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Isonicotinic acid hydrazide derivatives: synthesis, antimicrobial activity, and QSAR studies

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Abstract A series of isonicotinic acid hydrazide derivatives (**1–19**) was synthesized and tested in vitro for antimycobacterial activity against *Mycobacterium tuberculosis* and antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* and the results indicated that the compounds with OH, SCH₃, and OCH₃ groups were found to be active against the tested strains. None of the test compounds were active against a broad variety of RNA and DNA viruses at subtoxic concentrations, except **8**, that showed some selective anti-reovirus-1 activity. The multi-target QSAR models were found to be effective in predicting the antimicrobial activity of the isoniazid derivatives and indicated the importance of nuclear repulsion energy (Nu.E) in explaining the antimicrobial activity of isoniazid derivatives. The developed QSAR models were validated using the external test set of synthesized derivatives.

Keywords Isoniazid derivatives · Antimycobacterial · Antiviral · Antimicrobial · QSAR

Introduction

Tuberculosis (TB), caused by the infectious agent *Mycobacterium tuberculosis*, is one of the most important killing infectious diseases. According to alarming data from the World Health Organization (WHO), TB has spread to every corner of the globe. As much as one third of the world's population is currently infected, and more than 5,000 people die from TB everyday (Sriram *et al.*, 2009). The factors responsible for this are: (i) patient non-compliance to existing drug regimens which has resulted in the emergence of single drug-resistant strains to all major anti-TB drugs; (ii) emergence of multidrug-resistant TB (MDR-TB), which is defined as the disease caused by the strains of *M. tuberculosis* resistant to two mainstay first-line anti-TB drugs, isoniazid and rifampicin; and (iii) association of human immunodeficiency virus (HIV) with TB, in which TB is the leading cause of death among patients who are HIV-positive. Consequently, new drugs with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs are urgently required (Nayyar *et al.*, 2007).

AIDS is caused by a retrovirus, the human immunodeficiency virus (HIV). Two genetically distinct subtypes (HIV-1 and HIV-2) have been characterized, but it is known that the prime etiological agent of AIDS is HIV-1 (Canoa *et al.*, 2006). Combination therapy or highly active antiretroviral therapy (HAART) with two or more reverse transcriptase inhibitors and protease inhibitors (PIs) has dramatically improved the quality of life and survival of patients infected with human immunodeficiency virus

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(HIV) type 1 (HIV-1). However, the ability to provide effective long-term antiretroviral therapy for HIV-1 infection has become a complex issue, since 40–50% of those who initially achieved favorable viral suppression to undetectable levels have experienced treatment failure (Koh *et al.*, 2003).

In the past 25 years, the incidence of microbial infections has increased an alarming level over the world as result of antimicrobial resistance. A growing number of immuno-compromised patients are as a result of cancer chemotherapy, organ transplantation, and HIV infection which are the major factors contributing to this increase. The health problems demand to search and synthesize a new class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen (Bayrak *et al.*, 2009a).

Quantitative structure–activity relationship (QSAR) research has been considered a major tool in drug discovery to explore the ligand receptor/enzyme interactions, especially when the structural details of the target are not known. QSAR is an effective way for optimizing or correlating the biological activity within a congeneric series with certain structural features or with functional determinants, such as lipophilicity, polarizability, or electronic and steric properties (Sivaprakasam *et al.*, 2006). QSAR models are mathematical equations which construct a relationship between chemical structures and their biological activities as a linear regression model of the form $y = Xb + e$. This equation may be used to describe a set of predictor variables (X) with a predicted variable (y) by means of a regression vector (b). In a typical QSAR study one needs to find a set of molecular descriptors representing the higher impact on the biological activity of interest (Sabet *et al.*, 2010).

Isonicotinic acid hydrazide (Isoniazid; INH) and pyrazinamide (PZA) are widely applied as a first line drugs for the treatment of tuberculosis, usually in combination with other drugs. Modifying either of these molecules has been a challenge taken up by several research groups (Imramovsky *et al.*, 2007). Furthermore, the pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization to bioreversible forms of this drug, namely hydrazones (Sriram *et al.*, 2006). Isoniazid derivatives have been reviewed by Scior and Garces-Eisele (2006) and antitubercular evaluation of novel series of 3-benzofuran-5-aryl-1-pyrazolyl-pyridylmethanone analogs derived from isoniazid have been reported (Manna and Agrawal, 2010). Recently, antimicrobial properties of isoniazid have also been studied (Bayrak *et al.*, 2009a, b; Rodriguez-Arguelles *et al.*, 2007). In light of above facts and in continuation of our research endowed toward the synthesis, antimicrobial, antimycobacterial, antiviral, and QSAR studies (Judge

et al., in press; Kumar *et al.*, 2010a, b), we hereby report the synthesis, antitubercular, anti-HIV, antimicrobial, and QSAR studies of isoniazid derivatives.

Experimental

Melting points were determined in open capillary tubes 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 the purity of the compounds was ascertained by single spot on TLC sheet. ^1H nuclear magnetic resonance (^1H NMR) spectra were recorded in Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (δ , ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer.

General procedure for synthesis of ester of isonicotinic acid

The mixture of isonicotinic acid (0.1 mol) and ethanol (in excess) was refluxed with sulfuric acid (1–2 ml) till the completion of reaction monitored by TLC on silica gel G plates. Then the reaction mixture was added to 200 ml ice cold water and excess of acid was neutralized by a solution of sodium bicarbonate. The crude ester was extracted with ether. The ether layer was separated and ester was obtained on evaporation of ether layer.

General procedure for the synthesis of isonicotinic acid hydrazide

The ethanolic solution of ester (0.01 mol) and hydrazine-hydrate (0.015 mol) was refluxed for appropriate time. The reaction mixture was then cooled and the precipitated solid was washed with water, dried, and recrystallized from ethanol.

General procedure for the synthesis of schiff bases of isoniazid

The solution of isonicotinic acid hydrazide (0.01 mol) and appropriate substituted aldehyde (0.01 mol) in ethanol was refluxed for 4–5 h. The precipitates obtained were filtered off, washed, and recrystallized from ethanol.

Isonicotinic acid -3-ethoxy-4-hydroxybenzylidene hydrazide (**1**)

Mp ($^{\circ}\text{C}$) above 242; Yield—65.3%; ^1H NMR (400 MHz, CDCl_3) δ ppm: 3.87 (t, 3H, CH_3), 4.17–4.20 (q, 2H, CH_2),

6.89–6.91 (s, 1H, OH), 7.06–7.08 (s, 1H, CH of C₂ of phenyl ring), 7.97 (d, 2H, CH of C₅ and C₆ of phenyl ring), 8.37 (d, 2H, CH of C₂ and C₆ of pyridine ring), 8.79 (d, 2H, CH of C₃ and C₅ of pyridine ring), 11.71 (s, 1H, NH). IR (KBr pellets) ν cm⁻¹: 3254.34 (NH str., amide), 3093.31 (CH str., aromatic), 2973.58 (CH str., aliphatic), 1644.86 (C=O str., amide), 1593.74 (C=C str., skeletal phenyl nucleus), 1276.12 (C–O–C str., aralkyl ether), 1255.95 (OH bending), 845.53 (CH out of plane bending, 4-substituted pyridine).

Isonicotinic acid-3,4,5-trimethoxybenzylidene hydrazide (4)

Mp (°C) 205–208; Yield—80.7%; ¹H NMR (400 MHz, DMSO) δ ppm: 3.36 (s, 9H, OCH₃), 7.21–7.23 (s, 2H, CH of C₂ and C₆ of trimethoxy phenyl ring), 7.83–7.84 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.44 (s, 1H, CH of N=CH), 8.73–8.76 (d, 2H, CH of C₂ and C₆ of pyridine ring), 11.91 (s, 1H, NH). IR (KBr pellets) ν cm⁻¹: 3168.22 (NH str., amide), 2983.04 (CH str., aliphatic), 1682.00 (C=O str., amide), 1583.63 (C=C str., skeletal phenyl nucleus), 1248.96 (C–O–C str., aralkyl ether), 858.36 (CH out of plane bending, 4-substituted pyridine).

Isonicotinic acid-4-methylbenzylidene hydrazide (5)

Mp (°C) 198–201; Yield—84.6%; ¹H NMR (400 MHz, DMSO) δ ppm: 3.28 (s, 3H, CH₃), 6.72–6.95 (m, 4H, CH of phenyl ring), 7.76–7.77 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.32 (s, 1H, CH of N=CH), 8.68–8.69 (d, 2H, CH of C₂ and C₆ of pyridine ring), 11.90 (s, 1H, NH). IR (KBr pellets) ν cm⁻¹: 3177.86 (NH str., amide), 2935.78 (CH str., aliphatic), 1682.96 (C=O str., amide), 1583.63 (C=C str., skeletal phenyl nucleus), 1240.0 (C–O–C str., aralkyl ether), 835.21 (CH out of plane bending, 4-substituted pyridine).

Isonicotinic acid-1-H-indol-3lmethylene hydrazide (8)

Mp (°C) 225–228; Yield—61.9%; ¹H NMR (400 MHz, DMSO) δ ppm: 7.07–7.16 (m, 2H, CH of C₅ and C₆ of indole ring), 7.36–7.38 (m, 2H, CH of C₄ and C₇ of indole ring), 7.53–7.54 (m, 2H, CH of C₂ of indole ring), 7.80–7.81 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.60 (s, 1H, CH of N=CH), 8.68–8.71 (d, 2H, CH of C₂ and C₆ of pyridine ring), 11.24 (s, 1H, NH of indole), 11.57 (s, 1H, NH of hydrazide). IR (KBr pellets) ν cm⁻¹: 3173.04 (NH str., amide), 3052.48 (CH str., aromatic), 2940.61 (CH str., aliphatic), 1634.74 (C=O str., amide), 1428.35 (NH str., indole ring), 739.73 (CH out of plane bending, indole ring), 847.75 (CH out of plane bending, 4-substituted pyridine).

