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Synthesis and QSAR Study of Novel N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide derivatives as Anti mycobacterial Agents

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ABSTRACT

A series of N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide 2(a-j) have been synthesized under microwave irradiation and evaluated for their in vitro anti-mycobacterial activity against M. tuberculosis H37Rv using tube dilution method. Some of the compounds exhibited a significant anti mycobacterial activity when compared with first line drugs such as isoniazid (INH) and rifampicin (RIP).Stepwise multiple linear regression analysis was performed to derive QSAR models which were further evaluated for statistical significance and predictive power by internal and external validation. The QSAR model indicate that the thermodynamic descriptors (Molar refractivity), electronic descriptors (dipole moment), principal moment inertia, play an important role for anti mycobacterial activities.

Keywords: Isoniazid derivatives; Anti-mycobacterial; *Mycobacterium tuberculosis*, Quantitative structure-activity relationship.

INTRODUCTION

Over the last few years, tuberculosis is retrieving its place among these infectious diseases and today, nearly one-third of the world's population is infected with *Mycobacterium tuberculosis* with approximately three million patients deceasing every year [1]. A combination of factors has contributed to the observed increase in tuberculosis cases, including the worldwide AIDs epidemic, the general relaxation of public health policies in many countries, the increased overcrowding and homelessness in major cities and the increased emergence of multi drug-resistant strains of *M. Tuberculosis.* In spite of the availability of effective anti- tubercular drugs, such as isoniazid and rifampin, the emergence of resistant strains of *M. tuberculosis*, the pathogenic synergy of the tubercular and non tubercular mycobacterial infections with HIV infections [2-3], the scarce compliance with the complex therapeutic regimens, justify the effort directed to the design of new drugs [4, 5, 6,] for the treatment of tuberculosis and other atypical myco bacterioses [7].

2-Azetidinones (β-lactam) ring structure is a common structural feature of a number of broad spectrum β-lactam antibiotics which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases[8] and isoniazid is established anti tubercular drug. In the present investigation, with the aim to obtain new potent anti mycobacterial agents, we planned to synthesize novel coupled heterocyclic moiety containing

azetidinone and isoniazid, so as to get novel derivatives with enhanced anti tubercular activity. Novel isoniazid derivatives were synthesized by substitution on -N^2 of the pharmacophore -$CON^1HN^2H_2$; isoniazid was coupled via its Schiff's base[9] formation with 2-azetidinone under microwave irradiation, so as to get a N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide derivatives in good yields and in shorter reaction times. All the synthesized compounds were characterized and evaluated for their anti mycobacterial activity. QSAR study of the synthesized compounds was also performed.The objective of QSAR study was to develop a relationship between the structure of a set of compounds and the biological activity (BA) of interest [10].The relationship can be defined as

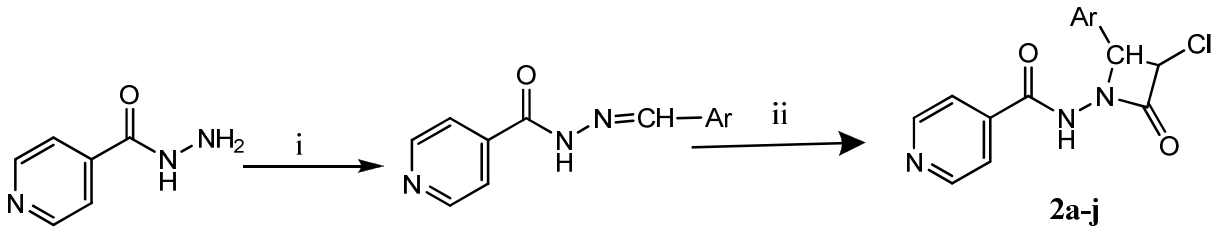
BA = *f* (molecular structure) = *f* (descriptors)

The ultimate objective of QSAR is prediction of either hypothesis on the mechanism of action for new analogs with high potency [11]. QSAR enables the investigators to establish a reliable quantitative structure-activity and structure-property relationship to derive an *in-silico* QSAR model to predict the activity of novel molecules prior to their synthesis.

