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QSAR Modeling of Antimicrobial Activity with Some Novel 1,2,4- Triazole Derivatives, Comparison with Experimental Study

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ABSTRACT

Our study performed upon an extended series of 28 compounds of 1,2,4-triazole derivatives that demonstrate substantial in vitro antimicrobial activities by serial plate dilution method, using quantitative structure-activity relationship (QSAR) methods that imply analysis of correlations and multiple linear regression (MLR); a significant collection of molecular descriptors was used e.g., Edge adjacency indices, GETAWAY , 3D-MoRSE , Burden eigenvalues and Constitutional descriptors. The obtained multi-parametric models when a different class of molecular descriptors was used led to three correlation coefficients closed to 0.900, 0.896 and 0.901 respectively. Results indicated this is no significant statistical differences between calculated activities of these compounds with laboratory methods thus, the obtained models allowed us to predict antimicrobial activity of substituted 1,2,4-triazole derivatives .

Keywords: Quantitative structure-activity relationship, Multiple linear regression, Antimicrobial activity, 1,2,4-triazole derivatives

1. INTRODUCTION

The steadily increasing bacterial resistance to existing drugs is a serious problem in antibacterial therapy and necessitates continuing research into new classes of antibacterials. Various 1,2,4-triazole derivatives have been reported to possess diverse types of biological properties such as antibacterial [1], antifungal [2], anti-inflammatory [3] antihypertensive [4], antiviral [5], antileishmanial [6] and antimigraine activities [7]. A thorough literature

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survey reveals that presence of 4-substituted thio phenoxy and 4-methyl sulphonyl phenoxy moieties is an important structural feature of wide variety of synthetic drugs [8]. It has been established that introduction of 4-methyl mercapto phenyl and 4-methyl sulphonyl phenyl groups to different heterocycles has yielded many biologically active compounds endowed with wide spectrum of pharmacological and antimicrobial activities [9]. It is well known that the N-bridged heterocycles derived from 1,2,4-triazoles find applications in the field of medicine agriculture and industry. A large number of triazolothiadiazoles and triazolothiadiazines have been reported to possess CNS depressant, antibacterial, antifungal, antitumour, anti-inflammatory, herbicidal, pesticidal and insecticida properties [10]. Therefore, it was envisaged that chemical entities with both 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles/1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines and 4-sulphursubstituted phenyl moieties, containing aryl ether linkage would result in compounds of interesting biological activities. In continuation of Karabasanagouda et al research program on the synthesis of novel heterocyclic compounds exhibiting biological activity, it was thought to be interesting to synthesize compounds containing the features, namely, 1,2,4-triazole moiety fused with the 1,3,4-thiadiazole/1,3,4-thiadiazine rings, in addition to have a sulphur substituted phenoxy group and to study their antimicrobial activities. The present study describes the synthesis of hither to unreported 6-aryl-3-{(4-thiosubstituted/methyl sulphonyl phenoxy) methyl}-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (6a-s) and 6-aryl-3-{(4-thiosubstituted/methyl sulphonyl phenoxy) methyl}-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (7a-i) and evaluation of their anti bacterial and antifungal activities [11].

Quantitative structure-activity relationships (QSAR), as a major factor in drug design, are mathematical equations relating chemical structure to their biological activity [12]. This work studies the role of different structural parameters in the case of a series of pyrimidinic congeners with antimicrobial activity: the objective was to assess electronic, transportation and topological effects of ideal substitutions to express while binding to target receptors. In the present study, we aimed to develop QSAR equations for the antimicrobial activity of a series of 1,2,4-triazole drugs. We therefore used six types of molecular descriptors to derive a quantitative relation between the antimicrobial activity and structural descriptors obtained by multiple linear regression (MLR) for the modeling and prediction of antimicrobial activities of 1,2,4-triazole derivatives.

2. ABOUT 1,2,4-TRIAZOLE DERIVATIVES

2.1 *Chemistry*

The reaction sequences employed for synthesis of title compounds are shown in Fig. 1. The key intermediate, ethyl [4-(thioalkyl) phenoxy] acetates (**2a-b**) was prepared by treating ethyl chloroacetate with 4-(thioalkyl) phenols (**1a- b**) in boiling dry acetone in presence of