General procedure for the synthesis of N₁-benzoylated schiff bases of isoniazid

The Schiff base (0.01 mol) obtained above was dissolved in dichloromethane to which an equimolar amount of benzoyl chloride, obtained by refluxing benzoic acid with thionyl chloride, was added with stirring for 2 h. The crude products were separated out by evaporating dichloromethane and recrystallized from ethanol to yield the pure compounds.

Benzoic acid N'-(4-hydroxy-3-methoxy-benzylidene)-N-(pyridine-4-carbonyl)-hydrazide (10)

Mp (°C) 230–233; Yield—57.8%; ¹H NMR (400 MHz, DMSO) δ ppm: 3.70 (s, 3H, OCH₃), 3.84 (s, 3H, OH), 7.28–7.38 (m, 5H, CH of phenyl ring), 7.46–7.50 (m, 3H, CH of benzylidene ring), 7.70 (s, 1H, CH of N=CH), 7.91–7.94 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.77 (d, 2H, CH of C₂ and C₆ of pyridine ring). IR (KBr pellets) ν cm⁻¹: 3414.15 (OH str.), 3036.09 (CH str., aromatic), 2904.92 (CH str., aliphatic), 1680.07 (C=O str., acyl group), 1597.13 (C=O str., amide), 1513.22 (C=C str., skeletal phenyl nucleus), 1285.61 (C–O–C str., aralkyl ether), 814.96 (CH out of plane bending, 4-substituted pyridine).

Benzoic acid N'-(4-methylsulfanyl-benzylidene)-N-(pyridine-4-carbonyl)-hydrazide (12)

Mp (°C) 226–229; Yield—45.8%; ¹H NMR (400 MHz, DMSO) δ ppm: 2.48 (s, 3H, SCH₃), 7.11–7.20 (m, 3H, CH of C₃, C₄ and C₅ phenyl ring), 7.34–7.37 (d, 2H, CH of C₂ and C₆ phenyl ring), 7.62–7.64 (d, 2H, CH of C₃ and C₅ of benzylidene ring), 7.91–7.93 (d, 2H, CH of C₂ and C₆ of benzylidene ring), 8.21–8.23 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.52 (s, 1H, CH of N=CH), 8.86–8.87 (d, 2H, CH of C₂ and C₆ of pyridine ring). IR (KBr pellets) ν cm⁻¹: 3014.87 (CH str., aromatic), 1667.53 (C=O str., acyl group), 1596.16 (C=O str., amide), 1497.79 (C=C str., skeletal phenyl nucleus), 609.53 (C–S str.), 732.98 (CH out of plane bending, 4-substituted pyridine).

Benzoic acid N'-(4-methyl-benzylidene)-N-(pyridine-4-carbonyl)-hydrazide (14)

Mp (°C) 220–223; Yield—62.7%; ¹H NMR (400 MHz, DMSO) δ ppm: 2.31 (s, 3H, CH₃), 7.07–7.49 (m, 5H, CH of phenyl ring), 7.59–7.61 (d, 2H, CH of C₃ and C₅ of benzylidene ring), 7.91–7.93 (d, 2H, CH of C₂ and C₆ of benzylidene ring), 8.56–8.57 (d, 2H, CH of C₃ and C₅ of pyridine ring), 8.68 (s, 1H, CH of N=CH), 8.93–8.94 (d, 2H, CH of C₂ and C₆ of pyridine ring). IR (KBr pellets)

ν cm⁻¹: 3004.26 (CH str., aromatic), 1653.07 (C=O str., acyl group), 1593.27 (C=O str., amide), 1486.22 (C=C str., skeletal phenyl nucleus), 841.00 (CH out of plane bending, 4-substituted pyridine).

General procedure for the synthesis of *N*₂-acyl isonicotinic acid hydrazides (**17–19**)

The corresponding carboxylic acids (0.01 mol) were refluxed with thionyl chloride (0.015 mol) for 3 h. After refluxing was complete, the excess thionyl chloride was distilled off to get the acyl chloride. The isonicotinic acid hydrazide (0.01 mol) was suspended in dichloromethane and respective acyl chloride (0.01 mol) was added dropwise to this solution with constant stirring on a magnetic stirrer for 3–4 h. The product obtained after evaporation of dichloromethane was recrystallized from ethanol.

2-Chloro-5-nitro-benzoic acid N'-(pyridine-4-carbonyl)-hydrazide (17)

Mp (°C) 200–203; Yield—56.30%; ¹H NMR (400 MHz, DMSO) δ ppm: 7.79–7.81 (d, 2H, CH of C₃ and C₄ of phenyl ring), 8.32 (s, 1H, CH of C₅ of phenyl ring), 8.51–8.53 (d, 2H, CH of C₃ and C₅ of pyridine ring), 9.14–9.17 (d, 2H, CH of C₂ and C₆ of pyridine ring), 10.44 (s, 1H, NH proton of N₂), 11.20 (s, 1H, NH proton of N₁). IR (KBr pellets) ν cm⁻¹: 3132.53 (NH str., amide), 3083.34 (CH str., aromatic), 1708.04 (C=O str., Nicotinoyl), 1677.18 (C=O str., Acyl), 1528.65 (NO₂ asymmetric str., aromatic nitro group), 1339.62 (NO₂ symmetric str., aromatic nitro group), 1273.07 (C–N str., coupled vibrations amide), 841.00 (CH out of plane bending, 4-substituted pyridine), 739.73 (OCN deformations, amide IV band), 667.40 (OCN deformations, amide VI band).

Evaluation of antimycobacterial activity

All compounds were screened for their in vitro antimycobacterial activity against MTB, in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate (1995). 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)

The antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus*

subtilis, Gram-negative bacterium: *Escherichia coli* and fungal strains: *Candida albicans* and *Aspergillus niger* by tube dilution method (Cappuccino and Sherman, 1999). Dilutions of test and standard compounds [norfloxacin (antibacterial) and fluconazole (antifungal)] were prepared in double strength nutrient broth I.P. (bacteria) and Sabouraud dextrose broth I.P. (Pharmacopoeia of India, 2007) (fungi). The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 7 days (*A. niger*), and at 37°C for 48 h (*C. albicans*), and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

QSAR studies

The structures of isonicotinyl hydrazide derivatives were 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). 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 (1999). The predictive powers of the equation were validated by determination of cross-validated r^2 (q^2) using leave one out (LOO) cross-validation method (Schaper, 1999).

Calculation of statistical parameters

The developed QSAR models were validated by the calculation of following statistical parameters: probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), standard error of prediction (SE_P), quality value (*Q*), and sum of squares of response values (SSY) (Mandloi *et al.*, 2005; Pinheiro *et al.*, 2004).

These parameters were calculated from the following equations:

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

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

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

where *Y*_{obs} and *Y*_{calc} are the observed and calculated values, respectively.

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

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.

$$SE_p = \sqrt{LSE/n}$$

The quality value, Q , is given by

$$Q = r/SE$$

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

The predictive ability of QSAR models was also quantified in terms of q^2 , which is defined as

$$q^2 = 1 - \left\{ \frac{\sum (Y_{\text{obs}} - Y_{\text{calc}})^2}{\sum (Y_{\text{obs}} - Y_{\text{mean}})^2} \right\}$$

The low value of PE, LSE, LOF, and SE_p and high value of Q and q^2 are the essential criteria for qualifying the model as the best one.

Evaluation of anti-HIV activity

The anti-HIV activity and cytotoxicity were evaluated against HIV-1 strain IIB 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 μ l of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-day incubation period at 37°C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

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, Cocksackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Cocksackie 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, ... μ g/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

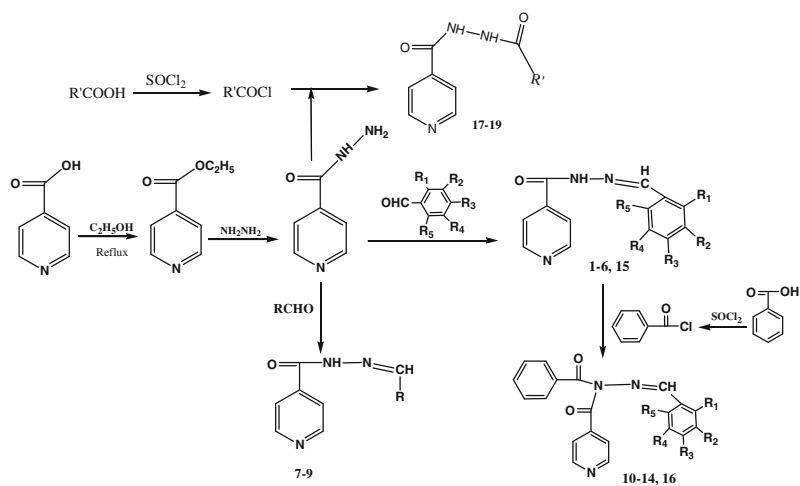
Chemistry

The synthesis of target compounds was carried out as depicted in Scheme 1. The isonicotinic acid was refluxed with ethanol in the presence of sulfuric acid to get the ethyl ester of isonicotinic acid. The ester thus obtained was refluxed with hydrazine hydrate to obtain the isonicotinic acid hydrazide. The isonicotinic acid hydrazide was refluxed with various substituted aldehydes to yield the target hydrazones (**1–9**, **15**). Furthermore, the reaction of compounds **1–3**, **5**, **6**, and **15** with benzoyl chloride, which was prepared by refluxing benzoic acid with thionyl chloride, resulted in the formation of benzoylated derivatives **10–14**, **16**. All the compounds were obtained in appreciable yield and their physicochemical characteristics are presented in Table 1.