MATERIALS AND METHODS

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled unless otherwise noted. Synthetic microwave oven Cata R , was used for the synthesis of final derivatives.Melting points were determined by open capillary method and are uncorrected. Infrared spectra were recorded on JASCO FT IR (PS-4000) using KBr powder technique and frequencies are expressed in cm^{-1}. Mass spectra were recorded on Micromasss Q-Tof Micro system mass spectrometer. ^{1}H-NMR spectra were recorded on Varian Mercury YH 300 FT-NMR Spectrometer operating at 300 MHz (^{1}H) and on BRUKER AVANCE II 400 spectrometer operating at 400 MHz (^{1}H) in deuterated dimethyl sulfoxide. Chemical shifts are reported in ppm (d) relative to tetramethylsilane. Proton spectra were typically obtained at room temperature. For TLC, plates coated with silica gel were run in chloroform/methanol mixture and spots were developed in iodine chamber. The anti-mycobacterial activities were assessed against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method. This methodology is nontoxic, uses a thermally- stable reagent. All the synthesized compounds were dissolved, separately, in dimethyl sulfoxide to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 500, 200, 100, and 50 and µg/mL so on were prepared in a similar manner up to 6 dilutions. A sweep of Mycobacterial tuberculosis H37Rv strain culture was discharged with the help of 22 S.G.W. nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water.

The synthesis of N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide derivatives **2(a-j)** was carried out as per the scheme of synthesis, Scheme1.



1a-j **2a-j**

	Ar		
a.	4- ClC_7H_5O	f.	4-OH, 3-MeO, C_7H_5O
b.	4- $NO_2C_7H_5O$	g.	C_7H_6O
c.	2- OHC_7H_5O	h.	Thiophenyl
d.	4- $MeOC_7H_5O$	i.	4-OHC_7H_5O
e.	Furfuryl	j.	$C_9H_{11}NO$

i = Ar-CHO / C_2H_5OH
ii = $ClCH_2COCl$/ Triethyl amine; MW

Scheme 1

The intermediate Schiff's bases (imines) **1(a-j)** were synthesized by refluxing isoniazid with aromatic and heterocyclic aldehydes in ethanol [12]. The N-(3-chloro-2-oxo-4-substitutedazetidin-1-yl) isonicotinamide derivatives **2(a-j)** were synthesized by cyclo condensation of intermediate Schiff's bases (imines) with chloroacetyl chloride and triethyl amine. The reaction was carried out under microwave irradiation, in dimethylformamide as solvent, with 70-85% yields. All the compounds were identified by spectral data. In general, IR spectra of Schiff's bases **1(a-j)** showed bands at 3448cm^{-1} (N-H stretch) and 1633cm^{-1} (C=N stretch). The ^{1}H-NMR showed signals at δ12.10 and 8.47 ppm due to exo cyclic NH and N=CH, respectively. The compounds of **2(a-j)** series exhibited bands at 1720 cm^{-1} (C=O stretch) and 751 cm^{-1} (C-Cl stretch). The ^{1}H-NMR presented signals at δ 8.6, 7.2-7.7 and 4.6 ppm due to -CONH, Ar-H and C-Cl groups, respectively.

4.1 Experiment

4.1.1. General procedures for the synthesis of N'-(substituted- aryl/heterylidene) isonicotinohydrazide ***1 (a-j)***

The isonicotinoyl hydrazide derivatives were prepared by reaction between equimolar quantities of isoniazid (1.0 equiv.) and substituted aldehydes (1.0 equiv.) in ethanol. The resulting mixture was refluxed for 2 to 2.5 h. The reaction mixture was then concentrated and cooled. Thus obtained solid was filtered and dried. The yield and mp of the product was recorded. The crude products were recrystallized from ethanol. The spectral data and elemental analysis data of **1 (a-j)** is given separately as Supporting Information**.**

4.1.2. General procedures for the synthesis of N-(3-chloro-2-oxo-4-substitutedazetidin-1- yl) isonicotinamide ***(2a-j)***

A mixture of Schiff base **1** (0.01mole) and chloroacetyl chloride (0.01mole) in dimethylformamide was taken in Erlenmeyer flask. Triethyl amine (0.01mole) was added to the reaction mixture, as a catalyst. The mixture was irradiated in microwave oven at 350W for about 5-7 min. Completion of reaction was monitored by TLC plates. The mixture was diluted with ice cold water and solid product precipitated. The crude compound was recrystallized from ethanol.

