Research Article

Qsar Studies of Novel 3-Substituted-5-(Pyridin-4-YL) -3H-1, 3, 4-Oxadiazol-2-One and 2-Thione Analogues as Antimycobacterial Agents

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ABSTRACT

QSAR study was performed on novel 3-substituted 5-(pyridin-4-yl)-3H-1, 3, 4-oxadiazol-2-one and 2-thiione analogues. 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 best QSAR model was selected, having correlation coefficient (r) = 0.904, standard error of estimation (SEE) = 81 %, and cross validated squired correlation coefficient (q2) = 0.73. The predictive ability of the selected model was also confirmed by leave one out cross validation. The QSAR model indicate that the thermodynamic descriptors (Molar refractivity), electronic descriptors (dipole moment), principal moment inertia, play an important role for antimycobacterial activities. The results of the present study may be useful on the designing of the more potent thionine analogues as antimycobacterial agents.

Keywords: Quantitative structure-activity relationship; Oxadiazole; Pyridine derivatives; Antimycobacterial activity

INTRODUCTION

The emergence of multidrug resistant strains (MDR) of Mycobacterium tuberculosis[1-4] together with the spread of severe opportunistic disseminated infections produced by mycobacteria other than tuberculosis (MOTT), particularly Mycobacterium avium in immune compromised patients prompted the search for new antimycobacterial agents.

Tuberculosis (TB), estimated to infect about one-third of the world's population, still remains the world-wide main cause of death among the infectious disease.

In spite of the availability of effective antitubercular drugs, such as isoniazid and rifampin, the emergence of resistant strains of M. tuberculosis, the pathogenic synergy of the tubercular and nontubercular mycobacterial infections with HIV infections, the scarce compliance with the complex therapeutic regimens, justify the e ort directed to the design of new drugs for the treatment of tuberculosis and other atypical mycobacterioses.

It has been reported[9] that conversion of isoniazid to oxadiazoles produced the corresponding 5-substituted 3H-1,3,4-oxadiazol-2-thione and 3H-1,3,4,-oxa-diazol-2-one and their 3-alkyl or aralkyl derivatives, characterized by antimycobacterial activity against M. tuberculosis $H_{37}Rv$.[5-8]

With the aim to obtain new potent antimycobacterial agents, we performed QSAR studies on a series of 5-(pyridin-4-yl)-3H-1,3,4-oxa-diazol-2-thione 1a–l and 5-(pyridin-4-yl)-3H-1,3,4-oxa-diazol-2-one 2-thione derivatives, in which the nitrogen at the 3 position is linked through a methylene bridge to a cyclic amine.

The objective of QSAR study is 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]

The nature of descriptors used and the extent to which they encode the structural feature of the molecules that are related to biological activity of drugs, depend on the types and magnitude of reaction between the receptor and drug molecules.

The descriptors may be physicochemical parameters (hydrophobic, steric or electronic), structural descriptors, topological indices geometric parameters (calculated from quantum mechanical method[9]) and are the determining factors regulating the interactions.[12]

A 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.

The overall process of QSAR model development can be divided into three stages namely, the data preparation, data analysis, and model validation, representing a standard practice of any QSAR modeling.

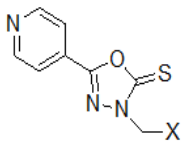
In this research, an attempt has been made to describe and deduce a correlation between structure and antimycobacterial activity of substituted 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-thione and 3H-1,3,4,-oxadiazol-2-one and their 3-alkyl or aralkyl derivatives.

MATERIALS AND METHODS

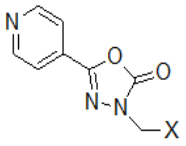
A set of 24 substituted 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-thione and 3H-1,3,4,-oxa-diazol-2-one exhibiting potent antimycobacterial activity was taken from the reported work of M. G. Mamolo.[13]

The biological activity was converted to -log (biological activity) to decrease the variance and to convert the data into free energy changes related value used as the response variable for the QSAR analysis.

The -log values of MIC along with the structure of compounds in the series are presented in Table 1.



1a-l



2a-l

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, supplied by Cambridge Software Company.[14] 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 A°.

Further geometry optimization was done using semiemperical 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 A°.

The low energy conformers obtained from the aforementioned procedure were used for the calculation of the ChemSAR descriptors.

The ChemSAR descriptors include physicochemical, thermodynamic, electronic and spatial descriptors available in the 'Analyze' option of the Chem3D package (Table 2).

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 indole (value of only those descriptors occurring in different equation is given in Table 3).

Table 1: Antimycobacterial activity of compounds 1a–l and 2a–l against M. tuberculosis $H_{37}Rv$[a]

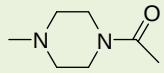
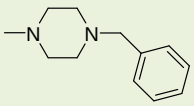
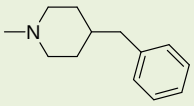
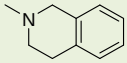
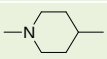
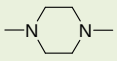
S. No.	X	Compound	MIC (lg/mL)	-Log MIC	Compound	MIC (lg/mL)	-Log MIC
1		1a	20	1.3	2a	2.5	0.39
2		1b	40	1.6	2b	2.5	0.39
3		1c	40	1.6	2c	2.5	0.39
4		1d	10	1	2d	1.25	0.09
5		1e	40	1.6	2e	2.5	0.39
6		1f	40	1.6	2f	1.25	0.09
7		1g	40	1.6	2g	2.5	0.39
8		1h	20	1.3	2h	2.5	0.39
9		1i	40	1.6	2i	2.5	0.39
10		1j	40	1.6	2j	2.5	0.39
11		1k	40	1.6	2k	2.5	0.39
12		1l	40	1.6	2l	2.5	0.39