The structures of synthesized compounds were ascertained on the basis of their consistent IR and NMR spectral characteristics. The presence of singlet signal above δ 11.50 ppm in compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** reveals the presence of the proton NH and it disappears in the compounds **10**, **11**, **12**, **13**, **14**, and **16**.

The presence of singlet signal around δ 8.0 ppm confirms the presence of N=CH linkage in the synthesized compounds. In all the synthesized compounds two doublet signals were observed at two different δ values, with the one at higher δ value corresponds to C_2 and C_6 CH protons as they are in close proximity of the electronegative nitrogen atom in the ring and the doublet signal at lower δ represents the remaining two protons of the isonicotinyl nucleus. The singlet signal due to NH at δ 11.24 ppm in compound **8** confirms the presence of the indole nucleus in the synthesized compounds. The appearance of multiplet signals in the aromatic region in compounds **10**, **12**, and **14** confirmed the existence of an acyl group in these compounds. The singlet signal at δ 3.28 ppm confirms the presence of a methyl group in compound **5**. The singlet signal at δ 3.36 ppm confirms the presence of a 3,4,5-trimethoxy group in compound **4**. The presence of CH_3 group in compound **5** was depicted by the singlet signal at δ 3.28 ppm. The singlet signal at δ 2.48 ppm corresponding to the SCH_3 group confirms the presence of thiomethyl group in compound **12**.

The presence of the C=O functional group was marked by the appearance of stretching band around 1680–1640 cm^{-1} , in compounds **1**, **4**, **5**, and **8** which is



Comp.	R ₁	R ₂	R ₃	R ₄	R ₅	R	R'
1	H	OC ₂ H ₅	OH	H	H	-	-
2	H	OCH ₃	OH	H	H	-	-
3	OCH ₃	H	H	H	OCH ₃	-	-
4	H	OCH ₃	OCH ₃	OCH ₃	H	-	-
5	H	H	CH ₃	H	H	-	-
6	H	H	SCH ₃	H	H	-	-
7	-	-	-	-	-		-
8	-	-	-	-	-		-
9	-	-	-	-	-		-
10	H	OCH ₃	OH	H	H	-	-
11	H	OC ₂ H ₅	OH	H	H	-	-
12	H	H	SCH ₃	H	H	-	-
13	OCH ₃	H	H	H	OCH ₃	-	-
14	H	H	CH ₃	H	H	-	-
15	OH	H	H	H	H	-	-
16	OH	H	H	H	H	-	-
17	-	-	-	-	-	-	
18	-	-	-	-	-	-	
19	-	-	-	-	-	-	

Scheme 1 Synthetic route followed for the synthesis of isoniazid derivatives

Table 1 Physicochemical properties and antimycobacterial activity of synthesized isoniazid derivatives

Compound	Mol. formula	Mol. wt.	Melting pt. (°C)	Rf ^a	% yield	MIC MTB-H37Rv ($\mu\text{M} \times 10^{-3}$)
Training set						
1	C ₁₅ H ₁₅ N ₃ O ₃	285	Above 242	0.78	65.3	11
2	C ₁₄ H ₁₃ N ₃ O ₃	271	238–241	0.74	71.2	12
3	C ₁₅ H ₁₅ N ₃ O ₃	285	193–196	0.69	68.5	11
4	C ₁₆ H ₁₇ N ₃ O ₄	315	205–208	0.71	80.7	20
5	C ₁₄ H ₁₃ N ₃ O	239	198–201	0.68	84.6	52
6	C ₁₄ H ₁₃ N ₃ OS	271	175–178	0.57	71.5	12
7	C ₁₇ H ₁₃ N ₃ O ₂	291	239–242	0.59	56.8	21
8	C ₁₅ H ₁₂ N ₄ O	264	225–228	0.64	61.9	24
9	C ₁₀ H ₁₁ N ₃ O	189	187–190	0.52	74.5	17
10	C ₂₁ H ₁₇ N ₃ O ₄	375	230–233	0.45	57.8	>67
11	C ₂₂ H ₁₉ N ₃ O ₄	389	234–237	0.43	60.4	>64
12	C ₂₁ H ₁₇ N ₃ O ₂ S	375	226–229	0.40	45.8	>67
13	C ₂₂ H ₁₉ N ₃ O ₄	389	215–218	0.51	63.9	>64
14	C ₂₁ H ₁₇ N ₃ O ₂	343	220–223	0.44	62.7	>73
Test set						
15	C ₁₃ H ₁₁ N ₃ O ₂	241	230–233	0.65	50.60	52
16	C ₂₀ H ₁₅ N ₃ O ₃	345	214–217	0.48	52.4	>72
17	C ₁₃ H ₉ N ₄ O ₄ Cl	320	200–203	0.71	56.30	4.9
18	C ₁₃ H ₁₀ N ₃ O ₂ Cl	275	216–219	0.63	71.40	22.7
19	C ₉ H ₁₀ N ₃ O ₂ Cl	227	235–238	0.69	51.80	55.1
INH						2.04
ETB ^b						15.31
CFL						9.4
RIF ^b						0.24

^a Mobile phase: ethanol^b Sriram *et al.* (2007)

characteristic of the amide linkage. The vibrational frequency C=O functional group shifts to a lower frequency around 1597–1593 cm⁻¹, in compounds **10**, **12**, and **14**, which may be due to substitution of NH proton with acyl group. The NH stretching of secondary amide was observed around 3200–3100 cm⁻¹ in compounds **1**, **4**, **5**, and **8** and this characteristic peak disappeared in the spectra of compounds **10**, **12**, and **14**. The presence of peaks slightly above and below 3000 cm⁻¹ indicates the presence of an aromatic and aliphatic portion in the synthesized compounds, respectively. The skeletal C=C-stretching bands were observed around 1500 cm⁻¹ in the spectra of the synthesized compounds which represents the presence of aromatic groups. In compounds **1**, **4**, and **5** the C–O–C stretching was observed at 1276.12, 1248.96, and 1240.0 cm⁻¹, which reveals the presence of a methoxy group in their structure. The CH out of plane bending observed around vibrational frequency of 860–814 cm⁻¹ indicated the presence of the 4-substituted pyridine ring in the structure of the synthesized compounds.

Antimycobacterial activity

The in vitro antitubercular activity of synthesized compounds against *M. tuberculosis* (MTB) was carried out in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method and the results are presented in Table 1. The results of antitubercular activity indicated that the synthesized derivatives were active against *M. tuberculosis* (MTB) with an MIC of 11 × 10⁻³ μM which approaches the antitubercular potential of ciprofloxacin. The antimycobacterial potential of compounds **1**, **2**, **3**, and **6** was found to be better than standard drug ethambutol (ETB). It was observed from the results of antimycobacterial activity that compounds with free OH group at *para* position (compounds **1** and **2**) in their molecular structure were more active as compared to other derivatives which may be due the fact that free OH group may be involved in the binding of the drug with the receptor site of the *Mycobacterium*. It can also be seen from the antimycobacterial activity data that when we replaced the OH group with electron donating CH₃

group (compound **5**), a fall in activity was observed ($52 \times 10^{-3} \mu\text{M}$) but if we substituted the CH_3 group with SCH_3 group (compound **6**), an abrupt rise in activity was observed ($12 \times 10^{-3} \mu\text{M}$). This may be attributed to the fact that, similar to the binding of the OH group with the target site, SCH_3 group containing free lone pair of electrons was involved in the binding of structure with the target site. The substitution of phenyl ring of the aldehydes with naphthalene nucleus (compound **7**) showed antimycobacterial potential but the replacement of the naphthalene with the heterocyclic indole nucleus (compound **8**) leads to a fall in antitubercular activity of the synthesized isoniazid derivatives. The substitution of NH proton of hydrazide with acyl group, $\text{C}_6\text{H}_5\text{CO}$, leads to decrease in antimycobacterial activity of synthesized derivatives (compounds **10**, **11**, **12**, **13**, and **14**) which shows that the NH proton is necessary for antimycobacterial activity.

The test set compounds **15–19** were also evaluated for their antimycobacterial activity and compound **17** was highly active antimycobacterial agent with MIC value $4.9 \times 10^{-3} \mu\text{M}$ and was twice active than ciprofloxacin and thrice active than ethambutol.

From the results of antimycobacterial activity the following conclusions regarding structure activity relationship (SAR) can be drawn:

1. From the appreciable antimycobacterial activity of compounds **1** and **2** it may be concluded that the presence of a free hydroxyl group at *para* position, OH, is necessary for antimycobacterial activity.
2. The substitution of the OH group with an electron donating CH_3 (compound **5**) decreases the antimycobacterial activity. These results are similar to the reports of Sriram *et al.* (2007) who observed that the addition of a methyl group decreases the antimycobacterial activity of *N*-hydroxythiosemicarbazones.
3. The replacement of the benzylidene ring with a naphthalene ring (compound **7**) or a heterocyclic indole ring (compound **8**) did not improve the antimycobacterial activity. This is similar to one of our previous reports (Kumar *et al.*, 2009).
4. The presence of the OH group at *ortho* position (compound **7**) increases the activity of the compounds. This is in concordance with Tripathi *et al.* (2006) who stated that the OH group at *ortho* position leads to a measurable change in activity of the compounds.
5. The substitution of the methyl group (compound **5**) with the more bulky thiomethyl group (compound **6**) increased the antimycobacterial activity. This is in accordance with the results of Desai *et al.* (2001) who reported that addition of a bulkier group at the 4-position positively contributes to the antimycobacterial activity.