Spectral data and elemental analysis data of the synthesized derivatives is given below

4.1.2.1. N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2a).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{15}H_{11}N_3O_2Cl_2$) Calc C 53.59, H 3.30, N 12.50 Found: C 53.14, H 2.96, N 11.87.

4.1.2.2. N-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2b).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{15}H_{11}N_4O_4Cl$) Calc C 51.96, H 3.20, N 16.16Found: C 51.59, H 2.90, N 15.78.

4.1.2.3. N-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2c).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{15}H_{12}N_3O_3Cl$) Calc C 56.70, H 3.81, N 13.23 Found: C 56.42, H 3.54, N 12.91.

4.1.2.4. N-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2d).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{16}H_{14}N_3O_3Cl$) Calc C 57.93, H 4.25, N 12.67Found: C 57.49, H 3.86, N 12.32.

4.1.2.5. N-(3-chloro-2-(furan-2-yl)-4-oxoazetidin-1-yl) isonicotinamide ***(2e).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{13}H_{10}N_3O_3Cl$) Calc C 53.53, H 3.46, N 14.41 Found: C 53.09, H 3.14, N 13.85.

4.1.2.6. N-(3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl isonicotinamide ***(2f).***

IR (KBr) in cm^{-}1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{16}H_{14}N_3O_4Cl$) Calc C 55.26, H 4.06, N 12.08Found: C 54.89, H 3.75, N 11.85.

4.1.2.7. N-(3-chloro-2-oxo-4-phenylazetidin-1-yl) isonicotinamide ***(2g).***
IR (KBr) in cm-1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{15}H_{12}N_3O_2Cl$) Calc C 59.71, H 4.01, N 13.93Found: C 59.35, H 3.77, N 13.33.

4.1.2.8. N-(3-chloro-2-oxo-4-(thiophen-2-yl) azetidin-1-yl) isonicotinamide ***(2h).***
IR (KBr) in cm-1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{13}H_{10}N_3O_2SCl$) Calc C 50.73, H 3.28, N 13.65 Found: C 50.29, H 2.95, N 13.27.

4.1.2.9. N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2i).***
IR (KBr) in cm-1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{15}H_{12}N_3O_3Cl$) Calc C 56.70, H 3.81, N 13.23Found: C 56.41, H 3.65, N 12.97.

4.1.2.10. N-(3-chloro-2-(4-(dimethylamino) phenyl)-4-oxoazetidin-1-yl) isonicotinamide ***(2j).***
IR (KBr) in cm-1: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. ($C_{17}H_{17}N_4O_2Cl$) Calc C 59.22, H 4.97, N 16.25Found: C 58.82, H 4.74, N 15.91.

4.2 Anti mycobacterial activity
All the newly synthesized derivatives 2(a-j) were assayed in vitro for anti tubercular activity against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method, using isoniazid and rifampicin as a reference standard. The screening was done as per the procedures [13, 14] . This methodology is nontoxic, uses a thermally- stable reagent. All the synthesized compounds were dissolved, 10 mg of each, separately in dimethyl sulfoxide to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 500, 250, 125, and 62.5 and so on were prepared in a similar manner up to 6 dilutions. A sweep of Mycobacterial tuberculosis H37Rv strain culture was discharged with the help of 22 S.G.W. nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water. Each loopful was supposed to deliver 4 mg moist weight of bacilli. The bottle was shaken with the help of a mechanical shaker for 2 min. Using S.W.G. nichrome wire loop, 3 mm diameter, a loopful of the suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing test compounds. Lowenstein-Jensen medium containing standard drug as well as control was also inoculated with Mycobacterial tuberculosis H37Rv strain. The medium inoculated were incubated at 37°C for six weeks. The presence or absence of growth of organism was observed after six weeks. The MIC (minimal inhibitory concentration) was defined as the lowest drug concentration, which prevented the microbial growth. Result of anti mycobacterial activities with standard drugs isoniazid and rifampicin are discussed in Table 1. Study revealed that **2d,** and **2e,** exhibited stronger anti-mycobacterial activity.