potassium carbonate. The compound, ethyl [4-(methyl sulphonyl phenoxy] acetate (**2c**) was obtained by the oxidation of ethyl [4-(methyl thio) phenoxy] acetate (**2a**) with 30% hydrogen peroxide in acetic acid. These esters (**2a-c**) were conveniently converted to 2-[4-(thioalkyl/methyl sulphonyl phenoxy] acetohydrazides (**3a-c**) by refluxing it with hydrazine hydrate in methanol. The compounds **3a-c** on reaction with carbon disulphide in methanolic potassium hydroxide yielded corresponding potassium dithiocarbazates (**4a-c**) in good yield. The required 4-amino-5-{[thioalkyl/methyl sulphonyl phenoxy) methyl}-4H-1,2,4-triazole-3-thiols (**5a-c**) were synthesized by refluxing **4a-c** with aqueous hydrazine hydrate. Condensation of **5a-c** with various aromatic carboxylic acids in presence of boiling phosphorous oxychloride yielded 6-aryl-3-{(4-thioalkyl/methyl sulphonyl phenoxy) methyl}-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (6a-s) and with various phenacyl bromides in refluxing ethanol gave 6-aryl-3-{(4-thioalkyl/methyl sulphonyl phenoxy) methyl}-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**7a-i**) in good yield. The structural assignments to new compounds were based on their elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR and mass) data. The characterization data of all the new compounds are summarized in Table 1[11].

2.2 *Biological Activities*

2.2.1 *Antibacterial Studies*

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterial stains by serial plate dilution method [13]. Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37ºC. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