6. The presence of electron withdrawing NO_2 and Cl groups in the molecular structure (compound **17**) leads to a steep increase in antimycobacterial activity.

Cytostatic, cytotoxic, and antiviral activities

The compounds were in general poorly cytotoxic against HEL, HeLa, and Vero cell cultures (except **7** that showed an MCC of $\geq 20 \mu\text{g/ml}$ for HEL, but 0.2 to $\geq 0.8 \mu\text{g/ml}$ for HeLa and Vero cell cultures). Instead, their cytostatic activities ranged between $0.10 \mu\text{g/ml}$ (compound **7**) and $\geq 100 \mu\text{g/ml}$ depending the nature of the compounds (Table 2). Generally, none of the compounds were inhibitory to HIV (Table 2) or any of the other RNA or DNA viruses tested at subtoxic concentrations. Compound **7** showed anti-HSV-1, -HSV-2, and -VV activity at EC_{50} values of $0.06\text{--}0.4 \mu\text{g/ml}$ (Table 3), but given the potent cytostatic activity of this compound, it is unclear whether this represents a direct antiviral effect or, more likely, an

Table 2 Anti-HIV potential of synthesized isoniazid derivatives in MT-4 cells

Compound	EC_{50} ($\mu\text{g/ml}$)		CC_{50}^a ($\mu\text{g/ml}$)
	HIV-1 (IIIb)	HIV-2 (ROD)	
1	>102	>102	102 ± 20
2	>89	>89	≥ 89
3	>9	>9	9 ± 4.5
4	>110	>110	110 ± 12
5	>25	>25	25 ± 9
6	>59	>59	59 ± 8
7	>0.10	>0.10	0.10 ± 0.01
8	>95	>95	95 ± 16
9	>11	>11	11 ± 10
10	>105	>105	105 ± 18
11	>92	>92	≥ 92
12	>69	>69	69 ± 8
13	>12	>12	12 ± 0.9
14	>42	>42	42 ± 11
15	>2.52	>2.52	2.52 ± 0.10
16	NT	NT	–
17	>115.67	>115.67	115.67 ± 6.03
18	>120.00	>120.00	>120.00
19	>77.47	>77.47	77.47 ± 58.50
Nevirapine	0.050 ± 0.011	>4.0	>4.0
Zidovudine	0.002 ± 0.001	0.002 ± 0.001	>25

EC_{50} 50% Effective concentration or compound concentration required to inhibit virus-induced cytopathicity by 50%

NT not tested

^a 50% Cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%

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

Compound	Minimum cytotoxic concentration ^a (μg/ml)	EC ₅₀ ^b (μg/ml)			
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Herpes simplex virus-1 TK ⁻ KOS ACV ^r
1	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100
7	≥20	0.06	0.4	0.14	0.4
8	>100	>100	>100	>100	>100
9	100	>20	>20	>20	>20
10	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100
14	>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%

Table 4 Cytotoxicity and anti-RNA virus activity of synthesized isoniazid 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	>100	>100	>100
2	100	>20	>20	>20
3	100	>20	>20	>20
4	≥100	>100	>100	>100
5	100	>20	9	>20
6	100	>20	>20	>20
7	0.2	>0.04	>0.04	>0.04
8	≥100	>100	>100	>100
9	100	>20	>20	>20
10	100	>20	>20	>20
11	>100	>100	>100	>100
12	>100	>100	>100	>100
13	20	>4	>4	>4
14	100	>20	>20	>20

^a Required to cause a microscopically detectable alteration of normal cell morphology

^b Required to reduce virus-induced cytopathicity by 50%

indirect cytostatic effect of the compound. Compounds **5**, **6**, **7**, and **12** were found to be inhibitory to Coxsackie B4 virus in one cell line but not in another (Tables 4, 5). Instead, compound **8** showed an EC₅₀ of 2.4 μg/ml against reovirus-1 in Vero cell cultures that is at a concentration well below its toxicity threshold. This observed activity is further under investigation.

Antibacterial and antifungal activities

The synthesized isoniazid derivatives (**1–19**) were evaluated in vitro for their antibacterial activity against Gram-

positive *S. aureus*, *B. subtilis*, Gram negative *E. coli* and antifungal activity against *C. albicans* and *A. niger* by the serial dilution method (Cappucino and Sherman, 1999) using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activities, respectively, and the results are presented in Table 6.

For antibacterial activity against *B. subtilis* all the synthesized derivatives were more active than the isoniazid. In particular, the compounds **4** and **13** were found to be the most effective antibacterial agents having pMIC_{bs} values 2.40 and 2.49 μM, respectively. The antibacterial activity value of these compounds approaches the antibacterial potential of

Table 5 Cytotoxicity and anti-RNA virus activity of synthesized isoniazid 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	>20	>20	>20	>20	>20
4	>100	>100	>100	>100	>100	>100
5	≥20	>20	>20	>20	>20	>20
6	≥100	>100	>100	>100	9	>100
7	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8
8	>100	>100	2.4	>100	45	>100
9	100	>20	>20	>20	>20	>20
10	≥100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	≥100	>100	>100	>100	9	>100
13	100	>20	>20	>20	>20	>20
14	≥20	>20	>20	>20	>20	>20

^a Required to cause a microscopically detectable alteration of normal cell morphology^b Required to reduce virus-induced cytopathicity by 50%**Table 6** Antibacterial and antifungal potentials (μM/ml) of synthesized isoniazid derivatives

Compound	pMIC _{bs}	pMIC _{sa}	pMIC _{ec}	pMIC _{ca}	pMIC _{an}	pMIC _b	pMIC _f	pMIC _{am}
Training set								
1	2.06	2.06	2.36	2.36	2.36	2.16	2.36	2.24
2	2.04	2.04	2.34	2.34	2.34	2.14	2.34	2.22
3	2.06	2.06	2.06	2.36	2.36	2.06	2.36	2.18
4	2.40	2.10	2.10	2.40	2.40	2.20	2.40	2.28
5	1.98	1.98	1.98	2.28	2.28	1.98	2.28	2.1
6	2.04	2.04	2.34	2.34	2.34	2.14	2.34	2.22
7	2.07	2.07	2.07	2.37	2.37	2.07	2.37	2.19
8	2.02	2.02	2.02	2.63	2.32	2.02	2.48	2.2
9	1.88	1.88	1.88	2.48	2.18	1.88	2.33	2.06
10	2.18	2.48	2.48	3.08	2.48	2.38	2.78	2.54
11	2.19	2.19	2.49	2.79	2.19	2.29	2.49	2.37
12	2.18	2.18	2.48	2.78	2.48	2.28	2.63	2.42
13	2.49	2.49	2.49	3.10	2.49	2.49	2.80	2.61
14	2.14	2.44	2.44	2.74	2.44	2.34	2.59	2.44
INH	1.74	2.04	1.74	2.34	2.04	1.84	2.19	1.98
SD	0.18	0.19	0.25	0.28	0.13	0.18	0.18	0.18
Test set								
15	1.98	1.98	2.29	2.29	2.29	2.08	2.29	2.17
16	2.44	1.84	2.44	3.04	2.44	2.24	2.74	2.44
17	2.11	2.11	2.11	2.41	2.41	2.11	2.41	2.23
18	2.34	2.34	1.74	2.64	1.74	2.14	2.19	2.16
19	1.96	2.26	2.26	2.26	2.26	2.16	2.26	2.20
Std.	2.61 ^a	2.61 ^a	2.61 ^a	2.64 ^b	2.64 ^b	2.61	2.64	2.62

SD standard deviation

^a Norfloxacin, ^b Fluconazole

the standard drug norfloxacin. In case of *S. aureus*, compounds **10** and **13** have shown marked antibacterial potential at pMIC_{sa} values 2.48 and 2.49 μ M, respectively. For antibacterial activity against *E. coli* compounds **10–13** had shown better antibacterial activity with pMIC_{ec} values of 2.48, 2.49, 2.48, and 2.49 μ M, respectively.

The antifungal activity against *C. albicans* revealed that compounds **10** and **13** were the potential candidates having pMIC_{ca} values 3.08 and 3.10 μ M, respectively. The compounds **10** and **13** have better antifungal activity than the standard drug fluconazole. In case of *A. niger* compounds **10**, **12**, and **13** were found to be active with pMIC_{an} values 2.48, 2.48, and 2.49 μ M, respectively.

The test set compounds (**15–19**) were also active against the tested microorganisms and compounds **16** and **18** were the most active among them, with their antifungal activity against *C. albicans* better than the standard drug fluconazole.

It can be observed from the results presented in Table 6 that the synthesized isoniazid derivatives have higher antifungal potential particularly against *C. albicans*. The compounds were also found to be more active against Gram-negative *E. coli* as compared to Gram-positive *B. subtilis* and *S. aureus*.