Table 1: Antimycobacterial activity of isonicotinamide derivatives (2a-j)

Compound No.	MIC µg/ml
2a	>500
2b	200
2c	>500
2d	50
2e	100
2f	>500
2g	100
2h	>500
2i	>500
Isoniazid	0.2
Rifampicin	40

4.3. QSAR study
QSAR studies have been widely used to understand the relationship between the structure of the molecule and biological activity[15].All the computations in the present study were performed on PIV workstation. The molecular structures of the training set were sketched using Chem. Draw Ultra module of CS Chem. Office 2004 molecular modeling software ver. 6.0 [16], supplied by Cambridge Software Company. The sketched structures were exported

to Chem3D module in order to create its 3D model. Each model was "cleaned up" and energy minimization was performed using Allinger's MM2 force field by fixing Root Mean Square Gradient (RMS) to 0.1 Kcal/mol Aº. Further geometry optimization was done using semi emperical AM1 (Austin Model) Hamiltonian method, closed shell restricted wave function available in the MOPAC module until the RMS value becomes smaller than 0.001 Kcal/mol Aº.

Table 2: Descriptors calculated for QSAR study

Sr. No.	Descriptor	Type
1	Heat of Formation (HF)	Thermodynamic
2	Boiling Point (BP)	Thermodynamic
3	Critical Pressure (CP)	Thermodynamic
4	Critical Temperature (CT)	Thermodynamic
5	Critical Volume (CV)	Thermodynamic
7	Henry's Law Constant (HLC)	Thermodynamic
8	Ideal Gas Thermal Capacity (IGTC)	Thermodynamic
9	Log P	Thermodynamic
10	Melting Point (MP)	Thermodynamic
11	Molar Refractivity (MR)	Thermodynamic
12	Standard Gibbs Free Energy (SGFE)	Thermodynamic
13	Connolly Accessible Area (CAA)	Steric
14	Connolly Molecular Area (CMA)	Steric
15	Connolly Solvent–Excluded Volume (CSEV)	Steric
16	Ovality (OVA)	Steric
17	Principal Moment of Inertia – X (PMI–X)	Steric
18	Principal Moment of Inertia – Y (PMI–Y)	Steric
19	Principal Moment of Inertia – Z (PMI–Z)	Steric
20	Dipole Moment (D)	Electronic
21	Dipole Moment –X Axis (DX)	Electronic
22	Dipole Moment –Y Axis (DY)	Electronic
23	Dipole Moment –Y Axis (DZ)	Electronic
24	Electronic Energy (EE)	Electronic
25	HOMO Energy (HOMO)	Electronic
26	LUMO Energy (LUMO)	Electronic
27	Repulsion Energy (RE)	Electronic
28	Bend Energy (E_b)	Thermodynamic
29	Charge–Charge Energy (CCE)	Thermodynamic
30	Charge–Dipole Energy (CDE)	Thermodynamic
31	Dipole–Dipole Energy (DDE)	Thermodynamic
32	Non–1, 4 VDW Energy (E_v)	Thermodynamic
33	Stretch Energy (SE)	Thermodynamic
34	Stretch–Bend Energy (SBE)	Thermodynamic
35	Torsion Energy (E_t)	Thermodynamic
36	Total Energy (E)	Thermodynamic
37	Van der Waals e 1,4 Energy (VDWE)	Thermodynamic
38	VDW 1,4 Energy (VDWE)	Thermodynamic
39	Partition coefficient	Thermodynamic

Table 3 Calculated descriptor values for the given series of compounds

Comp. No.	MR	SBE	D	PMI-Y
2a	8.6920	0.1643	1.76720	4713.26
2b	10.7037	0.1393	3.9113	6073.36
2c	7.8238	0.1334	3.8966	2942.12
2d	8.2876	7.5377	3.8309	3362.31
2e	10.7988	-6.7280	4.1692	6176.67
2f	9.4074	-0.2147	3.4333	4863.62
2g	7.5131	0.2056	2.6008	2957.32
2h	8.2876	0.1137	3.9402	3514.49
2i	8.1925	0.2744	3.8079	3507.35
2j	8.2876	0.1563	3.8929	3063.51

The low energy conformers obtained from the aforementioned procedure were used for the calculation of the Chem SAR descriptors. The Chem SAR descriptors include physicochemical, thermodynamic, electronic and spatial descriptors available in the 'Analyze' option of the Chem3D package, the data is presented in Table 2 given in Supporting information. The descriptors calculated for the present study accounts four important properties of the

molecules: physicochemical, thermodynamic, electronic and steric, as they represent the possible molecular interactions between the receptor and the synthesized molecules; value of only those descriptors occurring in different equation is given in Table 3 given in supporting information.