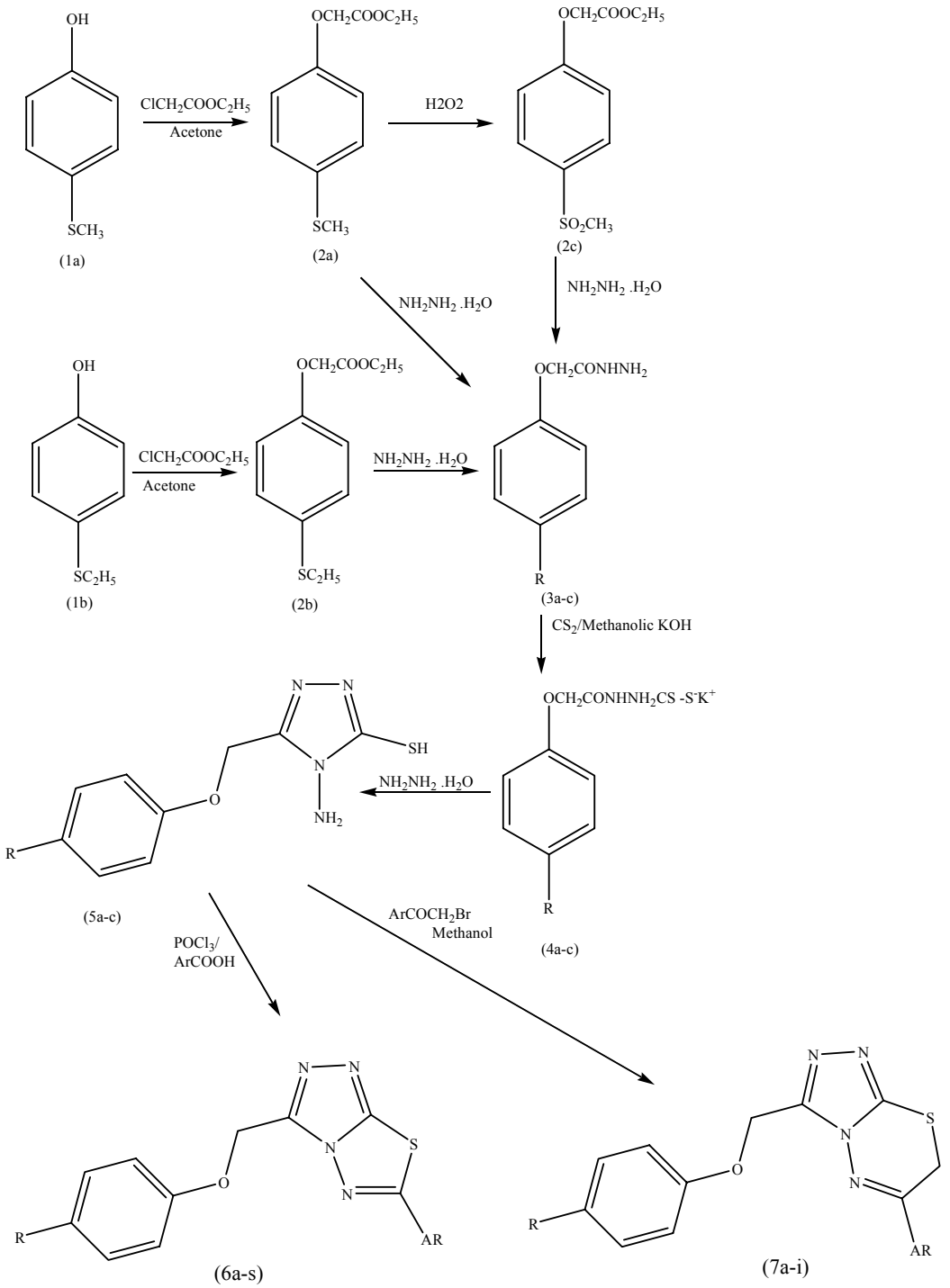

Figure 1. Preparation of 1,2,4-triazolothiadiazoles (6a-s) and 1,2,4-trazolothiadiazines (7a-i).

Table 1. Characterization data, antibacterial and antifungal activities of title compounds.

Compound	Ar	R	Mol. formula	Mol. mass	MP (ºC)	C in mg/ml		
						Antibacterial activity[A]	Antifungal activity[B]	Antifungal activity[C]
6a	C_6H_5	SCH_3	$C_{17}H_{14}N_4OS_2$	354	140	6.25	6.25	6.25
6b	2-ClC_6H_4	SCH_3	$C_{17}H_{13}ClN_4OS_2$	389	110	25.0	25.0	25.0
6c	4-$CH_3C_6H_4$	SCH_3	$C_{18}H_{16}N_4OS_2$	368	142	6.25	6.25	6.25
6d	4-$OCH_3C_6H_4$	SCH_3	$C_{18}H_{16}N_4O_2S_2$	384	110	6.25	6.25	6.25
6e	4-$NH_2C_6H_4$	SCH_3	$C_{17}H_{15}N_5OS_2$	369	120	6.25	6.25	6.25
6f	2,3-$(Cl)_2C_6H_4$	SCH_3	$C_{17}H_{12}Cl_2N_4OS_2$	371	106	6.25	6.25	6.25
6g	$C_6H_5CH_2$	SCH_3	$C_{18}H_{16}N_4O_2S_2$	384	112	6.25	6.25	6.25
6h	C_6H_5	SC_2H_5	$C_{18}H_{16}N_4OS_2$	368	129	6.25	6.25	6.25
6i	2-ClC_6H_4	SC_2H_5	$C_{18}H_{15}ClN_4OS_2$	402	102	25.0	25.0	25.0
6j	4-$CH_3C_6H_4$	SC_2H_5	$C_{19}H_{18}N_4OS_2$	382	105	6.25	6.25	25.0
6k	4-CH_3O-C_6H_4	SC_2H_5	$C_{19}H_{18}N_4O_2S_2$	398	98	25.0	25.0	25.0
6l	2-$NH_2C_6H_4$	SC_2H_5	$C_{18}H_{17}N_5OS_2$	383	120	25.0	25.0	25.0
6m	2,3-$(Cl)_2C_6H_4$	SC_2H_5	$C_{18}H_{14}Cl_2N_4OS_2$	437	118	25.0	25.0	25.0
6n	2-OHC_6H_4	SC_2H_5	$C_{18}H_{16}N_4O_2S_2$	384	185	25.0	25.0	25.0
6o	C_6H_5	SO_2CH_3	$C_{17}H_{14}N_4O_3S_2$	386	220	12.5	25.0	12.5
6p	2-ClC_6H_4	SO_2CH_3	$C_{17}H_{13}ClN_4O_3S_2$	420	200	12.5	25.0	12.5
6q	3-ClC_6H_4	SO_2CH_3	$C_{17}H_{13}ClN_4O_3S_2$	420	196	12.5	25.0	12.5
6r	$CH_2C_6H_5$	SO_2CH_3	$C_{18}H_{16}N_4O_3S_2$	400	170	12.5	25.0	12.5
6s	2,3-Dichloro-C_6H_4	SO_2CH_3	$C_{17}H_{12}Cl_2N_4O_3S_2$	400	110	6.25	6.25	6.25
7a	2-OH-benzamide	SCH_3	$C_{19}H_{17}N_5O_3S_2$	427	180	25.0	25.0	25.0
7b	2,4-Dichloro-C_6H_3	SCH_3	$C_{18}H_{14}Cl_2N_4OS_2$	437	110	6.25	6.25	6.25
7c	4-ClC_6H_4	SCH_3	$C_{18}H_{15}Cl_2N_4OS_2$	402	190	6.25	6.25	6.25
7d	2-OH-benzamide	SC_2H_5	$C_{20}H_{19}N_5O_3S_2$	441	175	6.25	6.25	6.25
7e	2,4-Dichloro-C_6H_3	SC_2H_5	$C_{19}H_{16}Cl_2N_4OS_2$	451	104	25.0	25.0	25.0
7f	4-ClC_6H_4	SC_2H_5	$C_{19}H_{17}ClN_4OS_2$	416	116	25.0	25.0	25.0
7g	2-OH-benzamide	SO_2CH_3	$C_{19}H_{17}N_5O_5S_2$	459	218	12.5	25.0	12.5
7h	2,4-Dichloro-C_6H_3	SO_2CH_3	$C_{18}H_{14}Cl_2N_4O_3S_2$	469	110	12.5	25.0	12.5
7i	4-ClC_6H_4	SO_2CH_3	$C_{18}H_{15}ClN_4O_3S_2$	434	180	25.0	25.0	25.0

