0.0001 Ele. E. − 0.502	15	0.846	0.592	0.106	32.96
15.	0.00012 Nu. E. − 0.415	15	0.834	0.577	0.102	31.10
Antimicrobial						
16.	0.221 $^{2}\chi$ − 0.311	15	0.804	0.469	0.071	24.75
17.	−0.0004 T_e + 0.076	15	0.801	0.480	0.072	23.37
18.	0.127 $^{0}\chi$ − 0.299	15	0.790	0.444	0.073	21.61

Further, recent literature reveals that the QSAR has been applied to describe the relationship between narrow range of biological activity and physicochemical properties of the molecules (Narasimhan *et al*., 2007; Sharma *et al*., 2006; Hatya *et al*., 2006; Kumar *et al*., 2006a, b). When biological activity data lies in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies (Narasimhan *et al*. 2007; Kumar *et al*., 2007). The minimum standard deviation (Table 6) observed in the antimicrobial activity data justifies its use in QSAR studies. Some of the other statistically important ot-QSAR models are given in Table 11. Further, the low values of PE, LSE, LOF, RMSE, SDEP, e, and high value of Q (Table 12) revealed the statistical significance of the models described by Eqs. 1–3. In case of *S. aureus* and *A. niger* no statistically significant models were obtained (Table 9).

In this study it is interesting to note that in many cases low interrelationship between different parameters was observed [e.g., $^{2}\chi^{v}$ and LUMO ($r = 0.003$), $^{1}\chi^{v}$ and $^{0}\chi$ ($r = 0.218$), Table 8], although, only one descriptor (LR) was used for modeling of developed QSAR equations, as we go for more than one descriptors (MLR) for generation of model development there is change of significant statistical parameters into insignificant one were observed, e.g., decrease in values of correlation coefficient (r), cross-validated r^2 obtained by LOO method (q^2) and Fischer statistics (F); increase in values of standard error of the estimate (s), root mean square of error (RMSE), absolute average error (e), and standard deviation of error of prediction (SDEP). This makes us to stick to a single parameter. This is similar to one of our previous results (Narasimhan *et al*., 2007).

Development of multi-target QSAR model (mt-QSAR)

According to the above 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 unpractical or at less complicated to use when we have to predict to each compound results for more than one target. In these cases we have to develop one ot-QSAR for each target. However, very recently the interest has been increased in development of multi-target QSAR (mt-QSAR) models. In opposition to ot-QSAR, the

Table 12 PE, LSE, LOF, SEP, VIF, and *Q* values calculated for the derived models for modeling antimicrobial activity of benzylidene hydrazide derivatives

S. no.	Descriptor	PE	LSE	LOF	SEP	VIF	*Q*
C. albicans							
1	$^{1}\chi^{v}$	0.011	0.001	0.0001	0.002	16.38	96.90
2	$^{0}\chi^{v}$	0.013	0.002	0.000	0.003	13.08	87.36
3	$^{2}\chi^{v}$	0.017	0.002	0.000	0.003	9.87	72.92
4	$^{3}\chi^{v}$	0.035	0.005	0.001	0.005	4.94	49.61
5	MR	0.041	0.005	0.001	0.005	4.24	43.70
6	log *P*	0.079	0.010	0.001	0.007	2.17	26.21
B. subtilis							
7	*B*	0.070	0.394	0.053	0.042	2.47	4.43
8	Ele.E	0.084	0.478	0.064	0.046	2.05	3.74
9	Nu.E	0.083	0.669	0.090	0.055	2.08	3.81
10	$^{1}\chi^{v}$, *B*	0.059	0.335	0.045	0.039	2.91	4.85
E. coli							
11	T.D.	0.063	0.346	0.046	0.039	2.72	4.91
12	LUMO	0.077	0.420	0.056	0.043	2.24	4.38
13	κ_3	0.077	0.422	0.056	0.043	2.23	4.12
Antibacterial							
14	$^{2}\chi$	0.045	0.122	0.016	0.023	3.82	8.95
15	T_e	0.046	0.124	0.017	0.023	3.74	8.82
16	$^{0}\chi$	0.049	0.133	0.018	0.024	3.50	8.45
17	$^{3}\chi$	0.052	0.140	0.019	0.025	3.30	8.11
18	Ele.E	0.049	0.132	0.018	0.024	3.52	7.98
19	Nu.E	0.052	0.137	0.018	0.025	3.28	8.18
Antimicrobial							
20	$\kappa\alpha_1$	0.060	0.066	0.009	0.017	2.87	11.37
21	$^{2}\chi$	0.061	0.065	0.009	0.017	2.83	11.32
22	T_e	0.062	0.067	0.009	0.017	2.79	11.13
23	$^{0}\chi$	0.065	0.071	0.010	0.018	2.66	10.82

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

In this study, we have 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 a common mt-QSAR model for describing the antimicrobial (overall antibacterial and antifungal) activity of substituted benzylidene hydrazides against all the above-mentioned microorganisms.

In order to develop mt-QSAR models, initially we have calculated the average antibacterial activity [$pMIC_b = (pMIC_{sa} + pMIC_{bs} + pMIC_{ec})/3$], antifungal activity [$pMIC_f = (pMIC_{ca} + pMIC_{an})/2$], and antimicrobial activity values [$pMIC_{am} = (pMIC_{sa} + pMIC_{bs} + pMIC_{ec} + pMIC_{ca} + pMIC_{an})/5$] of substituted hydrazide derivatives which are presented in Table 6. These average activity values were also correlated with the molecular descriptors of synthesized compounds (Table 7).

mt-QSAR model for antibacterial activity

$$pMIC_b = 0.369\,{}^{2}\chi - 1.500 \quad (4)$$

$$n = 15,\quad r = 0.859,\quad q^2 = 0.605,\quad s = 0.096,$$
$$F = 36.73,\quad RMSE = 0.008,\quad e = 0.073,$$
$$SDEP = 0.093$$

The mt-QSAR model for antibacterial activity displayed the importance of second order molecular connectivity index, $^{2}\chi$ (r = 0.859), in describing the antibacterial activity of substituted benzylidene hydrazides (Eq. 4).