To establish the correlation between physicochemical parameters as independent variable and anti mycobacterial activity as dependent variable, the data were transferred to statistical program VALSTAT [17]. Sequential multiple linear regression analysis method (in sequential multiple regression, the program searches for all permutations and combinations sequentially for the data set) was applied for the same. The best model was selected on the basis of statistical parameters viz., observed squared correlation coefficient (r^2), standard error of estimate (s), and sequential Fischer test (F). Z score (absolute difference between values of model and activity field, divided by the square root of mean square error of data set) was taken as a measure of outlier detection. To assess the self-consistency of derived models, they were validated using leave one out (LOO) and the predictive ability was checked using cross-validated squared correlation coefficient (r^2_{cv} or q^2), bootstrapping squared correlation coefficient (r^2_{bs}), chance statistics (evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.001 corresponds to 0.1% chance of fortuitous correlation), and outliers (on the basis of Z-score value). The ±data within parentheses are the standard deviation, associated with the coefficient of descriptors in regression equations. Each of the statistical parameters mentioned above were used for assessing the statistical significance of QSAR. Additionally the developed QSAR models were also checked for significance of the regression coefficients in the model and for multi colinearity problem by the calculation of Student's t-test values (t-value) using statistical software SYSTAT.

The generated QSAR models were validated for predictive ability inside the model (leave one out method) by using VALSTAT. The statistical program which is tailored specifically for QSAR statistics estimates the predictive potential of model by calculating the validation parameters squared cross-correlation coefficient (q^2), standard deviation of sum of square of difference between predicted and observed values (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}). Biological activity data and various physicochemical parameters were taken as dependent and independent variables, respectively and correlation's were established using sequential multiple regression analysis. Among the many correlations generated, two best quadratic and triparametric models were selected on the basis of statistical significance. The best models obtained are given below along with their statistical measures.

Model-I:
= -3.989(± 1.333) + 0.1364(± 0.032) MR - 0.001(± 0.0003) PMI-Y - 0.039(± 0.043) SBE - 0.417(± 0.202) D
n=24, r=0.905, r^2=0.820, variance=0.081, std=0.286, F=21.643

Model-II:
= -4.010(± 1.310) - 0.001(± 0.0003)] PMI-Y + 1.302(± 0.317) MR - 0.370(± 0.205) D
n=24, r=0.889, r^2=0.791, STD=0.300, F=25.269

Model–I show good correlation (r = 0.904) between descriptors (MR, PMI-Y, SBE, D) and the biological activity. Molar refractivity, thermodynamic descriptors is a corrected from of the molar volume, it reflects the effect of size, polarizalibility and steric bulk of molecules, as indicate in model-I, suggesting that MR plays a significant role towards the expressed biological activities, which is probably due to steric interaction occurring in the polar spaces. It has generally been assumed that a positive coefficient with an MR term in a correlation equation suggests a binding action via dispersion force. Such binding could produce a concomitant conformational change in a macromolecular binding site; however, if the conformational are detrimental, a negative coefficient could result for the MR term. Stretch bend energy, a thermodynamic parameter, deals with energy required to stretch the two bonds involved in a bond angle when that bond is severely compressed. The negative coefficient of the descriptor in model suggests that an increase in the stretch bend of the molecule is not conductive for activity. Moment of inertia is a steric parameter. The value of PMI depends on the total mass of the molecule, the distribution within the molecule and position of axis rotation of the molecule. Principal moment of inertia (PMI-Y) is a spatial descriptor which explains the significance of orientation and conformational rigidity of biological activity. The negative coefficient of PMI-Y suggests the presence of bulky substituents oriented towards Z-axis of the molecule will give better activity. Dipole moment indicates the strength and orientation behaviour of a molecule in an electrostatic field. It is a vector quantity with both additive and constitutive properties. The contribution of dipole moment illustrates the non–covalent, electronic interactions between the microtubule enzymes and inhibitor molecules. Thus, model-I suggests that molar refractivity is of significance having high value of t-test indicating statistical significance of calculated

regression coefficient. To confirm these results, the value of -Log MIC was estimated using leave one-out and correlated with observed value of -Log MIC. The value of r^2_{bs}, chance and q^2 in randomized biological activity indicates the statistical significance of the model as given below.