Table 2: Descriptors calculated for QSAR study

S. 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
1	8.6920	0.1643	1.76720	4713.26
2	10.7037	0.1393	3.9113	6073.36
3	7.8238	0.1334	3.8966	2942.12
4	8.2876	7.5377	3.8309	3362.31
5	10.7988	-6.7280	4.1692	6176.67
6	9.4074	-0.2147	3.4333	4863.62
7	7.5131	0.2056	2.6008	2957.32
8	8.2876	0.1137	3.9402	3514.49
9	8.1925	0.2744	3.8079	3507.35
10	8.2876	0.1563	3.8929	3063.51
11	8.2876	-9.9470	3.7803	3439.09
12	8.1663	0.2780	2.2940	3459.16
13	7.8358	0.4311	1.3844	4734.68
14	9.8475	0.4600	3.4820	5897.59
15	6.9676	0.4006	3.6016	2951.88
16	7.4314	0.3425	3.6448	3286.90
17	9.9426	0.3547	4.1813	5773.00
18	8.5512	0.2192	3.1752	4685.20
19	6.6569	0.4721	2.4547	2884.82
20	7.4314	0.3829	3.6397	3560.31
21	7.3363	0.5333	3.5835	3529.57
22	7.4314	0.4217	3.6393	3058.78
23	7.4314	0.3705	3.6256	3671.88
24	7.3101	0.5124	2.1447	3514.65

To establish the correlation between physicochemical parameters as independent variable and antimycobacterial activity as dependent variable, the data were transferred to statistical program VALSTAT.[15] 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 multicolinearity problem by the calculation of Student's t-test values (t-value) using statistical software SYSTAT.[16]

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}).

RESULTS AND DISCUSSION

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

P MIC = -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

P MIC = -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, polarizability 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 forces. 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 behavior 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.

Table 4: Predicted activity data of Model-I

S. No.	Observed–Log MIC	Predicted–Log MIC	Calculated–Log MIC
1	1.30	1.0993	1.1540
2	1.60	1.2999	1.3794
3	1.60	1.2641	1.3075
4	1.00	1.2463	1.1378
5	1.60	1.4663	1.5188
6	1.60	1.2982	1.3278
7	1.60	1.3711	1.4084
8	1.30	1.2052	1.2137
9	1.60	1.1483	1.1839
10	1.60	1.8049	1.7631
11	1.60	2.0391	1.7809
12	1.60	1.8256	1.7648
13	0.39	0.0558	0.1867
14	0.39	0.7382	0.6695
15	0.39	0.3052	0.3178
16	0.09	0.5496	0.5043
17	0.39	0.7049	0.6323
18	0.09	0.5860	0.5452
19	0.39	0.4691	0.4568
20	0.39	0.1468	0.1823
21	0.39	0.1076	0.1499
22	0.39	0.8106	0.7739
23	0.39	-0.0091	0.0600
24	0.39	0.7069	0.6599

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 above in Table 4.

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

S. No.	Observed–Log MIC	Predicted–Log MIC	Calculated–Log MIC
1	1.30	1.1571	1.1966
2	1.60	1.3962	1.4461
3	1.60	1.2868	1.3275
4	1.00	1.5303	1.4693
5	1.60	1.2647	1.3546
6	1.60	1.3091	1.3362
7	1.60	1.3452	1.3859
8	1.30	1.2477	1.2524
9	1.60	1.1518	1.1859
10	1.60	1.8414	1.7924
11	1.60	1.3772	1.3991
12	1.60	1.8307	1.7692
13	0.39	0.0773	0.1984
14	0.39	0.7639	0.6936
15	0.39	0.2965	0.3103
16	0.09	0.5557	0.5103
17	0.39	0.7886	0.7024
18	0.09	0.5638	0.5233
19	0.39	0.4122	0.4087
20	0.39	0.1633	0.1955
21	0.39	0.0801	0.1280
22	0.39	0.8136	0.7766
23	0.39	0.0070	0.0714
24	0.39	0.6893	0.6450

A plot of observed versus predicted -Log MIC for antimycobacterial activity using model-II is shown in Figure 1.

Although the intercorrelation between the two descriptors is within the acceptable range (< 0.8).

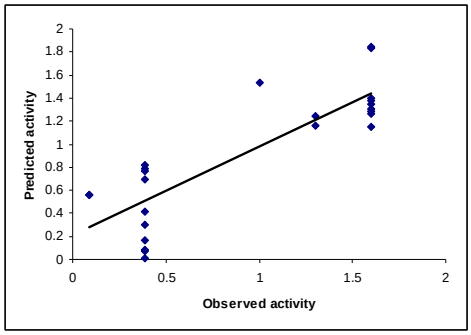


Figure 1: Observed versus predicted (LOO) pIC_{50} for anti-inflammatory activity using model-II

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 (Table 6 and Table 7). However, the model manifests moderate predictive potential as indicated by cross-validated correlation coefficient values.

Table 6: Correlation matrix for parameters in 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 matrix for parameters in model II

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

CONCLUSION

QSAR analysis was performed on a series of antimycobacterial 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-thione 1a–l and 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-one 2-thione 2a-l derivatives using molecular modeling program Chemoffice 2004. QSAR models were proposed for antimycobacterial activity of the thione using ChemSAR 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 antimycobacterial activity of thione increases in molar volume conducive for antimycobacterial activity.

Thus, the discussed models could be explored further to design potent antimycobacterial agents.

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