[A] -The antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

[B] - The antifungal activity against *Penicillium marneffei*.

[C] - The antifungal activity against *Aspergilus flavus*, *Aspergilusfumigatus*, and *Trichophyton mentagroph*ytes.

A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37ºC for an hour. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37ºC for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [14]. The minimum inhibitory concentration (MIC) was determined for 6a-7i and the results are summarized in Table 1.

2.2.2 *Antifungal Studies*

Newly prepared compounds were screened for their antifungal activity against *Aspergilus flavus*, *Aspergilus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* in DMSO by serial plate dilution method [14]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37ºC for 1 h. Using a punch, wells were made on these seeded agar plates minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37ºC for 3-4 days. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with cyclopiroxolamine as standard [15]. The minimum inhibitory concentration (MIC) was determined for 6a-7i and the results are summarized in Table 1.

3. MODELING

3.1 *QSAR Model*

QSAR expresses a multivariate mathematical relationship between a set of physicochemical properties or descriptors, $\{x_{ij}\}$, and a experimental function or biological activity, $\{y_i\}$. The QSAR relationship is expressed as a mathematical model, quantitative in the sense that it is

used to account for the observed activity. For a compound *i*, the linear equation that relates molecular properties, $x_1, x_2, \ldots$ to the desired activity, *y*, is:

$$y_i = x_{i1}b_1 + x_{i2}b_2 + \ldots + x_{in}b_n + e_i \quad (1)$$

Expressing the previous equation in a compact form for the general case of *n* selected descriptors, x_i, the QSAR equation results into:

$$y_i = \sum_{j=1}^{n} x_{ij}b_j + e_i \quad (2)$$

where *b* are the linear slopes that express the correlation of the particular molecular property x_{ij} with the activity y_i of the compound *i*; and e_i is a constant. The slopes and the constant are often calculated using regression analysis. Biological activity y_i is usually related to log (C), where C is the molar concentration which determines a constant biological response.

In this work, only the models with a single dependent variable, or *y* observation will be considered, although some models can deal with several biological activities. The strength of a QSAR model depends on the quality of this variable.

The independent variables, so-called descriptors, are usually physicochemical properties that describe some aspects of the chemical structure, which may be either experimentally or theoretically determined. The improper choice of independent variables can result in poor QSAR models. In a typical QSAR study, a large number of descriptors can be used; however, attention must be paid to overfitting, because with enough parameters any model can be successfully correlated. The final QSAR equation seeks to find the smallest number of descriptors that can adequately model the activity of the compounds in the study. The maximum recommended ratio is a single independent variable to compounds [16].

Actual drug design methods quantify biological activity depending on the molecular structure. Usually it is accomplished by modifying a reference structure through grafting X substituent. This leads to a series of bioactive compounds called effectors (E), or more recently ligands (L). L biological activities are determined and then correlated with the structure using correlation analysis methods, multi-linear regression equations like Equation 1 [17].

3.2 Computational Details

3.2.1 Moleculardescriptores

The considered structures to be analyzed starting from the general structure shown in Figure 1 presented in Table1. Upon these structures, molecular modeling was performed using the HyperChem 7.1 programme package (MM+ programme) [18] with 0.05 kcal/mol

RMS gradient. Optimization process comprised the optimized potentials for liquid simulations (OPLS). Conformational analysis was performed with Conformational Search from HyperChem 7.1 package program [18].

Antimicrobial activities will be correlated with molecular descriptors from Table 2. To calculate these descriptors, DRAGON 5.4 programme [19] was used; it allows importing 3D structures from the HyperChem package [18].

Table 2. Molecular descriptors for antimicrobial activity considered in this work.