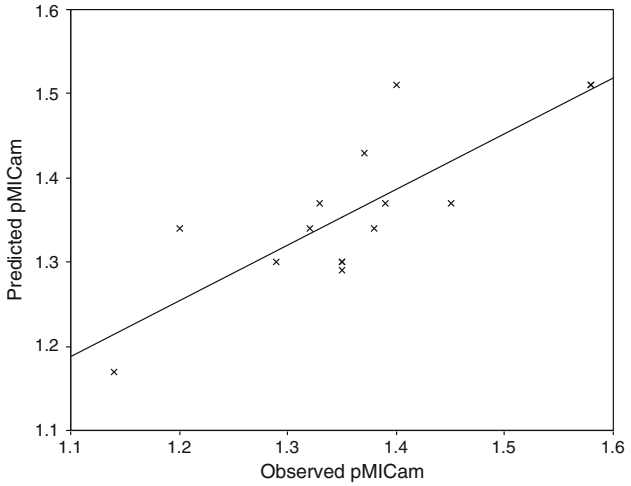

Fig. 4 Plot of predicted $pMIC_{am}$ values against observed $pMIC_{am}$ values for the linear regression developed model developed by Eq. 5

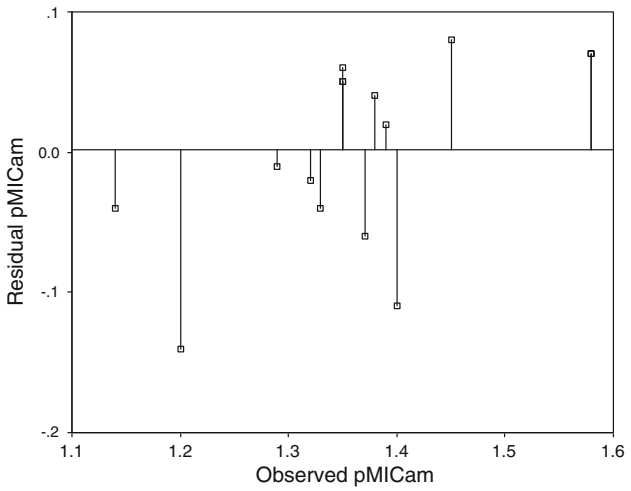

Fig. 5 Plot of residual $pMIC_{am}$ values against observed $pMIC_{am}$ values for the linear regression developed model developed by Eq. 5

mt-QSAR model for antimicrobial activity

$$pMIC_{am} = 0.135\,\kappa\alpha_1 - 0.435 \quad (5)$$

$$n = 15, \quad r = 0.807, \quad q^2 = 0.517, \quad s = 0.071,$$
$$F = 24.39, \quad \text{RMSE} = 0.004, \quad e = 0.057,$$
$$\text{SDEP} = 0.069$$

Further, the mt-QSAR model of antimicrobial activity depicted the importance of first order Kier's alpha shape index ($\kappa\alpha_1$) in describing the antimicrobial activity of synthesized substituted benzylidene hydrazide derivatives (Eq. 5).

The predictability of the Eq. 5 is further supported by the low residual values observed in Table 10 as well by the plot of predicted $pMIC_{am}$ against observed $pMIC_{am}$ (Fig. 4). The plot of observed $pMIC_{am}$ versus residual $pMIC_{am}$ (Fig. 5) depicted that there was no systemic error in model development as the propagation of residuals was observed on both sides of zero. Further, the low value of RMSE, SDEP, and *e* described the statistical significance of the developed models (Eqs. 4, 5). The statistical significance of the developed models (Eqs. 4, 5) was also supported by low LSE, LOF, PE, and high *Q* values (Table 12). In case of antifungal activity no statistically significant model was developed as value of regression coefficient is very low (Table 9).

Conclusion

In conclusion, a series of nicotinic acid benzylidene hydrazide derivatives (**1–18**) was synthesized and tested in vitro for antimycobacterial activity against *M. tuberculosis,* antiviral and antimicrobial activities against *S. aureus*, *B. subtilis*, *E. coli*, *C. albicans*, and *A. niger*. The antimycobacterial activity results showed that the presence of electron-withdrawing halogen groups (viz. chloro, bromo, and fluoro) at *para* position of the phenyl ring improved the antimycobacterial activity. The antiviral evaluation indicated that none of the synthesized derivatives inhibited viral replication. Further, the antimicrobial screening results indicated that compounds having dimethoxy (**16**) and nitro (**9**, **10**, and **11**) substituents were the most active ones against tested strains with some of them having antimicrobial activity comparable to standard drugs fluconazole and norfloxacin. To understand the relationship between physicochemical parameters and antimicrobial activity of substituted hydrazide derivatives, QSAR investigation was performed by the development of one-target and multi-target models. The multi-target model was more effective in describing the antimicrobial activity of substituted hydrazides than the one-target models. In addition, the QSAR studies demonstrated that the antibacterial and overall antimicrobial activity of synthesized substituted benzylidene hydrazides is governed by second order molecular connectivity index ($^2\chi$) and first order Kier's alpha shape index ($\kappa\alpha_1$), respectively. The statistical significance of developed models has been established by low LSE, LOF, PE, RMSE, SDEP, *e*, and high *Q* values.

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