Structure activity relationship

From the results of antimicrobial activity, the following structure activity the relationship can be drawn:

1. The compounds have shown marked antifungal potential as compared to antibacterial activity, which shows that there are different structural requirements are essential for antibacterial and antifungal activities. These results are similar to those of Sortino *et al.* (2007).
2. It can also be seen from the results of antibacterial and antifungal activities that the activity increases with the addition of a bulkier substituent on the phenyl nucleus of the synthesized compounds which can be easily seen from the higher activity of compound **1** in comparison to compounds **2** and **6** in comparison to compound **5**, respectively.
3. The results of antimicrobial activity depicted that the presence of electron donating group, OCH₃, enhanced the antimicrobial activity of the synthesized derivatives. It can be observed from the results of antimicrobial activity that compound **2** was active and the addition of OCH₃ group made it more active (compound **3**) and further addition of OCH₃ (compound **4**) made the structure of the molecule highly active against the tested strains. This is supported by results of Emami *et al.* (2008).

4. The synthesized derivatives were more active toward the Gram-negative bacterium *E. coli* than Gram-positive *B. subtilis* and *S. aureus*. This finding is in agreement with Sbardella *et al.* (2004).
5. The substitution of NH proton with acyl group (compounds **10–14**) leads to increase in activity of the compounds. This may be due to the increase in lipophilicity of the molecules which may allow them to easily penetrate the microbial membrane. These results are in agreement with the observations of Imramovsky *et al.* (2007). These results are in contrast with the results of antimycobacterial activity where the activity decreases due to the *N*₁-benzoylation.
6. The presence of substituent at *ortho* position increases the antibacterial and antifungal potentials of the compounds which can be seen from the antimicrobial activity of compounds **3**, **7**, and **13**. This fact is supported by the observations of Guven *et al.* (2007).
7. Compound **17** was found to be highly active which may be due to the presence of electron withdrawing groups NO₂ and Cl in its structure.
8. Compound **16** having OH group at *ortho* position and NH proton being replaced by benzoyl group was also found to be highly active that may be attributed to the free OH group and its increased lipophilicity.

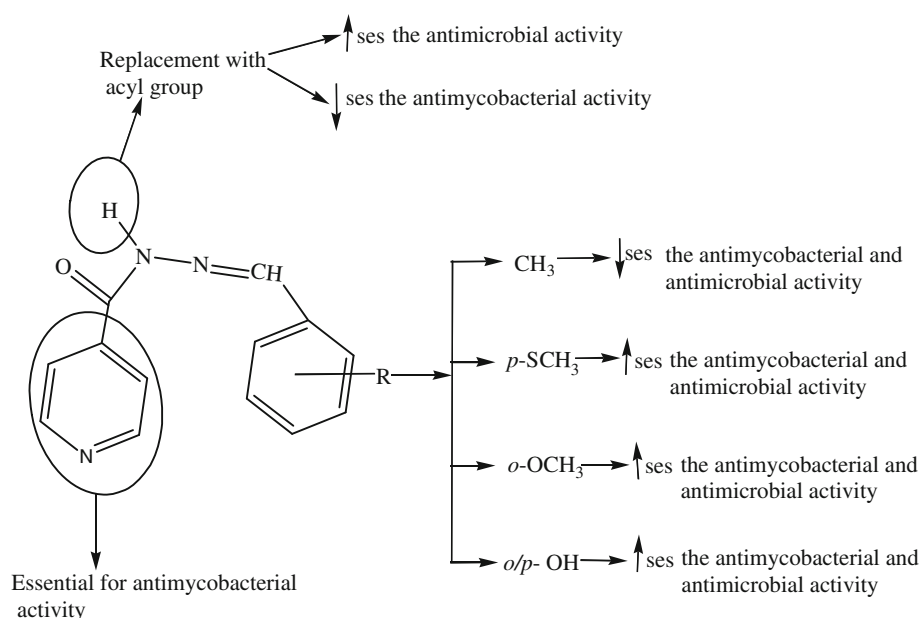
The SAR findings of the antimycobacterial and antimicrobial activities are summarized in Fig. 1.

QSAR studies

Development of one-target QSAR model

In order to identify the substituent effect on the antimicrobial activity, quantitative structure activity relationship (QSAR) studies between the in vitro activity and descriptors coding for lipophilic, electronic, steric, and topological properties of the 14 synthesized isoniazid derivatives (training set) were undertaken, using the linear free energy relationship model (LFER) described by Hansch and Fujita (1964). Biological activity data determined as MIC values were first transformed into pMIC values (i.e., $-\log \text{MIC}$) and used as dependent variable in 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 (*J*), Wiener topological index (*W*), total energy (*T_c*), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment (μ), nuclear repulsion energy (Nu.E) and electronic energy (Ele.E), calculated for

Fig. 1 Structural requirements for the antimycobacterial and antimicrobial activities of synthesized isoniazid derivatives



isoniazid derivatives are presented in Table 7 (Hansch *et al.*, 1973; Kier and Hall, 1976; Randic, 1975, 1993; Balaban, 1982; Wiener, 1947). Units of the energies and dipole were electron volts (eV) and atomic units (a.u.), respectively (Dai *et al.*, 1999).

In this study, a data set of 15 compounds (14 isoniazid derivatives along with isoniazid itself) was subjected to linear free energy regression analysis for model generation. Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity against *E. coli* is presented in Table 8. The correlations of different molecular descriptors with antibacterial and antifungal activities are presented in Table 9. In general, high colinearity ($r > 0.8$) was observed between different parameters. The high interrelationship was observed between ${}^0\chi$ and ${}^1\chi$ ($r = 0.998$) and low interrelationship was observed between T_e and μ ($r = 0.072$). The correlation matrix indicated the predominance of topological parameters and electronic parameters in describing the antimicrobial activity of the synthesized compounds.

The antibacterial activity of synthesized isoniazid derivatives against *E. coli* is governed by the valence first-order molecular connectivity topological index (${}^1\chi^v$) (Eq. 1).

ot-QSAR model for antibacterial activity against *E. coli*

$$\text{pMIC}_{\text{ec}} = 0.123^1\chi^v + 1.389 \quad (1)$$

$$n = 15, \quad r = 0.893, \quad q^2 = 0.756, \quad s = 0.118, \\ F = 50.93.$$

Here and thereafter, n is the number of data points, r is the correlation coefficient, q^2 is the cross-validated, r^2 is

obtained by LOO method, s is the standard error of the estimate, and F is the Fischer statistics.

For antibacterial activity against *E. coli*, the developed QSAR model (Eq. 1) describes the importance of valence first-order molecular connectivity topological index (${}^1\chi^v$). Topological indices 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 (Lather and Madan, 2005). The valence first-order molecular connectivity topological index (${}^1\chi^v$) represents the molecules with branched structure. In this case, the positive correlation was observed between ${}^1\chi^v$ and antibacterial activity against *E. coli* and the results presented in Table 9 are in concordance with the model expressed by Eq. 1 and values of ${}^1\chi^v$ shown in Table 10 which depict that compounds 10, 11, 12, and 13 having high ${}^1\chi^v$ values (8.35, 8.93, 9.53, and 8.74) have got the highest antibacterial potential (2.48, 2.49, 2.48, and 2.49).

The QSAR model expressed by Eq. 1 was cross-validated by its high q^2 values ($q^2 = 0.756$) obtained with 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 observed and predicted antibacterial activities is presented in Table 10. It can be seen from the results that the observed and predicted antibacterial activities lie close to each other as evidenced by their low residual values (Table 10). The plots of observed, predicted, and residual pMIC activity values were also developed to check the statistical validity of QSAR models. The plot of predicted pMIC_{ec} against observed pMIC_{ec} (Fig. 2) also favors the model expressed by Eq. 1. Further, the plot of observed pMIC_{ec} versus residual pMIC_{ec}