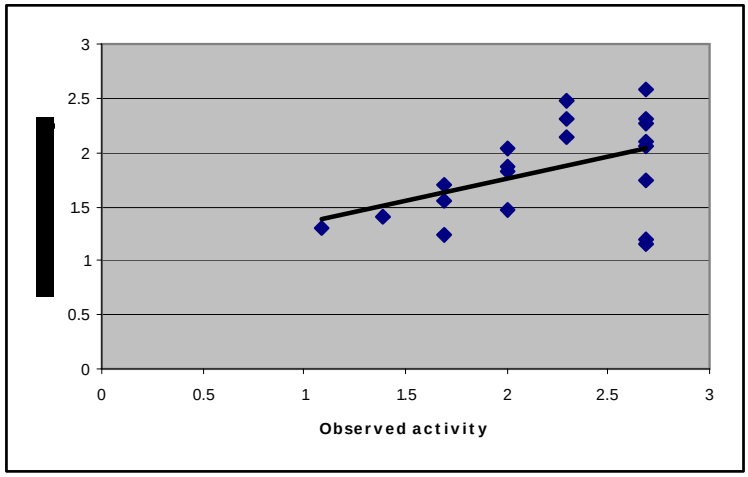
r^2_{bs} = 0.845, Chance = < 0.001, q^2 = 0.729, S_{PRESS} = 0.351, S_{DEP} = 0.312
The predicted activity data of model-I is shown in Table 4

Table 4 Predicted activity data of model-I

Sr.No.	Observed –Log MIC	Predicted –Log MIC	Calculated –Log MIC
2a	2.69	2.0993	2.1540
2b	2.30	2.2999	2.3794
2c	2.69	2.2641	2.3075
2d	1.69	1.2463	1.1378
2e	2	1.4663	1.5188
2f	2.69	2.2982	2.3278
2g	2	1.8711	1.4084
2h	2.69	1.2052	1.2137
2i	2.69	1.1483	1.1839
2j	1.39	1.4049	1.7631

A plot of observed versus predicted -Log MIC for anti mycobacterial activity using model-I is shown in Figure 1.

Fig. 1. Observed versus predicted (LOO) pIC_{50} for anti-mycobacterial activity using Model-I



Model-II shows good correlation (r =0.889) between descriptor (MR, PMI-Y, D) and the biological activity. Molar refractivity, thermodynamic parameters that contribute positively to the model means the groups which increases molar volume, may cause increase in biological activity. The negative coefficient of PMI-Y suggests the presence of bulky substituents oriented towards Z-axis of the molecule will give better activity. Thus, model suggests that MR is of significance having high value of t-test indicating statistical significance of calculated regression coefficient. To confirm these results, the value of -Log MIC was estimated using leave one-out and correlated with observed value of -Log MIC. The value of r^2_{bs}, chance and q^2 in randomized biological activity indicates the statistical significance of the model as follows.

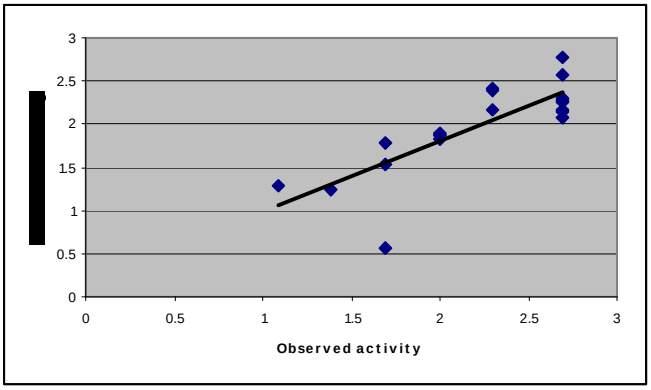
r^2_{bs} = 0.816, Chance= 0.001, q^2 = 0.713, S_{PRESS} = 0.352, S_{DEP} = 0.321
The predicted activity data of model-II is shown in Table 5