No.	Symbol	Definition	Class
1	eeig11r	Eigenvalue 11 from edge adj. matrix weighted by resonance integrals.	Edge adjacency indices
2	eeig12r	Eigenvalue 12 from edge adj. matrix weighted by resonance integrals.	
3	eeig09x	Eigenvalue 09 from edge adj. matrix weighted by resonance integrals.	
4	ish	Standardized information content on the leverage equality.	GETAWAY descriptors
5	rtm+	R maximal index / weighted by atomic masses.	
6	r6m+	R maximal autocorrelation of lag 6 / weighted by atomic masses.	
7	r3u+	R maximal autocorrelation of lag 3 / unweighted.	
8	mor20e	3D-MoRSE – signal 20 / weighted by atomic Sanderson electronegatives.	3D-MoRSE descriptors
9	mor18v	3D-MoRSE – signal 18 / weighted by atomic van der waals volumes.	
10	mor20p	3D-MoRSE – signal 20 / weighted by atomic polarizabilities.	
11	belm2	Lowest eigenvalue n. 2 of Burden matrix / weight by atomic masses.	Burden eigenvalues
12	behp5	Highest eigenvalue n. 5 of Burden matrix / weight by atomic polarizabilities.	
13	Mv	Mean atomic van der waals volume (scaled on Carbon atom)	Constitutional descriptors

3. 2.2 *Stepwise Multiple Linear Regression*

DRAGON provides 1664 molecular descriptors and in order to select the predominant parameters that significantly affect the antimicrobial activity of the compounds, we employed the statistic software SPSS, taking MIC as the dependent variable and every candidate descriptor calculated above as an independent variable to perform the stepwise multiple linear regression. Therefore we derived some molecular descriptors from the DRAGON programme, such as Edge adjacency indices, GETAWAY descriptors, 3D-MoRSE descriptors, Burden eigenvalues and Constitutional descriptors. The mentioned descriptors are presented in Table 2 and their numerical values are listed in Tables 3, 4 and 5 respectively.

In the next step, QSAR equation was made through the multiple linear regression method utilizing the three MLR models by thirteen calculated descriptors.

3.2.3 *QSAR Equation Analysis and Model Validation*

The attempt to obtain multi-linear mathematical models using molecular descriptors led to improvement of correlation coefficients and by investigation the biological activities it can been classified them in three groups (class A: the antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, class B: the antifungal activity against *Penicillium marneffei* and class C: the antifungal activity against *Aspergilus flavus, Aspergilus fumigatus*, and *Trichophyton mentagrophytes*. Finally, multiple linear regression was used to derive the QSAR equation and the three best QSAR models obtained (for classes A, B and C) with the molecular descriptors (Tables 3, 4 and 5 respectively) are given below together with the statistical parameters of the regression:

Model A:

$$Log\ X = -44.664(\pm 5.864) + 3.858(\pm 0.445)eeig11r - 1.415(\pm 0.137)mor20e + 23.672(\pm 3.043)belm2$$
$$-1.502(\pm 0.293)eeig12r - 1.210(\pm 0.373)mor18v - 2.834(\pm 0.879)ish$$
$$N = 28,\ R^2 = 0.900,\ SE = 0.097,\ F = 28.414,\ Q^2_{Loo} = 0.781$$

Model B:

$$Log\ Y = -38.305(\pm 8.356) + 2.424(\pm 0.307)eeig11r - 1.271(\pm 0.224)mor20p + 31.289(\pm 4.131)belm2$$
$$+ 4.092(\pm 0.704)rtm + -5.661(\pm 1.068)behp5 - 8.462(\pm 2.667)Mv$$
$$N = 28,\ R^2 = 0.896,\ SE = 0.112,\ F = 27.236,\ Q^2_{Loo} = 0.825$$

Model C:

$$Log\,Z = -40.190(\pm 6.475) + 4.032(\pm 0.436)eeig11r - 0.755(\pm 0.202)eeig09x + 8.983(\pm 1.294)r6m +$$
$$+ 26.671(\pm 4.565)r3u + + 19.182(\pm 3.189)belm2 - 1.600(\pm 0.305)eeig12r$$
$$N = 28,\ R^2 = 0.901,\ SE = 0.097,\ F = 28.839,\ Q^2_{Loo} = 0.798$$

where N is the number of compounds included in the model, R^2 is the square correlation coefficient, SE is the standard error, F is the Fisher statistic ratio and Q^2_{Loo} is the leave one out cross-validation.