Table 7 Value of selected descriptors used in the regression analysis

Compound	log <i>P</i>	MR	σ_χ	σ_χ^v	$^1\chi$	$^1\chi^v$	$^2\chi$	$^2\chi^v$	κ_1	$\kappa\alpha_1$	W	T_c	Ele.E	LUMO	HOMO	NuE
1	2.38	78.46	15.08	11.48	10.20	6.40	8.51	4.10	17.36	15.77	1100.00	−3706.88	−22404.60	−0.64	−8.60	18697.70
2	2.04	73.71	14.37	10.78	9.70	5.81	8.13	3.87	16.37	14.79	956.00	−3551.09	−20697.90	−0.66	−8.72	17146.80
3	2.07	78.48	15.08	11.74	10.26	6.20	8.24	4.01	17.36	15.77	1034.00	−3706.07	−23220.10	−0.57	−8.57	19514.10
4	1.82	84.95	16.66	13.07	11.19	6.73	9.05	4.39	19.33	17.70	1354.00	−4181.98	−26948.80	−0.72	−8.74	22766.90
5	3.05	70.60	12.79	10.00	8.75	5.56	7.43	3.85	14.41	12.91	727.00	−2910.53	−16804.60	−0.62	−8.84	13894.10
6	2.67	78.46	13.50	11.29	9.29	6.99	7.60	4.66	15.39	14.23	858.00	−3104.86	−18266.70	−0.64	−8.10	15161.80
7	3.30	83.70	15.36	11.60	10.75	6.70	9.23	4.65	16.84	14.83	1155.00	−3614.56	−22975.60	−0.72	−8.59	19361.00
8	2.35	76.64	13.79	10.58	9.84	6.14	8.35	4.22	14.92	13.17	927.00	−3230.67	−19617.70	−0.52	−8.17	16387.10
9	1.77	55.70	10.23	7.84	6.84	4.19	5.22	2.48	12.07	11.07	363.00	−2370.69	−12032.40	−0.74	−9.05	9661.69
10	3.95	103.45	19.93	15.02	13.60	8.35	11.58	5.70	22.68	20.03	2092.00	−4821.59	−35250.20	−0.56	−8.72	30428.60
11	4.30	108.20	20.64	15.73	14.10	8.93	11.96	5.93	23.66	21.00	2324.00	−4977.37	−37270.20	−0.55	−8.68	32292.80
12	4.58	108.20	19.06	15.53	13.19	9.53	11.05	6.49	21.70	19.47	1930.00	−4375.34	−32386.70	−0.57	−8.20	28011.40
13	3.98	108.22	20.64	15.98	14.13	8.74	11.77	5.88	23.66	21.00	2252.00	−4976.67	−38088.70	−0.43	−8.68	33112.00
14	4.96	100.34	18.36	14.24	12.65	8.09	10.88	5.68	20.73	18.16	1711.00	−4181.01	−30568.50	−0.54	−9.01	26387.50
INH	0.02	36.93	7.40	5.24	4.84	2.75	3.78	1.71	8.10	7.25	121.00	−1804.50	−8006.92	−0.51	−10.43	6202.42
Test set																
15	2.294	67.250	12.795	9.446	8.771	5.286	7.328	3.494	14.410	12.876	705.000	−3075.000	−17335.400	−0.677	−8.727	14260.4
16	4.205	96.989	18.355	13.688	12.665	7.824	10.777	5.327	20.727	18.121	1673.000	−4345.680	−31454.400	−0.657	−9.027	27108.7
17	2.037	77.618	16.113	11.764	10.486	6.343	9.388	4.506	18.340	16.254	1162.000	−4266.060	−26128.600	−1.660	−10.413	21862.50
18	2.083	70.294	13.665	10.578	9.165	5.838	7.950	4.123	15.390	13.745	808.000	−3435.390	−19870.300	−0.841	−9.919	16434.900
19	0.781	54.563	11.259	8.846	7.109	7.109	6.174	6.174	13.067	12.171	414.000	−2924.200	−15111.100	−0.818	−10.162	12186.900

Table 8 Correlation matrix for antibacterial activity of synthesized isoniazid derivatives against *E. coli*

	log <i>P</i>	$^0\chi$	$^0\chi^v$	$^1\chi$	$^1\chi^v$	$^2\chi$	$^2\chi^v$	$^3\chi$	κ_1	<i>W</i>	<i>T</i> _c	Ele.E	μ	Nu.E	pMIC _{ec}
log <i>P</i>	1.000														
$^0\chi$	0.851	1.000													
$^0\chi^v$	0.863	0.992	1.000												
$^1\chi$	0.865	0.998	0.992	1.000											
$^1\chi^v$	0.902	0.958	0.980	0.966	1.000										
$^2\chi$	0.884	0.992	0.982	0.997	0.963	1.000									
$^2\chi^v$	0.921	0.949	0.969	0.960	0.995	0.963	1.000								
$^3\chi$	0.853	0.970	0.949	0.970	0.920	0.981	0.928	1.000							
κ_1	0.835	0.997	0.991	0.991	0.951	0.980	0.935	0.957	1.000						
<i>W</i>	0.839	0.985	0.972	0.981	0.943	0.976	0.932	0.958	0.982	1.000					
<i>T</i> _c	−0.778	−0.991	−0.976	−0.985	−0.923	−0.974	−0.906	−0.959	−0.992	−0.975	1.000				
Ele.E	−0.820	−0.992	−0.978	−0.987	−0.937	−0.978	−0.924	−0.959	−0.991	−0.995	0.988	1.000			
μ	−0.306	−0.137	−0.189	−0.132	−0.256	−0.125	−0.271	−0.125	−0.141	−0.229	0.072	0.189	1.000		
Nu.E	0.824	0.991	0.977	0.986	0.937	0.977	0.925	0.958	0.989	0.996	−0.986	−1.000	−0.202	1.000	
pMIC _{ec}	0.794	0.869	0.878	0.864	0.893	0.864	0.878	0.863	0.873	0.857	−0.851	−0.848	−0.224	0.846	1.000

Table 9 Correlation of molecular descriptors with antimicrobial activity of synthesized derivatives

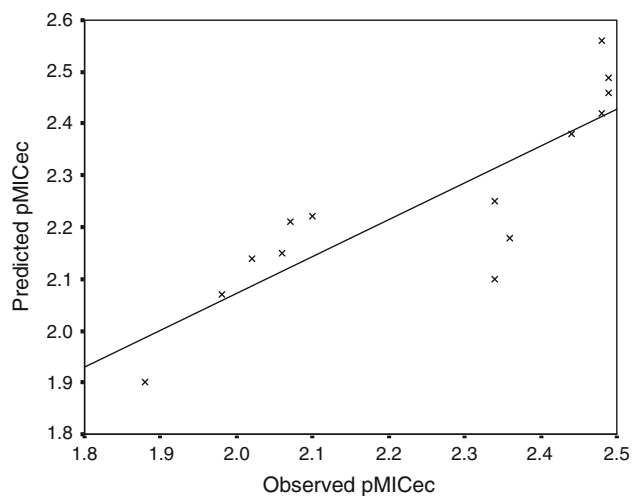
Mol. descriptor	pMIC _{bs}	pMIC _{sa}	pMIC _{ec}	pMIC _{ca}	pMIC _{an}	pMIC _b	pMIC _f	pMIC _{am}
log <i>P</i>	0.573	0.682	0.794	0.676	0.655	0.784	0.754	0.798
MR	0.813	0.743	0.877	0.739	0.759	0.923	0.841	0.919
$^0\chi$	0.847	0.773	0.869	0.750	0.737	0.941	0.842	0.931
$^0\chi^v$	0.854	0.744	0.878	0.725	0.763	0.937	0.831	0.924
$^1\chi$	0.837	0.767	0.864	0.752	0.746	0.933	0.846	0.928
$^1\chi^v$	0.775	0.700	0.893	0.706	0.748	0.903	0.811	0.895
$^2\chi$	0.808	0.771	0.864	0.747	0.739	0.925	0.840	0.920
$^2\chi^v$	0.755	0.715	0.878	0.702	0.761	0.894	0.812	0.890
$^3\chi$	0.780	0.798	0.863	0.706	0.708	0.924	0.796	0.903
$^3\chi^v$	0.698	0.704	0.851	0.615	0.753	0.859	0.741	0.840
κ_1	0.857	0.764	0.873	0.743	0.729	0.942	0.833	0.929
κ_2	0.851	0.710	0.869	0.717	0.712	0.921	0.806	0.905
$\kappa\alpha_1$	0.867	0.739	0.876	0.718	0.734	0.939	0.815	0.920
$\kappa\alpha_2$	0.854	0.658	0.860	0.668	0.709	0.900	0.767	0.876
<i>R</i>	0.837	0.767	0.864	0.752	0.746	0.933	0.846	0.928
<i>J</i>	−0.418	−0.226	−0.489	−0.188	−0.605	−0.439	−0.361	−0.420
<i>W</i>	0.814	0.810	0.857	0.818	0.649	0.937	0.864	0.937
<i>T</i> _c	−0.868	−0.759	−0.851	−0.728	−0.717	−0.935	−0.817	−0.917
Ele.E	−0.846	−0.812	−0.848	−0.798	−0.688	−0.944	−0.862	−0.941
LUMO	0.201	0.598	0.290	0.606	0.111	0.402	0.514	0.456
HOMO	0.465	0.049	0.521	0.171	0.647	0.409	0.361	0.402
μ	−0.126	−0.444	−0.224	−0.376	−0.062	−0.295	−0.313	−0.312
Nu.E	0.843	0.817	0.846	0.805	0.683	0.944	0.866	0.942

(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).

Equations 2–5 were developed to predict the antibacterial and antifungal activities of isoniazid derivatives against *B. subtilis*, *S. aureus*, *C. albicans*, and *A. niger*.

Table 10 Comparison of observed and predicted antibacterial and antifungal activities obtained by ot-QSAR model

