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Synthesis, Antimycobacterial, Antiviral, Antimicrobial Activity and QSAR Studies of N_2-acyl isonicotinic Acid Hydrazide Derivatives

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Abstract: A series of N_2-acyl isonicotinic acid hydrazides (**1-17**) was synthesized and tested for its *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* and the results indicated that the compound, isonicotinic acid N'-tetradecanoyl-hydrazide (**12**) was more active than the reference compound isoniazid. The results of antimicrobial activity of the synthesized compounds against *S. aureus, B. subtilis*, *E. coli, C. albicans* and *A. niger* indicated that compounds with dichloro, hydroxyl, tri-iodo and N_2 –tetradecanoyl substituent were the most active ones. The antiviral activity studies depicted that none of the tested compounds were active against DNA or RNA viruses. The multi-target QSAR model was found to be effective in describing the antimicrobial activity of N_2-acyl isonicotinic acid hydrazides.

Keywords: Antimicrobial, Antitubercular, Antiviral, N_2-acyl Isoniazid, QSAR.

INTRODUCTION

Tuberculosis (TB) is alarmingly on the rise. Approximately one third of the world's population is infected with TB bacillus, *Mycobacterium tuberculosis*, with more than 8 million people contracting the disease and 2 million people dying of it each year. A peculiar aspect of its pathogenicity comes from the fact that it remains quiescent and becomes active decades later. One of the most significant risk factor for developing tuberculosis is human immunodeficiency virus (HIV) infection. The current treatment of active TB includes a dosage regime of four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for at least six months. As a consequence of the prolonged duration, irregular treatment, and highly adaptive nature of the organisms to the surroundings, multidrug resistant (MDR) strains of *M. tuberculosis* have developed [1]. The emergence of AIDS, decline in socioeconomic standards and a reduced emphasis on TB control programs contribute to disease's resurgence in industrialized countries [2]. The search for novel antibacterial agents active against *Mycobacterium tuberculosis* and other atypical mycobacteria is urgent due to lack of effectiveness of known antitubercular agents against opportunistic pathogens as a consequence of rapidly emerging resistance. Fur thermore, immunocompromised patients observe with AIDS, or after transplantation, are easily infected by pathogenic fungi, protozoa and mycobacteria leading rapidly to death [3]. There are two basic approaches to develop new drugs for TB: (i) synthesis of analogues, modification or derivatives of existing compounds for shortening and improving TB treatment and (ii) searching for novel structures, that the TB organism has never been presented with before, for the treatment of MDR-TB [4].

The emergence of resistance in most of the pathogenic bacteria to the currently available antibacterial agents is the major problem in the treatment of serious bacterial infections caused by these organisms. These resistant strains curtail the life span of the drug [5]. During the past years an increasing interest has been devoted to the study of new and more selective antimicrobial agents. Due to this, not only have new synthetic methods been developed, but a greater amount of interest has been devoted to comprehension of their mechanism of action and structure activity relationships [6].

Acquired immunodeficiency syndrome (AIDS) is one of the most frightening syndromes worldwide. AIDS was first identified in 1983 in U.S.A. and the studies revealed that the virus entered the U.S. population sometime in the late 1980, Center for Disease Control and Prevention (CDCP) has defined AIDS as, all HIV infected people with fewer than 200 CD4+ T cells per cubic millimeter of blood (normal is 1000-1200). There are now more than 40 million people living with AIDS worldwide and globally 24.8 million people have died of AIDS since the beginning of epidemic and it is esti-

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mated that 68 million will die of AIDS by 2020 [7]. AIDS remains an enormous health threat, although chemotherapeutic agents have increased in number and effectiveness. Both nucleoside (AZT, DDI, DDC, D4T, 3TC) and non-nucleoside (nevirapine, delavirdine) HIV reverse transcriptase (RT) inhibitors and HIV protease (saquinavir, indinavir, ritonavir, nelfinavir) inhibitors have been licensed by the US FDA. Also, combination therapy of inhibitors of both groups results in undetectable levels of HIV in the blood of infected patients [8]. The US Food and Drug Administration (FDA) approved medications have limitations such as high cost, decreased sensitivity due to the rapid emergence of drug-resistant mutants, and adverse effects like peripheral neuropathy, bone marrow suppression, and anemia. Thus, more effective and less toxic anti-HIV agents are still needed. In addition, alternative approaches, including herbal therapies after long-term screening of plant extracts, particularly anti-infective or immunomodulating medicinal herbs and the structural modification of lead compounds, have been attempted [9].

Biological activities of the molecules are a function of their chemical and physical properties. A structure–activity relationship is a qualitative association between a chemical substructure and the potential of a chemical to exhibit a certain biological effect. A quantitative structure–activity relationship (QSAR) is a mathematical model that relates a quantitative measure of chemical structure to a biological effect. Thus, the structure–activity relationship of the molecules could be explained quantitatively [10]. Quantitative structure–activity relationships (QSARs) represent an attempt to correlate structural properties of the compounds with biological activities and chemical reactivity. These chemical descriptors, which include parameters to account for hydrophobicity, electronic, inductive, or polar properties, and steric effects, are determined empirically or by calculations [11].

Isoniazid (INH) is the most frequently prescribed primary prophylactic and chemotherapeutic drug against *M. tuberculosis*. Enzymatic acylation of the antitubercular isoniazid (INH) by arylamine *N*-acetyl transferases (NATs) reduces the therapeutic effectiveness of the drug. Because it represents a major metabolic pathway for INH in human beings, such acetylation has serious consequences for tuberculosis treatment regimens. Among patients in whom this process is efficient, the "rapid acetylators," the resultant chronic underdosing of INH may give rise to the development of resistance, as well as inadequate therapy. NATs occur in mycobacteria and in their mammalian hosts. It is thought that the resultant chemical change prevents the activation of INH that is required for proper drug action. A recombinant NAT from *M. tuberculosis* acetylates INH *in vitro*. When the corresponding *nat* gene is over expressed in a suitable INH-susceptible host, *Mycobacterium smegmatis*, the resultant organism becomes more resistant to INH. Resistance to INH in mycobacteria can thus be related to increased expression of NAT. Not much work has been done previously to characterize the antitubercular properties of other N_2- acyl isoniazid derivatives. The appreciable *in vitro* and *in vivo* antimycobacterial activity of acylated derivatives of isoniazid has been reported by Hearn and Cynamon [12]. The isoniazid derivatives have also been reported to possess antimicrobial activity [13-15]. In order to bring into sharper focus the fundamental issue of the activities of acylated derivatives of INH and in pursuit of achieving this goal, our research efforts focused on the synthesis, antimicrobial, antimycobacterial, antiviral and QSAR studies [16-18], herewith we report the synthesis, antimycobacterial, anti-HIV, antimicrobial and QSAR studies of N_2-acyl isonicotinic acid hydrazide derivatives.

MATERIAL AND METHODS

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. ^{1}H nuclear magnetic resonance (^{1}H 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 spectrophotometer.

General Procedure for Synthesis of Ester

The mixture of isonicotinic acid (0.1 mol) and ethanol (in excess) was refluxed with sulphuric 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 N_2-acyl Isonicotinic Acid Hydrazides (1-15)

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

Benzoic acid N'-(pyridine-4-carbonyl)-hydrazide (1): Mp (°C) 180-183; Yield– 89.4%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.42-7.58 (m, 3H, CH of C_3, C_4 and C_5 of phenyl ring), 7.97-8.01 (d, 2H, CH of C_2 and C_6 of phenyl ring), 8.33-8.35 (d, 2H, CH of C_3 and C_5 of pyridine ring), 8.91-8.93 (d, 2H, CH of C_2 and C_6 of pyridine ring), 10.55 (s, 1H, NH proton of N_2), 11.24 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3217.78 (NH str., amide), 3005.15 (CH

str., aromatic), 1611.24 (C=O str., Nicotinoyl), 1570.86 (C=O str., Acyl), 1466.363 (C=C str., skeletal phenyl nucleus), 1248.06 (C-N str., coupled vibrations amide), 839.47 (CH out of plane bending, 4-substituted pyridine), 720.11 (OCN deformations, amide IV band), 634.84 (OCN deformations, amide VI band).

3-Methyl-benzoic acid N'-(pyridine-4-carbonyl)-hydrazide (2): Mp (°C) 194-197; Yield– 85.68%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 2.38 (s, 3H, CH_3), 7.32-7.37 (m, 2H, CH of C_4 and C_5 of phenyl ring), 7.75-7.79 (d, 2H, CH of C_2 and C_6 of phenyl ring), 8.51-8.52 (d, 2H, CH of C_3 and C_5 of pyridine ring), 9.11-9.12 (d, 2H, CH of C_2 and C_6 of pyridine ring), 10.76 (s, 1H, NH proton of N_2), 11.43 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3209.44 (NH str., amide), 3074.98 (CH str., aromatic), 2967.28 (CH str., aliphatic), 1692.50 (C=O str., Nicotinoyl), 1654.68 (C=O str., Acyl), 1513.01 (C=C str., skeletal phenyl nucleus), 1279.97 (C-N str., coupled vibrations amide), 825.38 (CH out of plane bending, 4-substituted pyridine), 746.05 (OCN deformations, amide IV band), 632.01 (OCN deformations, amide VI band).

Isonicotinic acid N'-[2-(2,4-dichloro-phenyl)-acetyl]-hydrazide (5): Mp (°C) 222-225; Yield– 59.35%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 3.70 (s, 2H, CH_2), 7.26 (s, 1H, CH of C_6 of 2,4-dichlorophenyl ring), 7.33 (s, 1H, CH of C_5 of 2,4-dichlorophenyl ring), 7.42 (s, 1H, CH of C_3 of 2,4-dichlorophenyl ring), 8.46-8.48 (d, 2H, CH of C_3 and C_5 of pyridine ring), 9.09-9.10 (d, 2H, CH of C_2 and C_6 of pyridine ring), 10.65 (s, 1H, NH proton of N_2), 11.40 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3168.70 (NH str., amide), 3025.58 (CH str., aromatic), 1690.65 (C=O str., Nicotinoyl), 1625.24 (C=O str., Acyl), 1534.78 (C=C str., skeletal phenyl nucleus), 1280.68 (C-N str., coupled vibrations amide), 819.36 (CH out of plane bending, 4-substituted pyridine), 787.96 (OCN deformations, amide IV band), 638.16 (OCN deformations, amide VI band).

Isonicotinic acid N'-(2-chloro-pyridine-3-carbonyl)-hydrazide (8): Mp (°C) 160-163; Yield– 70.30%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.44-7.46 (t, 1H, CH of C_5 of 2-chloronicotinoyl ring), 7.98-8.02 (d, 2H, CH of C_3 and C_5 of pyridine ring), 8.49-8.52 (d, 1H, CH of C_6 of 2-chloronicotinoyl ring), 8.81-8.85 (d, 2H, CH of C_2 and C_6 of pyridine ring), 8.86-8.88 (d, 1H, CH of C_4 of 2-chloronicotinoyl ring), 9.90 (s, 1H, NH proton of N_2), 10.80 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3161.47 (NH str., amide), 1702.25 (C=O str., Nicotinoyl), 1585.55 (C=O str., Acyl), 1488.15 (C=C str., skeletal phenyl nucleus), 1256.68 (C-N str., coupled vibrations amide), 832.32 (CH out of plane bending, 4-substituted pyridine), 761.91 (C-Cl str., aromatic), 652.93 (OCN deformations, amide IV band), 542.02 (OCN deformations, amide VI band).