As can be seen, the MLR models have good statistical quality with low prediction error and although the equations in models A, B and C do not reproduce the absolute values of the experimental data, they can predict the activity of the drug.

The mentioned descriptors in Table 2 are usually used in QSAR analysis to judge how much the model is reliable. In order to check the reliability of the proposed equation, the observed versus predicted activities MIC values according to the QSAR equation using molecular descriptors in each class are plotted in Figures 2, 3 and 4 respectively. As can be seen, the experimental values are in good agreement with the predicted values and these figures show a linear regression between the predicted and observed values for log MIC using molecular descriptors, indicating the reliability of the equations.

Tables 3, 4 and 5 show the experimentally determined activity. The values of the key features of the molecular descriptors on the basis of our calculation in models A, B and C are also listed in Tables 3, 4 and 5 respectively.

In addition, with the number of the descriptors, various optimal combinations of the descriptors and some important statistics such as the correlation coefficient (R), R Square, Adjusted R Square and Standard Error of the Estimate are listed in Table 6.

Table 3. Experimentally determined activity and molecular descriptors used for multi-linear regressions used in model A. The descriptions of the codes are given in Table 2.

component	log X	eeig11r	mor20e	belm2	eeig12r	mor18v	ish
6a	0.80	1.682	1.875	1.898	1.327	-0.916	0.945
6b	1.40	1.684	1.369	1.897	1.327	-0.950	0.914
6c	0.80	1.687	1.993	1.898	1.327	-0.977	0.849
6d	0.80	1.689	1.893	1.898	1.338	-1.003	0.853
6e	0.80	1.687	1.605	1.898	1.327	-0.854	0.931
6f	0.80	1.687	1.370	1.873	1.327	-0.909	0.918
6g	0.80	1.693	1.905	1.898	1.327	-0.929	0.832
6h	0.80	1.870	1.831	1.899	0.631	-0.876	0.925
6i	1.40	1.885	1.749	1.898	1.633	-0.897	0.869
6j	0.80	1.964	2.290	1.899	1.635	-0.906	0.879
6k	1.40	1.974	1.940	1.898	1.636	-1.031	0.869
6l	1.40	1.962	1.659	1.899	1.634	-0.878	0.869
6m	1.40	1.962	1.387	1.873	1.635	0.946	0.907
6n	1.40	1.886	1.674	1.899	1.633	-0.858	0.873
6o	1.10	1.873	1.916	1.901	1.631	-0.933	0.869
6p	1.10	1.889	1.846	1.900	1.633	-0.917	0.891
6q	1.10	1.881	1.971	1.900	1.632	-0.945	0.838
6r	1.10	2.000	2.317	1.901	1.639	-0.994	0.838
6s	0.80	1.981	1.859	1.873	1.635	-0.888	0.870
7a	1.40	2.000	2.039	1.898	1.753	-1.025	0.856
7b	0.80	1.761	2.071	1.893	1.443	-0.037	0.827
7c	0.80	1.758	1.991	1.897	1.439	-0.954	0.847
7d	0.80	2.000	2.126	1.899	1.987	-1.038	0.867
7e	1.40	1.989	1.713	1.894	0.728	-1.018	0.876
7f	1.40	1.989	1.967	1.898	1.724	-1.010	0.907
7g	1.10	2.057	2.054	1.900	2.000	-1.033	0.847
7h	1.10	2.000	1.922	1.894	1.731	-0.860	0.867
7i	1.40	2.000	2.056	1.900	1.727	-0.976	0.816

Table 4. Experimentally determined activity and molecular descriptors used for multi-linear regressions used in model B. The descriptions of the codes are given in Table 2.