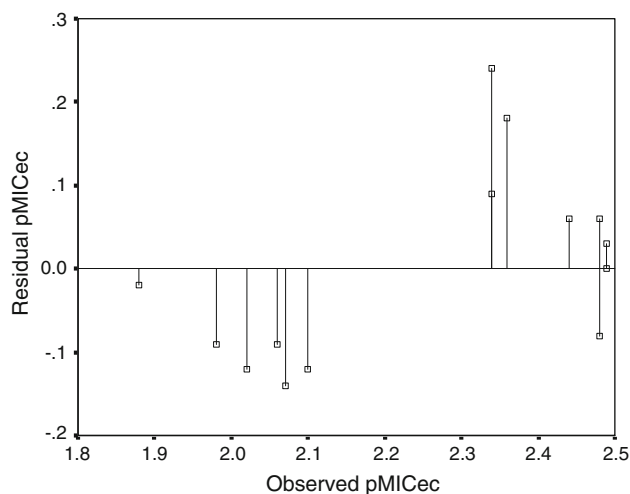
Compound	pMIC _{ec} (Eq. 1)			pMIC _{bs} (Eq. 2)			pMIC _{sa} (Eq. 3)			pMIC _{ca} (Eq. 4)			pMIC _{an} (Eq. 5)		
	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res
1	2.36	2.18	0.18	2.06	2.10	−0.04	2.06	2.10	−0.04	2.36	2.51	−0.15	2.36	2.32	0.04
2	2.34	2.10	0.24	2.04	2.07	−0.03	2.04	2.07	−0.03	2.34	2.46	−0.12	2.34	2.30	0.04
3	2.06	2.15	−0.09	2.06	2.10	−0.04	2.06	2.12	−0.06	2.36	2.48	−0.12	2.36	2.33	0.03
4	2.10	2.22	−0.12	2.40	2.18	0.22	2.10	2.18	−0.08	2.40	2.59	−0.19	2.40	2.37	0.03
5	1.98	2.07	−0.09	1.98	1.96	0.02	1.98	2.01	−0.03	2.28	2.38	−0.10	2.28	2.27	0.01
6	2.34	2.25	0.09	2.04	2.00	0.04	2.04	2.04	0.00	2.34	2.42	−0.08	2.34	2.32	0.02
7	2.07	2.21	−0.14	2.07	2.08	−0.01	2.07	2.11	−0.04	2.37	2.52	−0.15	2.37	2.33	0.04
8	2.02	2.14	−0.12	2.02	2.02	0.00	2.02	2.06	−0.04	2.63	2.45	0.18	2.32	2.29	0.03
9	1.88	1.90	−0.02	1.88	1.87	0.01	1.88	1.93	−0.05	2.48	2.26	0.22	2.18	2.21	−0.03
10	2.48	2.42	0.06	2.18	2.29	−0.11	2.48	2.32	0.16	3.08	2.84	0.24	2.48	2.43	0.05
11	2.49	2.49	0.00	2.19	2.32	−0.13	2.19	2.36	−0.17	2.79	2.92	−0.13	2.19	2.45	−0.26
12	2.48	2.56	−0.08	2.18	2.21	−0.03	2.18	2.28	−0.10	2.78	2.79	−0.01	2.48	2.45	0.03
13	2.49	2.46	0.03	2.49	2.32	0.17	2.49	2.37	0.12	3.10	2.89	0.21	2.49	2.46	0.03
14	2.44	2.38	0.06	2.14	2.18	−0.04	2.44	2.25	0.19	2.74	2.71	0.03	2.44	2.41	0.03
INH	1.74	1.73	0.01	1.74	1.77	−0.03	2.04	1.87	0.17	2.34	2.18	0.16	2.04	2.13	−0.09
Test set															
15	2.29	2.04	0.25	1.98	1.98	0.00	1.98	2.02	−0.04	2.29	2.37	−0.08	2.29	2.26	0.03
16	2.44	2.35	0.09	2.44	2.20	0.24	1.84	2.27	−0.43	3.04	2.72	0.32	2.44	2.39	0.05
17	2.11	2.17	−0.06	2.11	2.18	−0.07	2.11	2.16	−0.05	2.41	2.53	−0.12	2.41	2.33	0.08
18	1.74	2.11	−0.37	2.34	2.04	0.30	2.34	2.06	0.28	2.64	2.41	0.23	1.74	2.29	−0.55
19	2.26	1.97	0.29	1.96	1.96	0.00	2.26	1.98	0.28	2.26	2.28	−0.02	2.26	2.24	0.02

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

ot-QSAR model for antibacterial activity against *B. subtilis*

$$\text{pMIC}_{\text{bs}} = -0.00017T_e + 1.459 \quad (2)$$

$$n = 15, \quad r = 0.868, \quad q^2 = 0.660, \quad s = 0.095, \\ F = 39.79.$$

**Fig. 3** Plot of residual pMIC_{ec} against the experimental pMIC_{ec} for the linear regression model developed by Eq. 1

ot-QSAR model for antibacterial activity against *S. aureus*

$$\text{pMIC}_{\text{sa}} = 0.000019\text{Nu.E} + 1.750 \quad (3)$$

$$n = 15, \quad r = 0.817, \quad q^2 = 0.500, \quad s = 0.112, \\ F = 26.05.$$

ot-QSAR model for antifungal activity against *C. albicans*

$$\text{pMIC}_{\text{ca}} = 0.00034W + 2.134. \quad (4)$$

$$n = 15, \quad r = 0.818, \quad q^2 = 0.532, \quad s = 0.165, \\ F = 26.28.$$

ot-QSAR model for antifungal activity against *A. niger*

$$\text{pMIC}_{\text{an}} = 0.0315^0\chi^v + 1.960 \quad (5)$$

$$n = 15, \quad r = 0.763, \quad q^2 = 0.351, \quad s = 0.084, \\ F = 18.16.$$

The model expressed by Eq. 2 demonstrated that antibacterial activity against *B. subtilis* is governed by the total energy of the molecule (T_e). The total energy (T_e) calculated by semiempirical methods can be used as a measure of non-specific interactions of the drug with its target site, i.e., the total energies of protonated and neutral molecule, can be considered as a good measure of hydrogen bonds (the higher the energy, the stronger the bond) and can be used to determine the correct localization of the most favorable hydrogen bond acceptor site (Karelson *et al.*, 1996).

As the coefficient of total energy (T_e) in Eq. 2 is negative, therefore, the antibacterial activity against *B. subtilis* will increase with decreasing the T_e values. This is clearly evident from Table 6 that compounds 4, 11, and 13 having low T_e values -4181.98 , -4977.37 , and -4976.67 (Table 7) have highest antibacterial activity values of 2.40, 2.19, and 2.49 respectively. Similarly, compounds 5 and 9 having maximum T_e values -2910.53 and -2370.69 (Table 7) have minimum antibacterial activity against *B. subtilis* (Table 6).

In case of *S. aureus*, the developed QSAR model (Eq. 3) indicated the predominance of nuclear repulsion energy in describing the antibacterial activity. The nuclear repulsion energy between two atoms (A and B) is given by

$$E_{\text{Nu.E}}(\text{AB}) = Z_A Z_B / R_{\text{AB}}$$

where A is the given atomic species, B is the another atomic species, Z_A is the charge of atomic nucleus A, Z_B is the charge of atomic nucleus B, and R_{AB} is the distance between the atomic nuclei A and B (Clementi, 1980).

The coefficient of nuclear repulsion energy (Nu.E) is positive, which shows that the antibacterial activity will increase with the increase in nuclear repulsion energy of the synthesized compounds, which can be clearly seen from the results of antibacterial activity against *S. aureus* (Table 6) and values of nuclear repulsion energy presented in Table 7 that compounds 10, 13, and 14 had the highest antibacterial potential and compounds 5 and 9 had minimal antibacterial activity.

The model described by Eq. 4 depicted the importance of Wiener topological index (W) in describing the antifungal activity against *C. albicans*. The Wiener index (W) was introduced by Wiener (1947) to demonstrate the correlations between the physicochemical properties of organic compounds and the topological structure of their molecular graphs in terms of sum of distances between any two carbon atoms in the molecule.

The model described by Eq. 5 demonstrated the importance of valence first-order molecular connectivity topological index ($^1\chi^v$) in describing the antifungal activity against *A. niger*. The positive correlation of the molecular descriptor with antifungal activity reveals that increase in the value of $^1\chi^v$ (Table 7) will lead to an increase in antifungal activity against *A. niger*. The low residual values presented in Table 10 are in agreement with the fact that models expressed by Eqs. 2–5 are also valid ones.

As in case of Eq. 1, the high q^2 values ($q^2 > 0.5$) supported the validity of developed QSAR models described by Eqs. 2–4. The cross-validated correlation coefficient ($q^2 > 0.5$) values obtained for best QSAR model indicated their reliability in predicting the antimicrobial activity of synthesized compounds. In case of *A. niger* the q^2 (Eq. 5) value is less than 0.5, which shows that the developed model is an invalid one. But according to the recommendations of Kim *et al.* (2007), the regression models are acceptable if the value of standard deviation (SD, Table 6) is not much larger than 0.3. As the value of standard deviation in case of Eq. 5 is less than 0.3, i.e., 0.28, so the developed model is a valid one. It is important to note that Eqs. 1–5 were derived using the entire data set as there were no outliers in the data set.

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. It is important to note that the predictability of the QSAR models developed in this study is high evidenced by 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. Further, recent literature reveals that the QSAR have 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.*, 2006). 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 (Kumar *et al.*, 2007; Narasimhan *et al.*, 2007). The minimum standard deviation (Table 6) observed in the antimicrobial activity data justifies its use in QSAR studies. The low value of PE, LSE,

Table 11 PE, LSE, LOF, SE_P, Q , SD_{EP}, S_{press} , e^- , and RMSE values calculated for the derived models for modeling antimicrobial activity of synthesized isonicotinic acid-1-(substituted phenyl)-ethylidene/cycloheptylidene hydrazide derivatives

Equations	Descriptor	PE	LSE	LOF	SE _P	Q	e^-	RMSE	SD _{EP}	S_{press}
1	$^1\chi^v$	0.035	0.178	0.045	0.028	7.57	0.088	0.109	0.18	0.19
2	T_e	0.042	0.116	0.029	0.023	9.14	0.061	0.088	0.09	0.16
3	Nu.E	0.057	0.163	0.041	0.027	7.30	0.085	0.104	0.11	0.15
4	W	0.057	0.352	0.088	0.040	4.96	0.139	0.153	0.16	0.27
5	$^0\chi^v$	0.073	0.090	0.023	0.020	9.08	0.051	0.078	0.08	0.09
6	Ele.E	0.019	0.052	0.004	0.015	14.98	0.050	0.059	0.06	0.17
7	Nu.E	0.043	0.110	0.007	0.022	9.30	0.072	0.086	0.09	0.16
8	Nu.E	0.019	0.047	0.012	0.015	15.44	0.043	0.056	0.06	0.17

LOF, standard deviation of prediction (S_{press}), and standard deviation error of predictions (SD_{EP}) and SE_P and high value of r^2 and Q (Table 11) revealed the statistical significance of the developed QSAR models.