Isonicotinic acid N'-(3-phenyl-acryloyl)-hydrazide (9): Mp (°C) 208-211; Yield– 69.32%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.40-6.44 (d, 1H, CH of –CO-CH=), 6.83-6.87 (d, 1H, CH of =CH-C_6H_5), 7.55-7.74 (m, 5H, C_6H_5), 8.55-8.56 (d, 2H, CH of C_3 and C_5 of pyridine ring), 9.09-9.10 (d, 2H, CH of C_2 and C_6 of pyridine ring), 10.71 (s, 1H, NH proton of N_2), 11.63 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3195.22 (NH str., amide), 3011.98 (CH str., aromatic), 1680.07 (C=O str., Nicotinoyl), 1643.42 (C=O str., Acyl), 1501.65 (C=C str., skeletal phenyl nucleus), 1216.17 (C-N str., coupled vibrations amide), 842.93 (CH out of plane bending, 4-substituted pyridine), 643.29 (OCN deformations, amide VI band).

Isonicotinic acid N'-tetradecanoyl-hydrazide (12): Mp (°C) 194-197; Yield– 65.48%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 0.82-0.85 (t, 3H, CH_3), 1.21-1.27 (m, 20H, CH of CH_2 of C_4-C_{13} of Myristoyl chain), 1.54-1.56 (m, 2H, CH of CH_2 of C_3 of Myristoyl chain), 2.50-2.51 (m, 2H, CH of CH_2 of C_2 of Myristoyl chain), 8.13-8.14 (d, 2H, CH of C_3 and C_5 of pyridine ring), 8.92-8.94 (d, 2H, CH of C_2 and C_6 of pyridine ring), 10.09 (s, 1H, NH proton of N_2), 10.93 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3192.08 (NH str., amide), 2955.28 (CH str., aliphatic), 1700.23 (C=O str., Nicotinoyl), 1647.28 (C=O str., Acyl), 1495.93 (C=C str., skeletal phenyl nucleus), 1470.13 (CH bending, CH_3), 1281.55 (C-N str., coupled vibrations amide), 847.90 (CH out of plane bending, 4-substituted pyridine), 758.04 (OCN deformations, amide IV band), 652.47 (OCN deformations, amide VI band).

3-Hydroxy-benzoic acid N'-(pyridine-4-carbonyl)-hydrazide (13): Mp (°C) 182-185; Yield– 67.8%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.98 (s, 1H, OH), 7.21-7.24 (m, 1H, CH of C_4 of phenyl ring), 7.32-7.36 (m, 2H, CH of C_2 and C_6 of phenyl ring), 7.53-7.57 (m, 1H, CH of C_5 of phenyl ring), 8.51-8.53 (d, 2H, CH of C_3 and C_5 of pyridine ring), 9.14-9.18 (d, 2H, CH of C_2 and C_6 of pyridine ring), 9.80 (s, 1H, NH proton of N_2), 10.75 (s, 1H, NH proton of N_1). IR (KBr pellets) ν cm^{-1}: 3182.68 (NH str., amide), 3077.56 (CH str., aromatic), 1719.61 (C=O str., Nicotinoyl), 1681.04 (C=O str., Acyl), 1509.36 (C=C str., skeletal phenyl nucleus), 1283.68 (C-N str., coupled vibrations amide), 815.63 (CH out of plane bending, 4-substituted pyridine), 787.96 (OCN deformations, amide IV band), 632.68 (OCN deformations, amide VI band).

General Procedure for the Synthesis of Isonicotinic Acid (3-phenyl-allylidene)-hydrazide (16)

Isonicotinic acid hydrazide (0.05 mol) and cinnamaldehyde (0.06 mol) in ethanol was refluxed for appropriate time. At the completion of reaction the precipitates start to appear which were filtered off and recrystallized from ethanol.

Isonicotinic acid (3-phenyl-allylidene)-hydrazide (16): Mp (°C) 195-198; Yield– 61.9%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.97-7.05 (m, 2H, CH of –CH=CH-), 7.30-7.32 (t, 1H, CH of C_4 of phenyl ring), 7.37-7.40 (m, 2H, CH of C_3 and C_5 of phenyl ring), 7.50-7.52 (m, 2H, CH of C_2 and C_6 of phenyl ring), 7.82-7.83 (d, 2H, CH of C_3 and C_5 of pyridine ring), 8.25-8.27 (d, 1H, CH of N=CH), 8.75-8.76 (d, 2H, CH of C_2 and C_6 of pyridine ring), 11.94 (s, 1H, NH). IR (KBr pellets) ν cm^{-1}: 3228.98 (NH str., amide), 3037.05 (CH str., aromatic), 2988.83 (CH str., aliphatic), 1650.17 (C=O str., Nicotinoyl), 1573.02 (C=C str., skeletal phenyl nucleus), 1153.48 (C-N str., coupled vibrations amide), 830.39 (CH out of plane bending, 4-substituted pyridine), 676.08 (OCN deformations, amide VI band).

General Procedure for the Synthesis of Benzoic acid-N^2-(3-phenylallylidene)-N^1-(pyridine-4-carbonyl)-hydrazide (17):

Isonicotinic acid-3-phenylallylidene hydrazide (**16**) was dissolved in dichloromethane (0.001 mol) and a solution of benzoyl chloride (0.001 mol) in dichloromethane was added drop wise and kept for stirring on a magnetic stirrer till the evaporation of dichloromethane. The product obtained was checked for purity by TLC and recrystallized from ethanol.

Benzoic acid-N^2-(3-phenylallylidene)-N^1-(pyridine-4-carbonyl)-hydrazide (17): Mp (°C) 203-206; Yield– 67.5%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.33-7.58 (m, 10H, phenyl rings), 7.97-8.03 (m, 2H, CH of –CH=CH-), 8.44-8.46 (d, 2H, CH of C_3 and C_5 of pyridine ring), 8.65-8.67 (d, 1H, CH of N=CH), 8.93-8.96 (d, 2H, CH of C_2 and C_6 of pyridine ring). IR (KBr pellets) ν cm^{-1}: 3217.40 (NH str.), 3016.80 (CH str., aromatic), 2906.85 (CH str., aliphatic), 1605.81 (C=O str), 1474.64 (C=C str., skeletal phenyl nucleus), 1290.43 (C-N str., coupled vibrations amide), 840.04 (CH out of plane bending, 4-substituted pyridine), 674.15 (OCN deformations, amide VI band).

Evaluation of Antimycobacterial Activity

All compounds were screened for their *in vitro* antimycobacterial activity against MTB, in Middlebrook 7H11agar medium supplemented with OADC (Oleic Acid and Dextrose as Carbon source) by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate [19]. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth.

Evaluation of Antimicrobial Activity

The antimicrobial activity was performed against Gram-positive bacteria: *S. aureus*, *B. sublitis*, Gram-negative bacterium: *E. coli* and fungal strains: *C. albicans* and *A. niger* by tube dilution method [20]. 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. [21] (fungi). The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. niger*) and at 37 °C for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

QSAR STUDIES

Preparation of Data Set

An Intel (R) Core (TM) 2 Duo personal computer (CPU T 6600 at 2.20 GHz) with windows XP operating system was used. To obtain a QSAR model, the compounds must be represented by molecular descriptors that retain as much structure information as possible. The descriptors were generated as follows: The structures of N_2-acyl isonicotinic acid hydrazide derivatives were first pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in Hyperchem 6.03 [22] and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). We chose a gradient norm limit of 0.01 kcal/A˚ for the geometry optimization. The output files were used for producing constitutional, topological, electrostatic, and semiempirical descriptors using TSAR 3.3 software for Windows [23]. These quantitative descriptors contain information on the inter atom connections and the shape, branching, symmetry, distribution of charge and quantum-chemical properties of the molecule. Further, the regression analysis was performed using the SPSS software package [24]. 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 [25].

Calculation of Statistical Parameters [26, 27]

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 (SEP), quality value (*Q*) and SSY (sum of squares of response values)

These parameters were calculated from the following equations.

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

Where, r, correlation coefficient and n, number of compounds used.

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

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

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

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

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

The Quality value, *Q* is given by

$$Q = r/Se$$

Where, *r*, correlation coefficient and Se, standard error.

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

$$q^2 = 1- \{\Sigma(Y_{obs}-Y_{calc})^2/ \Sigma(Y_{obs}-Y_{mean})^2\}$$

The resubstitution test statistical parameters [28] *viz*. regression coefficient (r), root mean square error (RMSE) and absolute average error ($\bar{e}$) were calculated as:

$$\text{Regression coefficient (r)} = \sqrt{\{ 1 - (\Sigma(Y_{calc} - Y_{obs})^2/ \Sigma(Y_{calc} - Y_{mean})^2)\}}$$

The Absolute average error ($\bar{e}$) is used to illustrate the predictive accuracy more explicitly and given by

$$\text{Absolute average error } (\bar{e}) = \{\Sigma|Y_{calc} - Y_{obs}|\}/n$$

The root mean square error (RMSE) is calculated mathematically as:

$$\text{Root mean square error (RMSE)} = \sqrt{\{\Sigma(Y_{calc} - Y_{obs})^2\}}/n$$

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

Variation Inflation Factor [29] is employed to determine the multicolinearity between the physicochemical parameters. The VIF value is calculated as

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

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.

Theory of Stepwise Multiple Linear Regression (MLR) [30]

Multiple linear regression (MLR) is the most widely used multiple linear modeling techniques. Following is the regression equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + \ldots . + b_nX_n$$

In this equation, Y is the property, that is, the dependent variable; X_1–X_n represents the specific descriptors, while b_1–b_n represents the coefficient of those descriptors, and b_0 is the intercept of this equation. As is well known, MLR cannot be used to model complex data, since in most cases the number of explanatory variables exceeds the number of objects. Therefore, it is often used in combination with the stepwise procedure for variable selection. The forward stepwise regression procedure consists simply in a step-by-step addition of the best descriptors to the model that leads to the smallest standard deviation(s), until there is no other variable outside the equation that satisfies the selection criteria.

Evaluation of Anti-HIV Activity

The anti-HIV activity and cytotoxicity were evaluated against HIV-1 strain IIIB and HIV-2 ROD in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [31]. 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 x 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 a 4-d 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, 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.