Component	Log Y	eeig11r	mor20p	belm2	rtm+	behp5	Mv
6a	0.80	1.682	1.424	1.898	0.131	3.041	0.700
6b	1.40	1.684	1.149	1.897	0.283	3.053	0.720
6c	0.80	1.687	1.463	1.898	0.133	3.081	0.690
6d	0.80	1.689	1.375	1.898	0.134	3.064	0.800
6e	0.80	1.687	1.306	1.898	0.133	3.062	0.690
6f	0.80	1.687	1.188	1.873	0.365	3. 057	0.740
6g	0.80	1.693	1.361	1.898	0.149	3.091	0.690
6h	0.80	1.870	1.349	1.899	0.123	3.106	0.690
6i	1.40	1.885	1.390	1.898	0.281	3.106	0.710
6j	0.80	1.964	1.584	1.899	0.122	3.142	0.680
6k	1.40	1.974	1.376	1.898	0.118	3.119	0.670
6l	1.40	1.962	1.286	1.899	0.130	3.117	0.680
6m	1.40	1.962	1.196	1.873	0.368	3.110	0.720
6n	1.40	1.886	1.277	1.899	0.148	3.106	0.680
6o	1.40	1.873	1.305	1.901	0.172	3.084	0.690
6p	1.40	1.889	1.261	1.900	0.172	3. 084	0.710
6q	1.40	1.881	1.367	1.900	0.205	3.089	0.710
6r	1.40	2.000	1.464	1.901	0.190	3.147	0.680
6s	0.80	1.981	1.297	1.873	0.271	3.089	0.730
7a	1.40	2.000	1.474	1.898	0.169	3.112	0.680
7b	0.80	1.761	1.541	1.893	0.279	3.096	0.720
7c	0.80	1.758	1.448	1.897	0.232	3.096	0.710
7d	0.80	2.000	1.469	1.899	0.166	3.207	0.670
7f	1.40	1.898	1.144	1.894	0.234	3.170	0.710
7f	1.40	1.989	1.395	1.898	0.233	3.170	0.690
7g	1.40	2.057	1.326	1.900	0.167	3.186	0.670
7h	1.40	2.000	1.220	1.894	0.220	3.152	0.710
7i	1.40	2.000	1.304	1.900	0.232	3.152	0.700

Table 5. Experimentally determined activity and molecular descriptors used for multi-linear regressions used in model C. The descriptions of the codes are given in Table 2.

component	Log Z	eeig11r	eeig09x	r6m+	r3u+	belm2	eeig12r
6a	0.80	1.682	2.500	0.022	0.060	1.989	1.327
6b	1.40	1.684	2.500	0.081	0.059	1.897	1.327
6c	0.80	1.687	2.500	0.034	0.054	1.898	1.327
6d	0.80	1.689	2.500	0.041	0.056	1.898	1.338
6e	0.80	1.687	2.500	0.043	0.053	1.898	1.327
6f	0.80	1.687	2.539	0.079	0.059	1.873	1.327
6g	0.80	1.639	2.500	0.035	0.055	1.898	1.327
6h	0.80	1.870	2.500	0.020	0.057	1.899	1.631
6i	1.40	1.885	2.500	0.080	0.058	1.898	1.633
6j	1.40	1.964	2.500	0.034	0.052	1.899	1.635
6k	1.40	1.974	2.500	0.040	0.054	1.898	1.636
6l	1.40	1.962	2.500	0.044	0.052	1.899	1.634
6m	1.40	1.962	2.539	0.080	0.058	1.873	1.635
6n	1.40	1.886	2.500	0.025	0.066	1.899	1.633
6o	1.10	1.873	2.500	0.019	0.057	1.901	1.631
6p	1.10	1.889	2.761	0.052	0.057	1.900	1.633
6q	1.10	1.881	2.682	0.044	0.054	1.900	1.632
6r	1.10	2.000	2.643	0.028	0.044	1.901	1.639
6s	0.80	1.981	2.859	0.050	0.054	1.873	1.635
7a	1.40	2.000	2.643	0.046	0.052	1.898	1.753
7b	0.80	1.761	2.506	0.054	0.049	1.893	1.443
7c	0.80	1.758	2.500	0.054	0.047	1.897	1.439
7d	0.80	2.000	2.644	0.044	0.052	1.899	1.987
7e	1.40	1.989	2.506	0.052	0.049	1.894	1.728
7f	1.40	1.989	2.500	0.054	0.047	1.898	1.724
7g	1.10	2.057	20864	0.044	0.052	1.900	2.000
7h	1.10	2.000	2.756	0.050	0.053	1.894	1.731
7i	1.10	2.000	2.506	0.054	0.048	1.900	1.727

4. RESULTS AND DISCUSSION

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **6a-s** and **7a-i** showed moderate to good inhibition at 1.56-25 mg/ml in DMSO. The compounds **6a**, **6c**, **6d**, **6e**, **6f**, **6g**, **6h**, **6j**, **6s**, and **7b-d** showed comparatively good activity against all the bacterial strains. The good activity is attributed to the presence of pharmacologically active $-CH_3$, $-OCH_3$, $-NH_2$ and 2,3- dichloro groups attached to phenyl group at position 6 of the thiadiazole ring. Introduction of aryl moiety carrying phenyl, 2,3-dichloro, 4-chloro, and 2-hydoxy-4-amide groups at position 6 of thiadiazine caused enhanced activity. The presence of $-SCH_3$ and $-SC_2H_5$ groups at position 4 of phenoxy group caused good antibacterial activity while methyl sulphonyl group caused decrease in activity against most of the strains. The compounds **6o**, **6p**, **6q**, **6r**, and **7g-h**, exhibited moderate activity compared to that of standard against all the bacterial strains. This may be due to presence of methyl sulphonyl group in position 4 of phenoxy moiety [11].