Development of multi-target QSAR model

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 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, 2008; Cruz-Monteagudo *et al.*, 2007; Gonzalez-Diaz and Prado-Prado, 2008).

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 synthesized isoniazid derivatives against all the above-mentioned microorganisms.

In order to develop mt-QSAR models, initially we have calculated the average antibacterial, antifungal, and antimicrobial activity values of isoniazid derivatives which are presented in Table 6. These average activity values were also correlated with the molecular descriptors of synthesized compounds (Table 9).

The mt-QSAR model for antibacterial activity displayed the importance of electronic energy (Ele.E) in describing the antibacterial activity of synthesized isoniazid derivatives, represented as the model expressed by Eq. 6.

mt-QSAR model for antibacterial activity

$$\text{pMIC}_b = -0.0000192 \text{ Ele.E} + 1.684 \quad (6)$$

$$n = 15, \quad r = 0.944, \quad q^2 = 0.852, \quad s = 0.063, \\ F = 106.40.$$

The mt-QSAR model for antifungal and antimicrobial activities revealed the importance of nuclear repulsion energy (Nu.E) in describing antifungal activity and the overall antimicrobial activity represented by Eqs. 7 and 8, respectively.

mt-QSAR model for antifungal activity

$$\text{pMIC}_f = 0.0000191 \text{ Nu.E} + 2.057 \quad (7)$$

$$n = 15, \quad r = 0.865, \quad q^2 = 0.636, \quad s = 0.093, \\ F = 38.88.$$

mt-QSAR model for antimicrobial activity

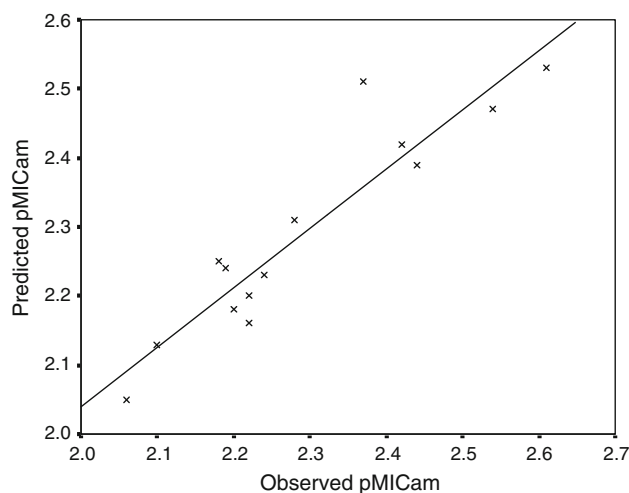
$$\text{pMIC}_{\text{am}} = 0.0000204 \text{ Nu.E} + 1.849 \quad (8)$$

$$n = 15, \quad r = 0.942, \quad q^2 = 0.835, \quad s = 0.061, \\ F = 03.09.$$

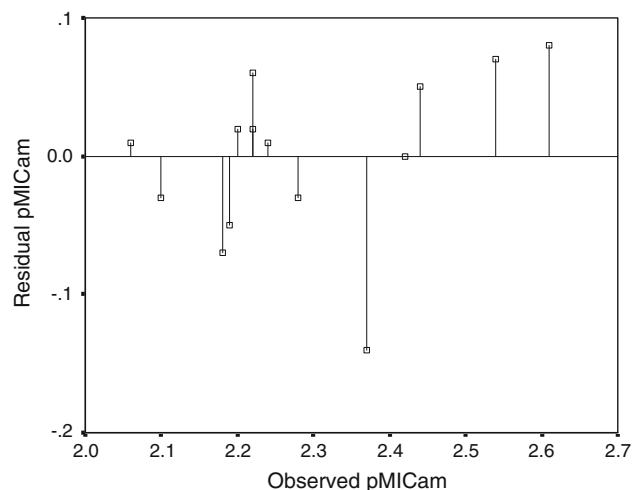
It was observed from mt-QSAR models (Eqs. 6–8) that the antibacterial, antifungal, and overall antimicrobial activities of synthesized isoniazid derivatives is governed by energy parameters, viz., nuclear repulsion energy (Nu.E) describing the antifungal and overall antimicrobial potential and electronic energy describing the antibacterial potential of the isoniazid derivatives. The developed mt-QSAR models were statistically valid as they had the high r and q^2 values and low residual values (Table 12). As in the case of ot-QSAR models, the plot of predicted pMIC_{am} against observed pMIC_{am} (Fig. 4) also favors the developed model

Table 12 Comparison of observed and predicted antibacterial, antifungal, and antimicrobial activities obtained by mt-QSAR model

Compound	pMIC _b (Eq. 6)			pMIC _f (Eq. 7)			pMIC _{am} (Eq. 8)		
	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res
Training set									
1	2.16	2.11	0.05	2.36	2.41	−0.05	2.24	2.23	0.01
2	2.14	2.08	0.06	2.34	2.38	−0.04	2.22	2.20	0.02
3	2.06	2.13	−0.07	2.36	2.43	−0.07	2.18	2.25	−0.07
4	2.20	2.20	0.00	2.40	2.49	−0.09	2.28	2.31	−0.03
5	1.98	2.01	−0.03	2.28	2.32	−0.04	2.10	2.13	−0.03
6	2.14	2.04	0.10	2.34	2.35	−0.01	2.22	2.16	0.06
7	2.07	2.13	−0.06	2.37	2.43	−0.06	2.19	2.24	−0.05
8	2.02	2.06	−0.04	2.48	2.37	0.11	2.20	2.18	0.02
9	1.88	1.92	−0.04	2.33	2.24	0.09	2.06	2.05	0.01
10	2.38	2.36	0.02	2.78	2.64	0.14	2.54	2.47	0.07
11	2.29	2.40	−0.11	2.49	2.67	−0.18	2.37	2.51	−0.14
12	2.28	2.31	−0.03	2.63	2.59	0.04	2.42	2.42	0.00
13	2.49	2.42	0.07	2.80	2.69	0.11	2.61	2.53	0.08
14	2.34	2.27	0.07	2.59	2.56	0.03	2.44	2.39	0.05
INH	1.84	1.84	0.00	2.19	2.17	0.02	1.98	1.98	0.00
Test set									
15	2.08	2.02	0.06	2.29	2.33	−0.04	2.17	2.14	0.03
16	2.24	2.29	−0.05	2.74	2.58	0.16	2.44	2.4	0.04
17	2.11	2.19	−0.08	2.41	2.48	−0.07	2.23	2.3	−0.07
18	2.14	2.07	0.07	2.19	2.37	−0.18	2.16	2.18	−0.02
19	2.16	1.84	0.32	2.26	2.17	0.09	2.20	1.97	0.23

**Fig. 4** Plot of observed pMIC_{am} against the predicted pMIC_{am} for the linear regression model developed by Eq. 8

expressed by Eq. 8. Further, the plot of observed pMIC_{am} versus residual pMIC_{am} (Fig. 5) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero, i.e., both positive and negative residual values were observed. The low value of

**Fig. 5** Plot of residual pMIC_{am} against the experimental pMIC_{am} for the linear regression model developed by Eq. 8

PE, LSE, LOF, standard deviation of prediction (S_{press}) and standard deviation error of predictions (SD_{EP}) and SE_P and high value of r^2 and Q (Table 11) revealed the statistical significance of the developed QSAR models.

Validation of predictability of developed QSAR models using test set

In order to validate the predictability of the developed QSAR models, we have synthesized a test set of five compounds (15–19) and their antimicrobial activity values were determined experimentally as well as predicted using the developed QSAR models and the results are presented in Tables 10 and 12. The results of the predicted antimicrobial activity indicated that the developed models were efficiently predicting the antimicrobial activity of the synthesized compounds with similar structure (compounds 15 and 16) as well as compound with slightly different structures (compounds 17–19). The low residual values for these compounds indicated that the developed QSAR models were valid as they were predicting the antimicrobial activity quite effectively. The mt-QSAR models were more precise in prediction as their residual values were very low.

Conclusion

A series of isoniazid derivatives (1–19) was synthesized and tested for their in vitro antimycobacterial activity against *M. tuberculosis* and the compounds with OH, SCH₃, and OCH₃ groups were found to be active ones and

compound **17** synthesized in the test set was twice active than ciprofloxacin and thrice active than ethambutol. The results of antiviral activity testing showed that none of the tested compounds was active against a broad variety of RNA and DNA viruses at subtoxic concentrations, except compound **8** that showed a selective anti-reovirus-1 activity. The synthesized compounds were also screened for their antimicrobial potential against *S. aureus*, *B. subtilis*, *E. coli*, *C. albicans*, and *A. niger* and the results of antimicrobial activity were similar to the antimycobacterial activity except that N_1 -acyl substituted derivatives (**10–14**) were the most active antimicrobial agents. To understand the relationship between physicochemical parameters and antibacterial and antifungal activities of isoniazid derivatives, QSAR investigation was performed by development of one target and multi target models. The multi-target model was found to be effective in describing the antimicrobial activity of isoniazid derivatives in comparison to the one target models and indicated the importance of nuclear repulsion energy (Nu.E). The external validation of QSAR models was done by the prediction of antimicrobial activity of test set compounds.

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