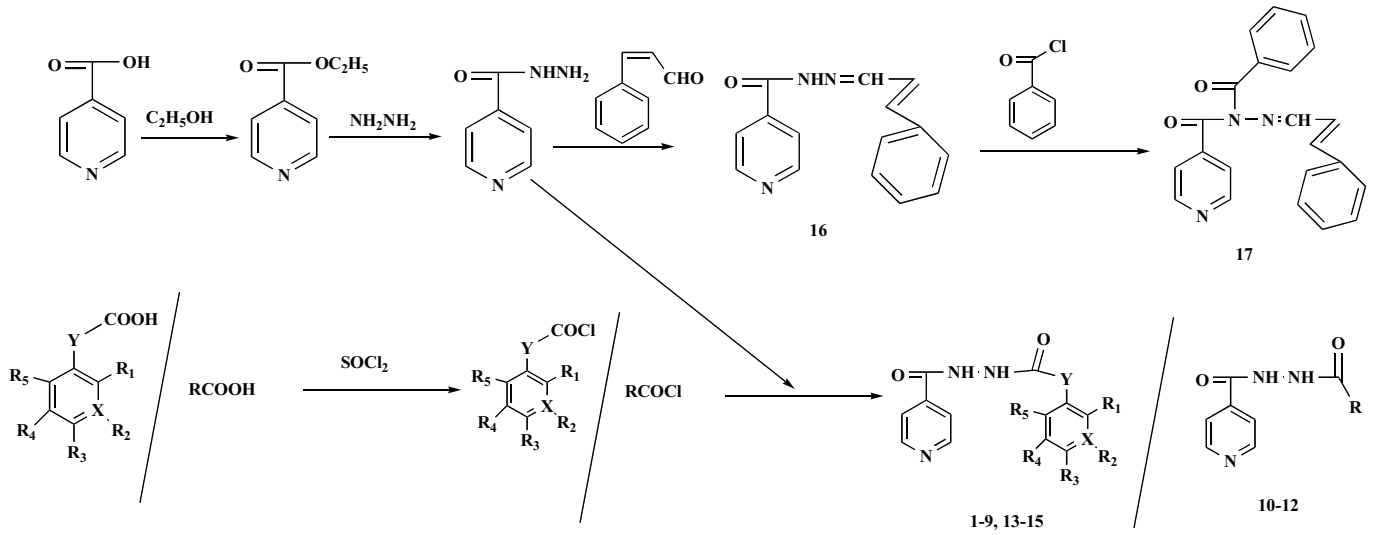
RESULTS AND DISCUSSION

Chemistry

The synthesis of N_2-acyl isonicotinic acid hydrazide derivatives was carried out as demonstrated by Scheme **1**. The isonicotinic acid was refluxed with ethanol in the presence of sulphuric 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 N_2-acyl isonicotinic acid hydrazide derivatives (**1-15**) were synthesized by the reaction of isonicotinic acid hydrazide, suspended in dichloromethane, with respective acyl chlorides which in turn were obtained by refluxing the respective acids with thionyl chloride. The isonicotinic acid (3-phenyl-allylidene)-hydrazide (**16**) was synthesized by refluxing isonicotinic acid hydrazide with cinnamaldehyde in ethanol and benzoic acid-N^2-(3-phenylallylidene)-N^1-(pyridine-4-carbonyl)-hydrazide (**17**) was prepared by the reaction of benzoyl chloride with Isonicotinic acid-3-phenylallylidene hydrazide (**16**). The yield of target compounds was appreciable and their physicochemical characteristics are presented in Table **1**.

The purity of N_2-acyl isonicotinic acid hydrazide derivatives was checked by single spot TLC and their IR and NMR spectra were found in agreement with their assigned molecular structures. The singlet signals observed around δ 10 ppm and δ 11 ppm represents the NH protons at N_2 and N_1 position of the hydrazide portion in compounds **1, 2, 5, 8, 9, 12** and **13**. This clearly signifies that the acyl group was attached to the isonicotinic acid hydrazide by replacing one of the two protons of NH_2 group. Similarly two doublet signals, in the region δ 7.90 – 9.20 ppm, each having an integral equivalent to two protons corresponds the presence of four CH protons of the pyridine ring in compounds **1, 2, 5, 8, 9, 12, 13, 16** and **17**. The presence of phenyl ring in the structure of compound **1** was confirmed by the multiplet signal having integral of five protons at δ 7.42 – 7.58 ppm. In compound **2**, a triplet at δ 2.38 ppm revealed the presence of a methyl group on the phenyl ring in its structure. The appearance of signals for three aromatic protons and a singlet at δ 3.70 ppm confirmed that 2,4-dichloro-phenyl)-acetyl group was attached at N_2 position in compound **5**. The doublet signals at δ 6.40 – 6.44 ppm and δ 6.83 – 6.87 ppm corresponding to CH protons of -CH=CH- group and multiplet of aromatic protons of phenyl nucleus at δ 7.55 – 7.74 ppm confirmed the successful synthesis of isonicotinic acid N'-(3-phenyl-acryloyl)-hydrazide (**9**). The structure of isonicotinic acid N'-tetradecanoyl-hydrazide (**12**) was confirmed by the presence of signals for CH_3 group and methylene protons at δ 0.82-0.85 ppm and above δ 1.20 ppm respectively. The singlet signal for NH proton of compound **16** was observed at δ 11.94 ppm and disappearance of this signal in compound **17** confirmed the formation compounds **16** and **17** respectively.

The IR spectra of compounds **1, 2, 5, 8, 9, 12** and **13** revealed the presence of vibrational frequencies corresponding to two C=O functional groups suggesting that the acyl resi-



Comp.	R_1	R_2	R_3	R_4	R_5	X	Y	R
1	H	H	H	H	H	C	-	-
2	H	CH_3	H	H	H	C	-	-
3	H	OCH_3	OCH_3	H	H	C	-	-
4	H	NO_2	H	NO_2	H	C	-	-
5	Cl	H	Cl	H	H	C	CH_2	-
6	OH	H	H	H	H	C	-	-
7	I	I	H	I	H	C	-	-
8	Cl	H	H	H	H	N	-	-
9	H	H	H	H	H	C	CH=CH	-
10	-	-	-	-	-	-	-	-CH=CH-CH_3
11	-	-	-	-	-	-	-	-$C_6H_{12}CH_3$
12	-	-	-	-	-	-	-	-$C_{12}H_{24}CH_3$
13	H	OH	H	H	H	C	-	-
14	H	OCH_3	H	H	H	C	-	-
15	H	H	H	H	H	N	-	-

Scheme 1. Synthetic route followed for the synthesis of n_2-acyl isonicotinic acid hydrazide derivatives.

due has been added to the isonicotinic acid hydrazide molecule. The amide I band was observed in the spectra of compounds **1, 2, 5, 8, 9, 12** and **13** in the region 3200-3100 cm^{-1}. The presence of peaks slightly above and below 3000 cm^{-1} indicated the presence of an aromatic and aliphatic portion in the synthesized compounds, respectively.

The OCN deformations corresponding to amide IV and amide VI bands were observed around 750-720 cm^{-1} and 650-630 cm^{-1} in the IR spectra of compounds **1, 2, 5, 8, 9** and **12**. In compound **8**, C-Cl stretching was observed at 761.91 cm^{-1} that depicted the presence of chloro group on aromatic nucleus in the molecular structure. The skeletal C=C-stretching bands (aromatic) were observed around 1513-1466 cm^{-1} in the spectra of the synthesized compounds which represents the presence of aromatic nucleus in their structure. Only one band corresponding to C=O function was observed in the IR spectra of compounds **16** and **17** which confirmed their synthesis as well as indicated that only one C=O function was present in their structure.

Antimycobacterial Activity

The *in vitro* antitubercular activity of synthesized N_2-acyl isonicotinic acid hydrazide derivatives against *Mycobacterium tuberculosis* (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, compound (**1**), isonicotinic acid hydrazide with an unsubsti-

Table 1. Physicochemical Properties and Antimycobacterial Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives

Comp.	Mol. Formula	Mol. Wt.	M.p. (°C)	Rf*	% Yield	MIC *MTB-H37Rv* (μM x 10^{-3})
Training Set						
1	$C_{13}H_{11}N_3O_2$	241	180-183	0.83	89.40	52
2	$C_{14}H_{13}N_3O_2$	255	194-197	0.87	85.68	49
3	$C_{15}H_{15}N_3O_4$	301	170-173	0.73	68.95	42
4	$C_{13}H_9N_5O_6$	331	198-201	0.68	66.28	9
5	$C_{14}H_{11}N_3O_2Cl_2$	324	222-225	0.64	59.35	10
6	$C_{13}H_{11}N_3O_3$	257	220-223	0.76	67.49	49
7	$C_{13}H_8N_3O_2I_3$	618	236-239	0.54	69.37	40
8	$C_{12}H_9N_4O_2Cl$	276	160-163	0.75	70.30	23
9	$C_{15}H_{13}N_3O_2$	267	208-211	0.62	69.32	23
10	$C_{10}H_{11}N_3O_2$	205	160-163	0.76	46.12	61
11	$C_{14}H_{21}N_3O_2$	263	Above 242	0.61	71.27	12
12	$C_{20}H_{33}N_3O_2$	347	194-197	0.54	65.48	2
Test Set						
13	$C_{13}H_{11}N_3O_3$	257	182-185	0.88	67.8	49
14	$C_{14}H_{13}N_3O_3$	271	206-209	0.81	64.8	92
15	$C_{12}H_{10}N_4O_2$	242	226-2229	0.67	67.8	52
16	$C_{15}H_{13}N_3O$	251	195-198	0.62	81.9	6
17	$C_{22}H_{17}N_3O_2$	355	203-206	0.38	67.5	70
INH						2.04
ETB[a]						15.31
CFL						9.4
RIF[a]						0.24

*Mobile phase: Ethanol
[a] D. Sriram, P. Yogeeswari, P. Dhakla, P. Senthilkumar, D., Banerjee, Bioorg. Med. Chem. Lett. 17 (2007) 1888-1891.

tuted phenyl ring attached at N_2 position through carbonyl function, displayed poor antimycobacterial activity with a MIC of 52 x 10^{-3} μM. Therefore, compound **1** was taken as a lead molecule and we planned to improve its antimycobacterial activity by altering the substituent on phenyl ring. The first step towards lead optimization was incorporation of electron donating group (CH_3) on phenyl ring. The addition of electron donating group slightly improved the antitubercular activity evidenced by the small increase in the activity of compound **2** (MIC = 49 x 10^{-3} μM) in comparison to **1**. Then we replaced the methyl group with 3,4-dimethoxy substituents (Compound **3**) on phenyl nucleus, which marginally increased the antimycobacterial activity (MIC = 42 x 10^{-3} μM). As the increment of antimycobacterial activity was not highly appreciable that changed our mind to add some electron withdrawing groups as substituent on the phenyl nucleus and compound **4** having 3,5-dinitro substitution was synthesized. The compound **4** was found to be highly active with MIC of 9 x 10^{-3} μM. The next modification in the structure was addition of electronegative chloro substituent on the phenyl nucleus that resulted in the synthesis of compound **5**, which has 2,4-dichloro substitution on phenyl nucleus with phenyl nucleus attached to carbonyl function with a methylene linker. Compound **5** was also found to be active antimycobacterial agent with MIC of 10 x 10^{-3} μM. In compound **6**, the phenyl nucleus was substituted with OH group at ortho position, but this modification lead to decrease in activity with MIC falling to 49 x 10^{-3} μM. Therefore, the idea of addition of OH group was dropped and next change was addition of iodine group on the phenyl nucleus that resulted in the synthesis of compound **7** having 2,3,5-triiodo substitution and this molecule showed better antimycobacterial activity, with MIC of 40 x 10^{-3} μM, than compound **6**. Then we planned to add some hetero atom in the phenyl nucleus and

synthesized compound **8** having hetero atom nitrogen at 3rd position and electronegative chloro group as substituent at 2nd position on the phenyl nucleus. This idea also worked and compound **8** was also found to be active with MIC of 23 x 10^{-3} μM. In compound **9**, the -CH=CH- group was inserted between the phenyl nucleus and carbonyl group and this compound was also equally active with MIC of 23 x 10^{-3} μM. The next modification was the replacement of phenyl nucleus with aliphatic chain of carbon atoms that resulted in compound **10**, which has 2-propene as substituent attached to the carbonyl function. This change leads to a steep fall in activity with MIC falling to 61 x 10^{-3} μM. Then we added heptane as side chain (octanoyl as N_2 substituent) in compound **11**, and got a highly active molecule with MIC of 12 x 10^{-3} μM. In compound **12,** myristoyl group (acyl group of myristic acid) was taken as substituent on N_2 of hydrazide and the resultant molecule was the most active antimycobacterial agent with MIC of 2 x 10^{-3} μM, which was lowest among the synthesized derivatives. The compound **12** was found to be more active antitubercular agent than isoniazid.