Table 5 Predicted activity data of model-II

Sr. No.	Observed –Log MIC	Predicted –Log MIC	Calculated –Log MIC
2a	2.69	2.1571	2.1966
2b	2.30	2.3962	2.4461
2c	2.69	2.2868	2.3275
2d	1.69	1.5303	1.4693
2e	2	1.8647	1.3546
2f	2.69	2.3091	2.3362
2g	2	1.8952	1.7859
2h	2.69	2.2477	2.2524
2i	2.69	2.1518	2.1859
2j	1.39	1.2414	1.2924

A plot of observed versus predicted -Log MIC for anti mycobacterial activity using model-II is shown in Figure 2.

Fig. 2. Observed versus predicted (LOO) pIC50 for anti- mycobacterial activity using Model- II



Although the inter correlation between the two descriptors is within the acceptable range (< 0.8).Comparison of model-I and model-II revels that model-I shows better correlation (r = 0.905) between descriptors and biological activity than model-II (r = 0.889). The bootstrapping r^2 (r^2_{bs} = 0.845) results reflect the significance of the model-I when compared to model-II. The cross validate (q^2) values reflect predictive power of the model-I. Low standard error of estimation (<0.4) suggests a high degree of confidence in the analysis. Moreover, the descriptors used to construct the model are not correlated with each other as suggested by their correlation matrix values, respectively as presented in Table 6 and Table 7. However, the model manifests moderate predictive potential as indicated by cross-validated correlation coefficient values.

Table 6 Correlation coefficient values of model-I

Parameters	MR	PMI-Y	SBE	DM
MR	1.000			
PMI-Y	0.899	1.000		
SBE	0.252	0.201	1.000	
DM	0.344	0.090	0.139	1.000

Table 7 Correlation coefficient values of model-II

Parameters	PMI-Y	MR	DM
PMI-Y	1.000		
MR1	0.893	1.000	
DM	0.090	0.336	1.000

RESULTS AND DISCUSSION

In the present investigation, new series of isoniazid derivatives, 2(a-j) have been synthesized and evaluated for anti mycobacterial activity against *M. Tuberculosis* H_{37}Rv. The compounds have shown moderate anti mycobacterial activity and compound namely, 2j have shown good potency and significant in vitro anti mycobacterial activity against *M. Tuberculosis*. Comparative study of the substitution pattern of the aryl and heteryl group towards antimicrobial activity has shown that electron donating groups gives the better activity while electron withdrawing groups causes less activity. The details are given as follows: When substituent on phenyl ring is $–OCH_3$, the compound **2d**, has shown comparable activity with standard drug (rifampicin). The compound containing $(–N(CH_3)_2$ group on phenyl ring, the compound **2j** has shown excellent activity when compared with standard drug (rifampicin). However, when compared with the isoniazid, the standard drug, only compound no. **2j** have shown moderate activity.

CONCLUSION

The presence of isoniazid and azetidinone moieties in the final derivatives have contributed towards better anti mycobacterial activity. However, the synthesized **N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide** derivatives, which were modified isoniazid derivatives obtained by substitution on N^2 of the pharmacophore $–CON^1HN^2H_2$ of the standard anti mycobacterial drug, isoniazid, resulted in decrease in anti mycobacterial activity.QSAR analysis was performed on a series of N-(3-chloro-2-oxo-4-substituted azetidin-1-yl) isonicotinamide using molecular modelling program Chem Office 2004. QSAR models were proposed for anti mycobacterial activity using Chem SAR descriptors employing sequential multiple regression analysis method. The selected models were checked for multicolinearity and autocorrelation. The predictive power of each model was estimated with bootstrapping r^2 method and leaves one out cross validation method. The result of the study suggests involvement of molar refractivity and dipole moment in anti mycobacterial activity increases in molar volume conducive for anti mycobacterial activity. Thus, as the literature suggests, substitution of N^2 of the standard drug isoniazid has resulted in the overall decrease in anti mycobacterial activity, but two of the synthesized compounds **2d** and **2e** have shown the promising anti mycobacterial activity.

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