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

1. The replacement of the phenyl nucleus with long aliphatic chain (Compound **11** and **12**) gave the molecules with highly improved antimycobacterial activity. This is similar to one of our previous report in which an increase in antimicrobial activity was observed with long aliphatic chain compounds [32].
2. In contrast with Tripathi *et al.* [33] who stated that the OH group at ortho position leads to a measurable change in activity of the compounds, the presence of the OH group at ortho position (Compound **6**) decreased the activity of the compounds.
3. The presence of electron withdrawing groups on the phenyl ring increases the antitubercular activity as evidenced by the high antimycobacterial activity of compounds **4** and **5**. The role of electron withdrawing group in improving activity is supported by the studies of Sharma *et al.* [34].
4. Among the different electron withdrawing groups, nitro group is most effective in conferring the antimycobacterial activity to potential.
5. The compounds **5, 7, 11** and **12** having high log P values were found to be more active than other synthesized derivatives which is similar to the findings of Sriram *et al.* [38] who observed that increased lipophilicity of 1-[(4-sub)phenyl]-3-(4-{1-[(pyridine-4-carbonyl)hydrazono]ethyl}phenyl thiourea rendered them more capable of penetrating various biomembranes.

Based on the SAR results, we have synthesized a test set of isonicotinic acid hydrazide derivatives (**13-17**) so as to validate the SAR findings by the results of antimycobacterial activity of test set compounds. First of all, OH group was added, with an idea that the electronegative oxygen atom of OH group may facilitate the binding of N_2-acyl isonicotinic acid hydrazide derivatives by hydrogen bonding at the target site, at 3rd position of the phenyl nucleus (Compound **13**) and the resultant compound was not active. This is similar to the results of antimycobacterial activity of compound **6**, which confirms the fact that OH group decreases the antimycobacterial activity. It was observed from the SAR studies that electron donating as well as electron withdrawing groups increase the antimycobacterial activity which is contradictory and in order to find out the real fact compound **14**, having OCH_3 group at 3rd position of phenyl nucleus, was synthesized and from the results of antimycobacterial activity it was clear that OCH_3 group decrease the antimycobacterial activity as compound **14** was found to be least active with MIC 92 x 10^{-3} μM. The increased antimycobacterial activity of compound **3** may be due to the presence of an additional OCH_3 group in its structure and it can be concluded that OCH_3 reduces activity only in position 3 and it increases the activity in position 4. The presence of pyridine ring in the structure confers antimycobacterial activity to the isoniazid, keeping this fact in mind compound **15**, having 3-pyridyl ring in place of phenyl nucleus, was synthesized but the activity was found to be very less (MIC = 52 x 10^{-3} μM) and it can be concluded that an additional pyridine ring in the isonicotinic acid hydrazide structure has no impact on the activity. This clearly indicates that the increase in antimycobacterial activity of compound **8** may be due to the presence of the electron withdrawing chloro group and not due to presence of an additional pyridine nucleus. The hydrazones of isonicotinic acid hydrazide have been reported to be active antimycobacterial agents and appreciable antimycobacterial activity of compound **9** (Isonicotinic acid N'-(3-phenyl-acryloyl)-hydrazide) derived by acylation of isonicotinic acid hydrazide with cinnamic acid inspired us for synthesis of hydrazone of isoniazid with cinnamaldehyde and the resultant compound **16** was highly active with MIC of 6 x 10^{-3} μM. The fact that presence of phenyl substituent increased the antimycobacterial activity lead us to the synthesis of compound **17** in which the N_1-proton of **16** was replaced with benzoyl nucleus and this substitution decreased the antimycobacterial activity (MIC) to 70 x 10^{-3} μM. It can be seen from this result that presence of at least one NH group is necessary for a molecule to be active antimycobacterial agent.

Summarizingly, it can be concluded that long alkyl chain substituted derivatives were highly active antimycobacterial agents with compound **12** having myristoyl substituent on N_2-position was more active than isoniazid. This may be due to the fact that mycolic acid that constitutes the mycobacterial cell wall contains long chain stearyl group in its molecular structure and compounds derived from long alky chains may act as false substrate for the *Mycobacterium* and get incorporated in the bacterial cell wall and give rise to a pseudo cell wall and also carries the isonicotinic acid hydrazide attached to its structure in to the bacterial cell thus leading to an increased uptake of isonicotinic acid hydrazide by the *Mycobacterium* which may be cause of lysis of *Mycobacterium* and increased antimycobacterial potential of saturated long chain N_2-acyl isonicotinic acid hydrazide derivatives.

Cytostatic, Cytotoxic and Antiviral Activity

Generally, none of the compounds were inhibitory to DNA, RNA and retroviruses at subtoxic concentrations. The

Table 2. Anti-HIV Potential of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives in MT-4 Cells[a)]

Compound	EC_{50} (μg/ml)		CC_{50}[b] (μg/ml)
	HIV-1 (IIIb)	HIV-2 (ROD)	
Training Set			
1	>125.00	>125.00	>125.00
2	>125.00	>125.00	>125.00
3	>105.15	>105.15	105.15 ± 8.59
4	>111.75	>111.75	111.75 ± 8.81
5	>104.53	>104.53	104.53 ± 5.22
6	>2.43	>2.43	2.43 ± 0.35
7	>61.85	>61.85	61.85 ± 3.02
8	>109.33	>109.33	109.33 ± 8.39
9	>25.83	>25.83	25.83 ± 3.92
10	>103.53	>103.53	103.53 ± 14.68
11	>83.65	>83.65	83.65 ± 10.66
12	>38.32	>38.32	38.32 ± 56.48
Test Set			
13	>94.38	>94.38	94.38 ± 29.33
14	>125.00	>125.00	>125.00
15	>113.00	>113.00	>113.00 ± 8.68
16	>12.14	>12.14	12.14 ± 1.86
17	>24.60	>24.60	24.60 ± 15.44
Nevirapine	0.050 ± 0.011	>4.0	>4.0
Zidovudine	0.002 ± 0.001	0.002 ± 0.001	>25

[a]50% Effective concentration or compound concentration required to inhibit virus-induced cytopathicity by 50%.
[b]50% Cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%.

results of antiviral activity against the various viruses are presented in Tables **2**-**5**. Compound **6** proved highly cytostatic (CC_{50} for MT-4 cells: 2.4 μg/ml), followed by compounds **16**, **9** and **12** (CC_{50}: 12-38 μg/ml). The other compounds were not toxic at ≥ 100 μg/ml.

Antibacterial and Antifungal Activity

The synthesized N_2-acyl isonicotinic acid hydrazide derivatives [**1-17**] were evaluated, *in vitro*, for their antibacterial activity against Gram positive *Staphylococcus aureus, Bacillus subtilis,* Gram negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger* by the serial dilution method [20] using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activity, respectively, and the results are presented in Table **6**.

The results of antibacterial activity against *B. subtilis* indicated that all the synthesized derivatives have higher antibacterial potential than isoniazid. In particular the compounds **6** and **12** were found to be the most effective antibacterial agents having $pMIC_{bs}$ values 2.31 and 2.14 μM, respectively. In case of *S. aureus* compounds **5** and **7** have shown marked antibacterial potential at $pMIC_{sa}$ values 2.41 and 3.30 μM, respectively. For antibacterial activity against *E. coli* compounds **7** and **12** had shown better antibacterial activity with $pMIC_{ec}$ values of 3.00 and 2.44 μM, respectively. The antibacterial potential of compound **12** was found to be better than the standard drug norfloxacin.

The antifungal activity against *C. albicans* revealed that compounds **5** and **7** were the potential candidates having $pMIC_{ca}$ values 2.71 and 3.00 μM, respectively. The antifungal activity of compound **7** was higher than the standard drug fluconazole against *C. albicans*. In case of *A. niger* compounds **4** and **12** were found to be active with $pMIC_{an}$ values 2.42 and 2.44 μM, respectively.

It can be observed from the results presented in Table **6** that the synthesized N_2-acyl isonicotinic acid hydrazide derivatives have higher antifungal potential particularly against

Table 3. Cytotoxicity and Anti-DNA Virus Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide 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
Training Set					
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	≥0.8	>0.8	>0.8	>0.8	>0.8
7	100	>20	>20	>20	>20
8	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100
12	100	>20	>20	>20	>20
Test Set					
13	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100
17	>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%.

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 antifungal potential of N_2-acyl isonicotinic acid hydrazide derivatives was higher than the antibacterial activity, which shows that different structural requirements are essential for antibacterial and antifungal activity. These results are similar to those of Sortino *et al*. [39].

2. Similar to the observations of Guven *et al*. [37], the presence of substitutent at ortho position increased the antibacterial and antifungal potential of the compounds which can be seen from the antimicrobial activity of compounds **5, 6, 7** and **8**.

3. The results of antimicrobial activity depicted that the presence of electron donating OCH_3 group enhanced the antimicrobial activity of the synthesized derivatives (Compound **3**). This fact is supported by the results of Emami *et al*. [40].

4. The synthesized derivatives were more active towards the Gram-negative bacterium *E. coli* than Gram-positive *B. subtilis* and *S. aureus*. This finding is in agreement with Sbardella *et al.* [41].

5. It was observed from the results of antimicrobial activity that the introduction of *m*-methyl group in compound **2** in comparison to unsubstituted derivatives (compound **1**) did not appreciably enhanced the growth inhibition potential of synthesized derivatives.

6. The compound **9,** derived from cinnamic acid, has better antibacterial activity as compared to unsubstituted derivative (compound **1**). This may be due to the presence of extended conjugation in cinnamic acid residue which may be involved in effective binding of synthesized compound with the target site. This fact is supported by one of our earlier studies [42].

Table 4. Cytotoxicity and Anti-RNA Virus Activity of Synthesized N_2-acyl Isonicotinic 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
Training Set				
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	≥4	>4	>4	>4
7	100	>20	>20	>20
8	>100	>100	>100	>100
9	100	>20	>20	>20
10	>100	>100	>100	>100
11	>100	>100	>100	>100
12	100	>20	>20	>20
Test Set				
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

[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 N_2-acyl Isonicotinic 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
Training Set						
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	≥4	>4	>4	>4	>4	>4
7	100	>20	>20	>20	>20	>20
8	>100	>100	>100	>100	>100	>100

Table 5. contd….

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
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	20	>4	>4	>4	>4	>4
Test Set						
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

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathicity by 50%.

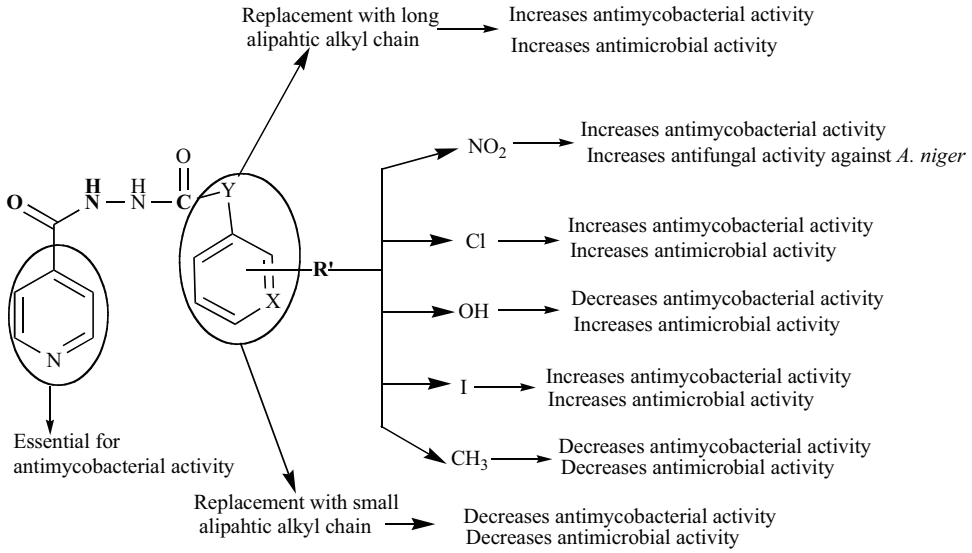


Fig. (1). Structural requirements for the antimycobacterial and antimicrobial activity of synthesized N_2-acyl isonicotinic acid hydrazide derivatives.

7. Compound **7** having 2,3,5-triiodo substitution on phenyl nucleus was found to be most active antimicrobial agent among the synthesized derivatives. This may be attributed to the presence of iodine atom in the molecular structure.

The SAR findings of the antimycobacterial and antimicrobial activity are summarized in Fig. (**1**).

The test set synthesized after the QSAR studies was also evaluated for antimicrobial activity against all the strains tested above. The results of antimicrobial activity studies revealed that the synthesized test set was more active than isonicotinic acid hydrazide except compound **15** which was less active against Gram positive *Staphylococcus aureus,* Gram negative *Escherichia coli* and fungus *Candida albicans* and *Aspergillus niger* and compound **13** which was less active against Gram positive *Bacillus subtilis*. Compound **14** was equipotent to standard drug fluconazole for antifungal activity against *C. albicans*. Compound **17** was highly active antimicrobial agent against Gram negative *Escherichia coli* and fungus *Candida albicans* and *Aspergillus niger*.

QSAR STUDIES

Development of One-target (ot) QSAR Model

QSAR analysis is an area of computational research which builds models of biological activity using physicochemical properties of a series of compounds. QSAR approach is based on the assumption that the behavior of a compound, expressed by any measured activities, is corre-

lated with the molecular features of the compound, termed descriptors. After calculation of the molecular descriptors, the commonly used linear methods, such as linear regression (LR) and multiple linear regression (MLR), can be used in the development of a relationship between the structure descriptors and the activity [30]. By definition, a model is not reality and does not replace reality (e.g. experiments and observations). A model considers some selected and relevant elements of a phenomenon and attempts to mimic their behavior in such a way that predictions can be made. A model is useful in those situations in which either we do not want or we cannot carry out direct experiments. Thus, the key features of a model are (a) the phenomenological elements that it represents and (b) the accuracy of the representation [43]. To obtain suitable equations two factors were taken into account [44].

1. A ratio of compounds to variables greater than 5.
2. An intercorrelation among the independent variables smaller than 0.6.

In order to identify the substituent effect on the antimicrobial activity, quantitative structure activity relationship (QSAR) studies between the *in vitro* antimicrobial activity and descriptors coding for lipophilic, electronic, steric and topological properties of the 12 synthesized N_2-acyl isonicotinic acid hydrazide derivatives were undertaken, using the linear free energy relationship model (LFER) described by Hansch and Fujita [45]. Biological activity data determined as MIC values was first transformed into pMIC values (*i.e.* – log MIC) to get all the values positive, normal distribution of errors and to get linear free energy relationship of these data with physicochemical properties and used as dependent variable in QSAR study.

The thermodynamic, spatial, electronic and topological parameters calculated for QSAR analysis are presented in Table **7**. Thermodynamic parameters describe free energy change during drug receptor complex formation. Spatial parameters are the quantified steric features of drug molecules required for its complimentary fit with receptor. Electronic parameters describe weak non-covalent bonding between drug molecules and receptor [46]. 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 (Te), 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 isoniazid derivatives are presented in Table **7** [47-52].

For Hansch analysis, regression was performed using pMIC values as dependent variables and calculated parameters as independent variables. Out of hundreds of equations generated, some of the best QSAR equations are given below. 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.

In the present study, a training data set of 13 compounds (12 N_2-acyl isonicotinic acid hydrazide derivatives along with isoniazid itself) was subjected to linear free energy regression analysis for model generation. During the regression analysis studies it was observed that the response values of compound **6** were outside the limits of response values of other synthesized N_2-acyl isonicotinic acid hydrazide derivatives. Thus compound **6** was designated as an outlier and it was not involved in the data set for QSAR model generation. In multivariate statistics, it is common to define three types of outliers [53].

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

A compound was considered as an outlier for deriving a particular model when the residual value exceeded twice the standard error of estimate of the model [54]. In light of the above guidelines, Compound **6** was considered as outliers because its response values (antimicrobial activity) were outside the range in comparison to the other compounds included in the present study.

Based on the colinearity problem among the descriptors and their contribution towards the biological activity, different descriptors and/or a combination of descriptors were subjected to linear regressions using SPSS. Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity against *S. aureus* is presented in Table **8**. The correlations of different molecular descriptors with antibacterial and antifungal activity are presented in Table **9**. In general, high colinearity ($r > 0.8$) was observed between different parameters. The highest interrelationship was observed between $^0\chi$ and $^1\chi$ ($r = 0.994$) and lowest interrelationship was observed between Cosmic E and $^3\chi^v$ ($r = -0.103$). The correlation matrix indicated the predominance of topological parameters in describing the antimicrobial activity of the synthesized compounds. The statistical quality of the models was gauged by the parameters like correlation coefficient (r) or squared correlation coefficient (r^2), standard error of estimate (s), variance ratio (F). In order to corroborate the validity of the derived QSAR models, leave-one-out (LOO) method was used. Each compound is eliminated once, a model is derived from the remaining compounds and the eliminated compounds are predicted from this model. The same procedure is repeated after elimination of another compound until all the compounds have been eliminated once. Sum of squared prediction errors called predictive residual sum of squares (PRESS) statistic is calculated as the sum of squares of the differences between predicted and observed values of the activity. Standard deviation of prediction (Spress), the cross-validated correlation coefficient (q^2) and standard deviation error of predictions

Table 6. Antibacterial and Antifungal Potential (μM/mL) of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives

Comp.	$pMIC_{bs}$	$pMIC_{sa}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{b}$	$pMIC_{f}$	$pMIC_{am}$
Training Set								
1	1.98	1.98	2.29	2.59	2.29	2.08	2.44	2.23
2	2.01	2.01	2.31	2.31	2.31	2.11	2.31	2.19
3	1.78	2.08	2.38	2.68	2.38	2.08	2.53	2.26
4	1.82	2.12	2.12	2.42	2.42	2.02	2.42	2.18
5	2.11	2.41	2.11	2.71	2.41	2.21	2.56	2.35
6	2.31	2.01	2.31	2.61	2.31	2.21	2.46	2.31
7	1.79	3.30	3.00	3.00	2.09	2.70	2.55	2.64
8	2.04	2.04	2.34	2.34	2.34	2.14	2.34	2.22
9	2.03	2.03	2.33	2.33	2.03	2.13	2.18	2.15
10	1.91	1.91	2.21	1.91	1.91	2.01	1.91	1.97
11	2.02	2.02	2.02	2.32	2.32	2.02	2.32	2.14
12	2.14	2.14	2.44	2.44	2.44	2.24	2.44	2.32
INH	1.74	2.04	1.74	2.34	2.04	1.84	2.19	1.98
S.D.[a]	0.15	0.38	0.25	0.27	0.17	0.19	0.18	0.16
Test Set								
13	1.71	2.31	2.31	2.61	2.31	2.11	2.46	2.25
14	2.04	2.34	2.04	2.64	2.34	2.14	2.49	2.28
15	1.99	1.68	1.68	1.68	1.68	1.78	1.68	1.74
16	2.00	2.00	2.30	2.30	2.30	2.10	2.30	2.18
17	2.15	2.15	2.45	2.45	2.45	2.25	2.45	2.33
Std.	2.61*	2.61*	2.61*	2.64**	2.64**	2.61*	2.64**	2.62

*Norfloxacin **Fluconazole [a] Standard deviation

(SDEP) were calculated for each model and taken as an estimate of the predictability of the models.

The QSAR model represented by Eq. 1 correlates the antibacterial activity of synthesized N_2-acyl isonicotinic acid hydrazide derivatives against *S. aureus* with molecular descriptors and demonstrated that the antibacterial potential is governed by the valence third order molecular connectivity topological index ($^3\chi^v$) (Eq. 1).

QSAR Model for Antibacterial Activity Against S. aureus

$$pMIC_{sa} = 1.058\ ^3\chi^v + 1.727 \quad \text{Eq. 1}$$

$n = 12$ $r = 0.950$ $q^2 = 0.640$ $s = 0.123$ $F = 93.05$

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

For antibacterial activity against *S. aureus*, the developed QSAR model (Eq. 1) describes the importance of valence third order molecular connectivity index ($^3\chi^v$). 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 [55]. The valence third order molecular connectivity index ($^3\chi^v$) represents the molecules with highly branched structure. In this case, the positive correlation was observed between $^3\chi^v$ and antibacterial activity against *S. aureus* which means the activity will increase with increase in value of molecular descriptor $^3\chi^v$. The results presented in the Table **6** are in concordance with the model expressed by Eq. 1 and values of $^3\chi^v$ shown in Table **7** which depicts that compounds **5** and **7** having high $^3\chi^v$ values (0.680 and 1.375) have got the highest antibacterial potential (2.41 and 3.30). The value of r^2 for Eq. 1 is 0.903 which means the QSAR model is able to predict the 90.30% of variance.

To systematically assess a prediction algorithm, a reliable validation is required. Usually, a predictive method is evaluated by the predictive results for a training data set and testing data set, respectively. According to the statistical terminology, the former is called a test of resubstitution, and the latter is a test of cross-validation. By the test of resubstitu-

tion, the value of each compound in a training data set is predicted using the rules derived from the same set. Although this test gives somewhat optimistic error estimate because the same compounds are used to derive the prediction rules and to predict themselves, the resubstitution test is absolutely necessary due to its ability of reflecting the self-consistency of a given method. On the other hand, a cross-validation test for an independent testing data set is also needed because it can reflect the generalizing effectiveness of a predictive method. Of various cross-validation tests, the LOO test is thought to be a reliable one. Both tests of resubstitution and LOO [28] were used to evaluate the prediction of models developed in the current study. In the resubstitution test, three statistical parameters *viz.* regression coefficient (r), root mean square error (RMSE) and absolute average error (e^-) were used to evaluate the performance. The QSAR model expressed by Eq. 1 was cross validated by its high q^2 values (q^2 = 0.640) obtained with leave one out (LOO) method. The value of q^2 greater than 0.5 is the basic requirement for qualifying a QSAR model to be valid one [56]. 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 $pMIC_{sa}$ against observed $pMIC_{sa}$ (Fig. **2**) also favors the model expressed by Eq. 1. Further, the plot of observed $pMIC_{sa}$ Vs residual $pMIC_{sa}$ (Fig. **3**) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero [29].

Equations 2-9 were developed to predict the antibacterial and antifungal activity of N_2-acyl isonicotinic acid hydrazide derivatives against *B. subtilis, E. coli, C. albicans* and *A. niger*.

QSAR Model for Antibacterial Activity Against *B. Subtilis*

$$pMIC_{bs} = - 0.00693 \text{ Cosmic E} + 1.997 \quad \text{Eq. 2}$$

n = 12 r = 0.779 q^2 = 0.168 s = 0.089 F = 15.47

QSAR Model for Antibacterial Activity Against *B. Subtilis* Developed by MLR

$$pMIC_{bsMLR} = - 0.0072 \text{ Cosmic E} - 0.128\ {}^3\chi^v + 2.053 \quad \text{Eq. 3}$$

n = 12 r = 0.841 q^2 = 0.262 s = 0.081 F = 10.87

QSAR Model for Antibacterial Activity Against *E. Coli*

$$pMIC_{ec} = 0.0762\ {}^0\chi^v + 1.419 \quad \text{Eq. 4}$$

n = 12 r = 0.783 q^2 = 0.362 s = 0.195 F = 15.89

QSAR Model for Antibacterial Activity Against *E. Coli* Developed by MLR

$$pMIC_{ecMLR} = 0.166\ {}^0\chi^v - 0.083\ \kappa\alpha_1 + 1.646 \quad \text{Eq. 5}$$

n = 12 r = 0.884 q^2 = 0.616 s = 0.154 F = 16.16

QSAR Model for Antifungal Activity Against *C. Albicans*

$$pMIC_{ca} = 0.609\ {}^3\chi^v + 2.192 \quad \text{Eq. 6}$$

n = 12 r = 0.757 q^2 = 0.431 s = 0.186 F = 13.43

QSAR Model for Antifungal Activity Against *C. Albicans* Developed by MLR

$$pMIC_{caMLR} = 0.090\ {}^0\chi^v - 0.063\ \kappa_2 + 1.920 \quad \text{Eq. 7}$$

n = 12 r = 0.819 q^2 = 0.354 s = 0.172 F = 9.17

QSAR Model for Antifungal Activity Against *A. Niger*

$$pMIC_{an} = 0.0000236 \text{ NuE} + 1.832 \quad \text{Eq. 8}$$

n = 12 r = 0.712 q^2 = 0.338 s = 0.133 F = 10.34

QSAR Model for Antifungal Activity Against *A. Niger* Developed by MLR

$$pMIC_{anMLR} = - 0.0076 \text{ MR} + 0.126\ {}^1\chi + 1.627 \quad \text{Eq. 9}$$

n = 12 r = 0.763 q^2 = 0.298 s = 0.129 F = 6.27

The model expressed by Eq. 2 demonstrated that antibacterial activity against *B. subtilis* is governed by the cosmic energy of the molecule (Cosmic E). The model expressed by Eq. 2 is a monoparametric one and if we go for multiple linear regression model generation we got the biparametric QSAR models with higher r values which is represented by Eq. 3 and indicated that addition of molecular descriptor valence third order molecular connectivity index ($^3\chi^v$) increased the r value from 0.779 to 0.841. The model expressed by Eq. 3 has good predictability as evidenced by the low residual values in Table **10**.

In case of *E. coli*, the model expressed by Eq. 4 depicts the importance of valence zero order molecular connectivity index ($^0\chi^v$) in describing the antibacterial activity of synthesized derivatives. Similarly in this case also, the biparametric model expressed by Eq. 5, obtained by multiple linear regression demonstrated that a combination of valence zero order molecular connectivity index ($^0\chi^v$) and Kier's first order alpha shape topological index ($\kappa\alpha_1$) best describes the antibacterial activity of the synthesized derivatives which can be seen from the low residual values obtained by Eq. 5 (Table **10**).

The model described by Eq. 6 demonstrated the importance of valence third order molecular connectivity index ($^3\chi^v$) governs the antifungal activity of N_2-acyl isonicotinic acid hydrazide derivatives against *C. albicans*. The positive correlation of the molecular descriptor with antifungal activity reveals that increase in $^3\chi^v$ value will lead to an increase in antifungal activity against *C. albicans* (Table **7**). In this case also, the multiple linear regression analysis was found to be superior and demonstrated that model represented by Eq. 7 obtained by combining valence zero order molecular connectivity index ($^0\chi^v$) and Kier's third order shape topological index (κ_3) is effective in describing the antifungal activity of N_2-acyl isonicotinic acid hydrazide derivatives against *C. albicans*.

Table 7. Value of Selected Descriptors Used in the Regression Analysis

Comp.	Cosmic E	log P	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{2}\chi$	$^{3}\chi$	$^{3}\chi^{v}$	κ_3	$\kappa\alpha_1$	$\kappa\alpha_3$	Te	Ele.E	Nu.E
Training Set															
1	9.772	1.565	65.489	12.795	9.460	8.771	7.328	0.805	0.269	4.566	12.486	3.510	-3075.320	-18003.100	14927.800
2	6.076	2.033	70.530	13.665	10.383	9.165	7.962	1.093	0.435	4.795	13.461	3.744	-3231.190	-19813.000	16581.800
3	17.813	1.060	78.415	15.949	12.122	10.651	8.840	1.138	0.387	5.263	16.323	4.226	-4026.950	-26410.100	22383.100
4	17.235	1.473	80.138	17.690	11.833	11.380	10.405	1.805	0.492	6.046	17.503	4.573	-4736.730	-29718.900	24982.100
5	-5.663	2.534	79.648	15.242	12.403	10.059	8.949	1.382	0.680	5.606	15.989	4.828	-3951.280	-23122.800	19171.500
6*	2.965	1.281	67.183	13.665	9.830	9.182	7.856	1.010	0.322	4.500	13.422	3.480	-3395.970	-20149.000	16753.000
7	7.990	5.338	102.713	15.405	16.918	9.986	9.007	1.491	1.375	4.733	17.572	4.845	-4075.950	-23729.600	19653.700
8	-3.267	1.607	69.167	13.665	10.448	9.182	7.856	1.010	0.391	4.500	14.068	3.804	-3500.280	-20321.400	16821.100
9	-0.866	1.973	75.732	14.209	10.615	9.754	8.139	0.895	0.290	6.120	14.185	4.763	-3358.530	-20192.300	16833.800
10	-3.726	0.756	55.637	11.096	8.228	7.236	5.847	0.691	0.193	5.040	11.624	4.059	-2691.500	-14183.900	11492.400
11	-3.546	2.262	72.948	13.924	11.316	9.236	7.261	0.691	0.207	8.000	15.866	7.086	-3343.390	-20721.200	17377.800
12	-3.525	4.640	100.554	18.167	15.558	12.236	9.382	0.691	0.207	12.907	21.852	11.901	-4278.400	-30018.100	25739.700
INH	47.800	0.020	36.930	7.400	5.240	4.840	2.750	3.780	1.710	8.100	7.250	121.00	-1804.500	-8006.920	6202.420
Test Set															
13	2.411	1.281	67.183	13.665	9.830	9.165	7.962	1.093	0.343	4.795	13.422	3.724	-3395.920	-20514.500	17118.600
14	7.970	1.313	71.952	14.372	10.791	9.703	8.131	1.009	0.337	5.058	14.400	3.991	-3551.220	-22178.700	18627.400
15	2.532	0.718	63.300	12.795	9.330	8.771	7.328	0.805	0.269	4.566	12.808	3.680	-3140.290	-18120.700	14980.400
16	13.515	2.987	75.800	13.339	10.231	9.360	7.517	0.606	0.231	5.878	13.637	4.765	-3037.820	-17453.300	14415.500
17	37.021	4.898	105.538	18.899	14.473	13.254	10.967	1.058	0.404	6.780	18.878	5.288	-4308.300	-31158.400	26850.100

*Outlier

Table 8. Correlation Matrix for Antibacterial Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives Against *S. Aureus*

Cosmic E	Cosmic E	log P	$^0\chi$	$^1\chi$	$^2\chi$	$^3\chi$	$^3\chi^v$	κ_1	Te	Ele.E	Nu.E	$pMIC_{sa}$
	1.000											
log P	-0.449	1.000										
$^0\chi$	-0.491	0.617	1.000									
$^1\chi$	-0.519	0.614	0.994	1.000								
$^2\chi$	-0.457	0.575	0.972	0.961	1.000							
$^3\chi$	-0.136	0.306	0.642	0.586	0.783	1.000						
$^3\chi^v$	-0.103	0.645	0.345	0.296	0.462	0.701	1.000					
κ_1	-0.520	0.636	0.983	0.982	0.914	0.508	0.237	1.000				
Te	0.408	-0.578	-0.975	-0.951	-0.980	-0.773	-0.465	-0.931	1.000			
Ele.E	0.405	-0.577	-0.993	-0.983	-0.960	-0.645	-0.328	-0.976	0.978	1.000		
Nu.E	-0.403	0.575	0.992	0.983	0.953	0.625	0.307	0.979	-0.971	-1.000	1.000	
$pMIC_{sa}$	-0.031	0.734	0.278	0.232	0.344	0.511	0.950	0.213	-0.378	-0.267	0.250	1.000

Table 9. Correlation of Molecular Descriptors with Antimicrobial Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives

Mol. Descriptor	$pMIC_{bs}$	$pMIC_{sa}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_b$	$pMIC_f$	$pMIC_{am}$
Cosmic E	-0.779	-0.031	-0.378	0.105	-0.192	-0.371	-0.016	-0.269
log P	0.318	0.734	0.762	0.571	0.217	0.883	0.522	0.847
MR	0.284	0.611	0.768	0.598	0.442	0.803	0.649	0.845
$^0\chi$	0.321	0.278	0.510	0.416	0.674	0.484	0.625	0.609
$^0\chi^v$	0.225	0.685	0.783	0.642	0.418	0.843	0.669	0.882
$^1\chi$	0.380	0.232	0.511	0.404	0.681	0.470	0.620	0.597
$^1\chi^v$	0.367	0.563	0.699	0.544	0.467	0.759	0.621	0.801
$^2\chi$	0.264	0.344	0.538	0.486	0.651	0.525	0.666	0.656
$^2\chi^v$	0.243	0.744	0.773	0.667	0.378	0.878	0.668	0.906
$^3\chi$	-0.160	0.511	0.416	0.523	0.390	0.476	0.568	0.579
$^3\chi^v$	-0.234	0.950	0.732	0.757	-0.001	0.880	0.553	0.858
κ_1	0.385	0.213	0.459	0.329	0.661	0.434	0.556	0.544
κ_2	0.546	-0.001	0.286	0.081	0.543	0.256	0.319	0.317
κ_3	0.589	-0.055	0.186	-0.033	0.449	0.185	0.191	0.211
$\kappa\alpha_1$	0.369	0.361	0.548	0.412	0.608	0.565	0.591	0.651
$\kappa\alpha_2$	0.519	0.144	0.368	0.166	0.491	0.379	0.356	0.419
$\kappa\alpha_3$	0.561	0.067	0.257	0.047	0.417	0.287	0.234	0.302
R	0.380	0.232	0.511	0.404	0.681	0.470	0.620	0.597
J	-0.246	-0.187	-0.436	-0.503	-0.373	-0.376	-0.544	-0.501
W	0.398	0.160	0.374	0.298	0.647	0.364	0.527	0.481
Te	-0.184	-0.378	-0.509	-0.490	-0.657	-0.515	-0.672	-0.650
Ele.E	-0.245	-0.267	-0.473	-0.431	-0.708	-0.443	-0.653	-0.592
Nu.E	0.253	0.250	0.466	0.421	0.713	0.432	0.648	0.581

Table 10. Comparison of Observed and Predicted Antibacterial and Antifungal Activity Obtained by ot-QSAR Model

Comp.	$pMIC_{sa}$ (Eq. 1)			$pMIC_{bsMLR}$ (Eq. 3)			$pMIC_{ecMLR}$ (Eq. 5)			$pMIC_{caMLR}$ (Eq. 7)			$pMIC_{anMLR}$ (Eq. 9)		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
Training Set															
1	1.98	2.01	-0.03	1.98	1.95	0.03	2.29	2.18	0.11	2.59	2.37	0.22	2.29	2.24	0.05
2	2.01	2.19	-0.18	2.01	1.95	0.06	2.31	2.25	0.06	2.31	2.44	-0.13	2.31	2.25	0.06
3	2.08	2.14	-0.06	1.78	1.88	-0.10	2.38	2.30	0.08	2.68	2.57	0.11	2.38	2.38	0.00
4	2.12	2.25	-0.13	1.82	1.87	-0.05	2.12	2.16	-0.04	2.42	2.49	-0.07	2.42	2.45	-0.03
5	2.41	2.45	-0.04	2.11	2.01	0.10	2.11	2.38	-0.27	2.71	2.57	0.14	2.41	2.29	0.12
7	3.30	3.18	0.12	1.79	1.82	-0.03	3.00	3.00	0.00	3.00	3.03	-0.03	2.09	2.11	-0.02
8	2.04	2.14	-0.10	2.04	2.03	0.01	2.34	2.21	0.13	2.34	2.47	-0.13	2.34	2.26	0.08
9	2.03	2.03	0.00	2.03	2.02	0.01	2.33	2.23	0.10	2.33	2.38	-0.05	2.03	2.28	-0.25
10	1.91	1.93	-0.02	1.91	2.06	-0.15	2.21	2.05	0.16	1.91	2.23	-0.32	1.91	2.12	-0.21
11	2.02	1.95	0.07	2.02	2.05	-0.03	2.02	2.21	-0.19	2.32	2.32	0.00	2.32	2.24	0.08
12	2.14	1.95	0.19	2.14	2.05	0.09	2.44	2.41	0.03	2.44	2.37	0.07	2.44	2.41	0.03
INH	2.04	1.87	0.17	1.74	1.69	0.05	1.74	1.91	-0.17	2.34	2.15	0.19	2.04	1.96	0.08
Test Set															
6	2.01	2.07	-0.06	2.31	1.99	0.32	2.31	2.14	0.17	2.61	2.39	0.22	2.31	2.27	0.04
13	2.31	2.10	0.21	1.71	1.99	-0.28	2.31	2.14	0.17	2.61	2.37	0.24	2.31	2.27	0.04
14	2.34	2.09	0.25	2.04	1.96	0.08	2.04	2.22	-0.18	2.64	2.44	0.20	2.34	2.30	0.04
14	1.68	2.02	-0.34	1.99	2.00	-0.01	1.68	2.11	-0.43	1.68	2.34	-0.66	1.68	2.25	-0.57
16	2.00	1.98	0.02	2.00	1.93	0.07	2.30	2.19	0.11	2.30	2.34	-0.04	2.30	2.23	0.07
17	2.15	2.16	-0.01	2.15	1.74	0.41	2.45	2.47	-0.02	2.45	2.65	-0.20	2.45	2.50	-0.05

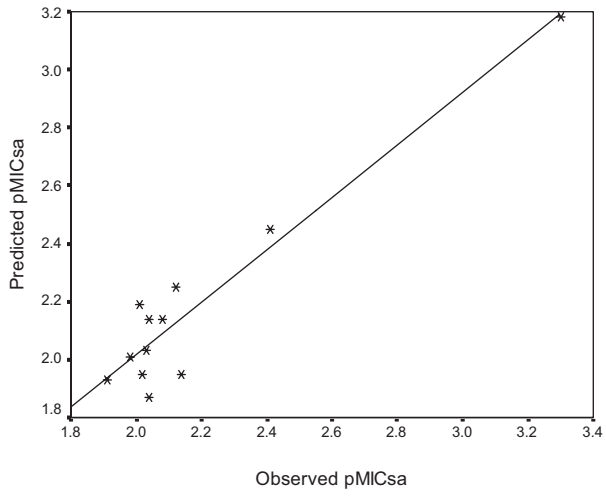


Fig. (2). Plot of observed pMICsa against the predicted pMICsa for the linear regression model developed by Eq. 1.

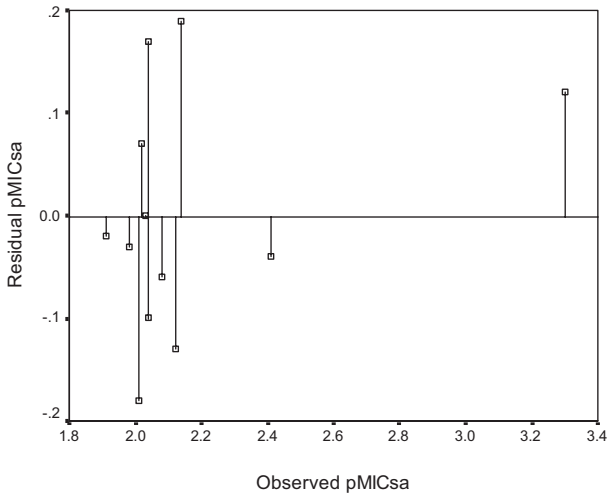


Fig. (3). Plot of residual pMICsa against the experimental pMICsa for the linear regression model developed by Eq. 1.

For antifungal activity against *A. niger*, the developed QSAR model (Eq. 8) indicated the predominance of nuclear repulsion energy in describing the antifungal activity. The nuclear repulsion energy between two atoms (A and B) is given by

$$E_{NuE}(AB) = Z_A Z_B / R_{AB}$$

where A – given atomic species; B – another atomic species; Z_A - charge of atomic nucleus A; Z_B - charge of atomic nucleus B; R_{AB} - distance between the atomic nuclei, A and B [57].

The coefficient of nuclear repulsion energy (NuE) is positive, which shows that the antifungal activity will increase with the increase in nuclear repulsion energy of the synthesized compounds, which can be clearly seen from the results of antifungal activity against *A. niger* (Table **6**) and values of nuclear repulsion energy presented in Table **7** that compounds **4** and **12** having high NuE values (24982.100 and 25739.700 respectively) had got the highest antifungal activity of 2.42 and 2.44 μM. Further, the biparametric model obtained by MLR analysis (Eq. 9) revealed that molar refractivity (MR) and first order molecular connectivity index ($^1\chi$) effectively describes the antifungal activity of synthesized derivatives which can be seen from low residual values of Eq. 9 (Table **10**).

The molar refractivity is a constitutive additive property that is calculated by Lorenz-Lorentz formula [58], as given below

$$MR = (n^2 - 1 * MW)/(n^2 + 2 * d)$$

Where MW is molecular weight, n is `the refraction index and d is the density, and its value depends on the wave longitude of the light used to measure the refraction index.

The low residual values presented in Table **10** are in agreement with the fact that models expressed by Eq. 3, Eq. 5, Eq. 7 and Eq. 9 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 Eq. 5. 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 all other developed QSAR models the value of q^2 is less than 0.5, which shows that the developed model is an invalid one. But according to the recommendations of Kim *et al.* [59], 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 all of these cases is less than 0.3, so the developed QSAR models are valid.

Generally for QSAR studies, the biological activities of compounds should span 2-3 orders of magnitude. But in the present 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 the present study is high which is evidenced by their low residual values. This is in agreement with results suggested by the Bajaj *et al.* [60], 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 [42, 61-63]. 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 [42, 29]. The minimum standard deviation (Table **6**) observed in the antimicrobial activity data justifies its use in QSAR studies. The value of PRESS (predictive residual sum of squares which is also termed as least square error (LSE)) < sum of squares of response values (SSY) is an indicative of statistical significance of prediction of QSAR models [64] which can be easily clarified by their values listed in Table **11**. In 2D-QSAR, the internal consistency of the models using q^2 is assessed with the ratio RQR = q^2/r^2 [65]. Considering RQR for the QSAR models represented by Eq. 1, 3, 5, 7 and 9 (Table **12**), it is pleasing that the lowest value is RQR = 0.371 for QSAR Model 3 and there is a gradual, roughly monotonic increase across the series to RQR = 0.788. Thus the QSAR models we present are statistically robust. The low value of probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), standard error of prediction (SEP), and SSY (sum of squares of response values), standard deviation of prediction (Spress), and standard deviation error of predictions (SDEP), root mean square error (RMSE) and absolute average error (e^-) and high value of quality value (Q), r^2 and the cross-validated correlation coefficient (q^2) (Table **12** and Table **13**) revealed the statistical significance of the model described by Eq. 1, 3, 5, 7 and 9. Also the value VIF < 5 indicates that MLR models expressed by Eq. 3, 5, 7 and 9 are statistically valid [29]. The validity of the developed ot-QSAR models was also confirmed the prediction of antimicrobial activity of the test set compounds by using these developed ot-QSAR models. The low residual values of compounds **13-17** clearly indicated that the developed ot-QSAR models are statistically valid.

Development of Multi-target (mt) 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 activity [66-70].

In the present 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 anti-

Table 11. PE, LSE, LOF, SEP, *Q* and SSY Values Calculated for the Derived Models for Modeling Antimicrobial Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives

S. No.	Descriptor	PE	LSE	LOF	SEP	*Q*	SSY
Eq. 1	$^3\chi^v$	0.018	0.0150	0.0122	0.0322	7.724	0.558
Eq. 3	Cosmic E, $^3\chi^v$	0.056	0.0621	0.0031	0.0208	10.383	0.150
Eq. 5	$^0\chi^v$, $\kappa\alpha_1$	0.042	0.0179	0.0009	0.0112	5.740	0.376
Eq. 7	$^0\chi^v$, κ_2	0.063	0.2656	0.0131	0.0429	4.762	0.517
Eq. 9	$^1\chi$, MR	0.081	0.1485	0.0073	0.0321	5.915	0.252
Eq. 11	log P, $\kappa\alpha_3$	0.012	0.0276	0.0014	0.0138	17.304	0.057
Eq. 12	Te	0.106	0.2160	0.0180	0.0387	4.634	0.306
Eq. 14	$^2\chi^v$, κ_3	0.009	0.0162	0.0008	0.0106	23.240	0.040

Table 12. RMSE, e^-, S_{Press}, SDEP, VIF, r^2 and RQR Values Calculated for the Derived Models for Modeling Antimicrobial Activity of Synthesized N_2-acyl Isonicotinic Acid Hydrazide Derivatives

S.No.	Descriptor	RMSE	e^-	S_{Press}	SDEP	VIF	r^2	RQR
Eq. 1	$^3\chi^v$	0.112	0.093	0.356	0.117	-	0.903	0.709
Eq. 3	Cosmic E, $^3\chi^v$	0.072	0.059	0.114	0.075	3.41	0.707	0.371
Eq. 5	$^0\chi^v$, $\kappa\alpha_1$	0.134	0.112	0.265	0.140	4.59	0.782	0.788
Eq. 7	$^0\chi^v$, κ_2	0.149	0.122	0.223	0.155	3.04	0.671	0.528
Eq. 9	$^1\chi$, MR	0.111	0.084	0.137	0.116	2.39	0.583	0.511
Eq. 11	log P, $\kappa\alpha_3$	0.048	0.038	0.200	0.050	16.39	0.939	0.937
Eq. 12	Te	0.134	0.105	0.125	0.140	-	0.452	0.469
Eq. 14	$^2\chi^v$, κ_3	0.037	0.030	0.170	0.038	21.09	0.953	0.925

bacterial 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 activity, antifungal activity and antimicrobial activity values of N_2-acyl isonicotinic acid hydrazide 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 depicted the importance of lipophilic parameter (log P) in describing the antibacterial activity of synthesized N_2-acyl isonicotinic acid hydrazide derivatives, and presented in the model expressed by Eq. 10.

mt-QSAR Model for Antibacterial Activity

$$pMIC_b = 0.121 \log P + 1.878 \qquad \text{Eq. 10}$$

$n = 12 \quad r = 0.883 \quad q^2 = 0.457 \quad s = 0.102 \quad F = 35.39$

In this case also the MLR analysis gave the valid QSAR modelwith high r and q^2 values and elaborated that correlation of logP with Kier's third order shape topological index ($\kappa\alpha_3$) best describes the antibacterial potential of synthesized derivatives and given by the model expressed by Eq.11.

mt-QSAR Model for Antibacterial Activity Developed by MLR

$$pMIC_{bMLR} = 0.169 \log P - 0.044\ \kappa\alpha_3 + 1.994 \qquad \text{Eq. 11}$$

$n = 12 \quad r = 0.969 \quad q^2 = 0.880 \quad s = 0.056 \quad F = 70.08$

The mt-QSAR model for antifungal activity revealed the importance of total energy (Te) in describing antifungal activity of synthesized derivatives and represented by Eq. 12.

mt-QSAR Model for Antifungal Activity

$$pMIC_f = -0.00016\ Te + 1.785 \qquad \text{Eq. 12}$$

$n = 12 \quad r = 0.672 \quad q^2 = 0.212 \quad s = 0.145 \quad F = 8.23$

The mt-QSAR model for antimicrobial activity depicted that valence second order molecular connectivity index ($^2\chi^v$) effectively describes the overall antimicrobial activity of the synthesized derivatives and MLR models developed by the combination of valence second order molecular connectivity index ($^2\chi^v$) with Kier's third order shape topological index (κ_3) and the models are represented by Eq. 13 and Eq. 14 respectively.

mt-QSAR Model for Antimicrobial Activity

$$pMIC_{am} = 0.104\ {}^{2}\chi^{v} + 1.771 \qquad \text{Eq. 13}$$

$n = 12 \quad r = 0.906 \quad q^2 = 0.685 \quad s = 0.078 \quad F = 45.74$

mt-QSAR Model for Antimicrobial Activity Developed by MLR

$$pMIC_{amMLR} = 0.133\ {}^{2}\chi^{v} - 0.0298\ \kappa_3 + 1.821 \qquad \text{Eq. 14}$$

$n = 12 \quad r = 0.976 \quad q^2 = 0.882 \quad s = 0.042 \quad F = 91.41$

It was observed from mt-QSAR models [Eq. 10-14] that the antibacterial, antifungal and overall antimicrobial activity of synthesized N_2-acyl isonicotinic acid hydrazide derivatives is governed by energy parameters, *viz.* total energy (Te) describing the antifungal activity and combination of log P and Kier's third order shape topological index ($\kappa\alpha_3$) describes the antibacterial potential of the N_2-acyl isonicotinic acid hydrazide derivatives and the combination of valence second order molecular connectivity index (${}^{2}\chi^{v}$) with Kier's third order shape topological index (κ_3) best describes the overall antimicrobial activity. The developed mt-QSAR models were statistically valid as they had the high r and q^2 values and low residual values (Table **13**). As in the case of ot-QSAR models, the plot of predicted $pMIC_{amMLR}$ against observed $pMIC_{amMLR}$ (Fig. **4**) also favors the developed model expressed by Eq. 14. Further, the plot of observed $pMIC_{amMLR}$ Vs residual $pMIC_{amMLR}$ (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 probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), standard error of prediction (SEP), and SSY (sum of squares of response values), standard deviation of prediction (Spress), and standard deviation error of predictions (SDEP), root mean square error (RMSE) and absolute average error ($\bar{e}$) and high value of quality value (Q) and the cross-validated correlation coefficient (q^2) (Table **11** and Table **12**) revealed the statistical significance of the model described by Eq. 11 and 14. Similarly Considering RQR for the QSAR models represented by Eq. 11, and 14 (Table **12**), it is observed that QSAR models form Eq. 11 and Eq. 14 has got the RQR value of 0.933 and 0.925. Thus, here also the QSAR models are statistically robust. The validity of the developed mt-QSAR models was also confirmed the prediction of antimicrobial activity of the test set compounds by using these models and the low residual values of compounds **13-17** clearly indicated that the developed mt-QSAR models are statistically valid.

CONCLUSION

A series of N_2-acyl isonicotinic acid hydrazide derivatives (**1-17**) was synthesized and tested for its *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* and the compounds with dinitro, dichloro, N_2-octanoyl and N_2-tetradecanoyl groups were found to be the most effective and compound **12** was more active antitubercular agent than isoniazid. The results of antiviral activity testing showed that none of the tested compounds was active against any of the viruses investigated. The synthesized compounds were also screened for their antimicrobial potential against *S. aureus,*

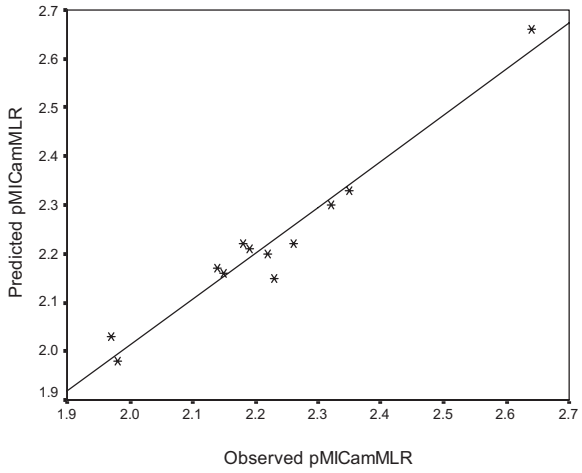


Fig. (4). Plot of observed pMICamMLR against the predicted pMICamMLR for the multiple linear regression model developed by Eq. 14.

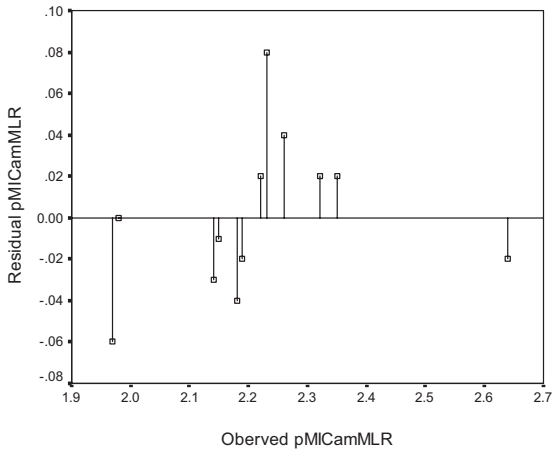


Fig. (5). Plot of residual pMICamMLR against the experimental pMICamMLR for the multiple linear regression model developed by Eq. 14.

B. subtilis, *E. coli, C. albicans* and *A. niger* and the results indicated that compounds with dichloro, hydroxyl, triiodo and N_2 –tetradecanoyl substituents were the most active antimicrobial agents. Compound **6** proved most cytostatic in cell culture. To find out the correlation between physicochemical parameters and antibacterial and antifungal activity of N_2-acyl isonicotinic acid hydrazide 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 N_2-acyl isonicotinic acid hydrazide derivatives in comparison to the one target models and indicated that the combination of valence second order molecular connectivity index (${}^{2}\chi^{v}$) with Kier's third order shape topological index (κ_3) best describes the overall antimicrobial activity.

DISCLOSURE

Some part of the information included in this paper has been previously published in Drug Design & Discovery Volume 8, Number 9, November 2011.

Table 13. Comparison of Observed and Predicted Antibacterial, Antifungal and Antimicrobial Activity Obtained by mt-QSAR Model

Comp.	$pMIC_{bMLR}$ (Eq. 11)			$pMIC_{f}$ (Eq. 12)			$pMIC_{amMLR}$ (Eq. 14)		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
Training Set									
1	2.08	2.10	-0.02	2.44	2.28	0.16	2.23	2.15	0.08
2	2.11	2.17	-0.06	2.31	2.30	0.01	2.19	2.21	-0.02
3	2.08	1.99	0.09	2.53	2.43	0.10	2.26	2.22	0.04
4	2.02	2.04	-0.02	2.42	2.55	-0.13	2.18	2.22	-0.04
5	2.21	2.21	0.00	2.56	2.42	0.14	2.35	2.33	0.02
7	2.70	2.68	0.02	2.55	2.44	0.11	2.64	2.66	-0.02
8	2.14	2.10	0.04	2.34	2.35	-0.01	2.22	2.20	0.02
9	2.13	2.12	0.01	2.18	2.33	-0.15	2.15	2.16	-0.01
10	2.01	1.94	0.07	1.91	2.22	-0.31	1.97	2.03	-0.06
11	2.02	2.06	-0.04	2.32	2.32	0.00	2.14	2.17	-0.03
12	2.24	2.25	-0.01	2.44	2.47	-0.03	2.32	2.30	0.02
INH	1.84	1.92	-0.08	2.19	2.08	0.11	1.98	1.98	0.00
Test Set									
6	2.21	2.05	0.16	2.46	2.32	0.14	2.31	2.17	0.14
13	2.11	2.05	0.06	2.46	2.32	0.14	2.25	2.16	0.09
14	2.14	2.04	0.10	2.49	2.35	0.14	2.28	2.18	0.10
15	1.78	1.98	-0.20	1.68	2.27	-0.59	1.74	2.13	-0.39
16	2.10	2.23	-0.13	2.30	2.25	0.05	2.18	2.13	0.05
17	2.25	2.46	-0.21	2.45	2.48	-0.03	2.33	2.36	-0.03

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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