The compounds **6a**, **6c**, **6d**, **6e**, **6f**, **6g**, **6h**, **6j**, **6s**, and **7b-d** showed comparatively good activity against all the fungal strains. The structure of these compounds contains biologically active $-CH_3$, $-OCH_3$, $-NH_2$, 2,3-dichloro groups attached to phenyl group in position 6 of the thiadiazole ring and aryl moiety carrying phenyl, 2,4-dichloro, 4-chloro, and 2-hydoxy- 4-amide groups, in position 6 of thiadiazine. The compounds **6o**, **6p**, **6q**, **6r**, and **7g-h** exhibited moderate activity compared to that of standard against *T. mentagrophytes*, *A. flavus*, and *A. fumigatus*. Results of antifungal screening showed that the presence of $S-CH_3$ and $S-C_2H_5$ groups at position 4 of phenoxy group caused increased activity. It has been observed that the thiadiazole derivatives are found to be more active than thiadiazines [11].

In addition, this work shows an extensive study performed by means of molecular modeling upon a series of 28 compounds of 1,2,4-triazole derivatives with antimicrobial activities. Molecular modeling methods (molecular mechanics and conformational analysis) and QSAR methods based on correlation analysis and multiple linear regression use a large number of molecular descriptors calculated with the HyperChem and DRAGON programme packages.

The correlations performed for the whole set provided the optimal equations for different numbers of descriptors in the range of 1-6. Table 6 shows the values of R, R Square, Adjusted R Square and Standard Error of the Estimate corresponding to the number of variables in the regression model.

It suggests that the best one-descriptor model with highest impact is eeig11r which is defined as Eigenvalue 11 from edge adj. matrix weighted by resonance integrals representing Edge adjacency indices. Subsequent addition of variables produces monotonously increasing

values of R, R Square, Adjusted R Square and decreasing values Standard Error of the Estimate and the break point is not clearly defined. We decided to select the best models to be the one having the smallest number of parameters and satisfactory statistical parameters (the models A, B and C with 6 descriptors). They allowed obtaining essential data regarding the imposed structural requirements at molecular level in order to improve the antimicrobial potentiality of the studied compounds.

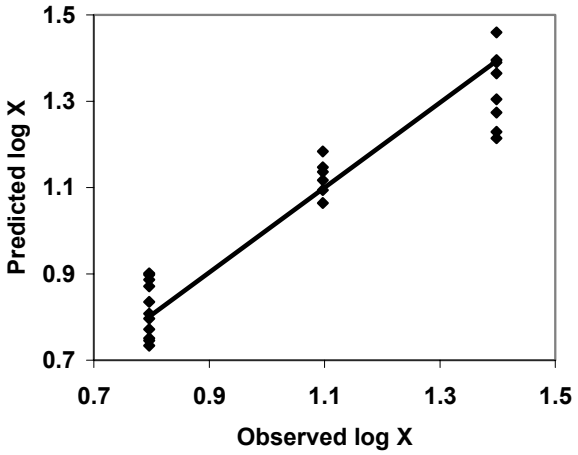

Figure 2. The plot of calculated vs. observed antimicrobial activity of 1,2,4-triazole derivatives in model A.

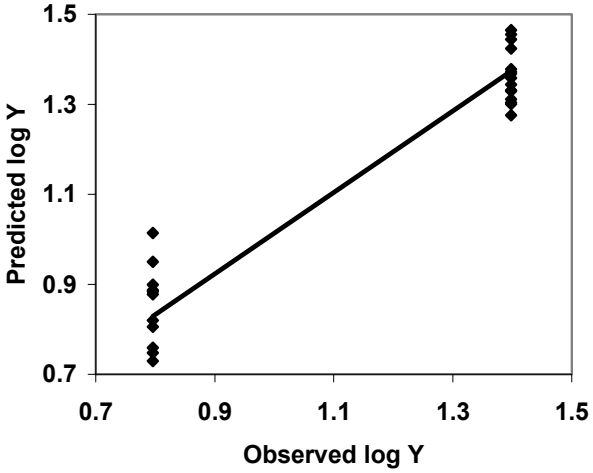

Figure 3. The plot of calculated vs. observed antimicrobial activity of 1,2,4-triazole derivatives in model B.

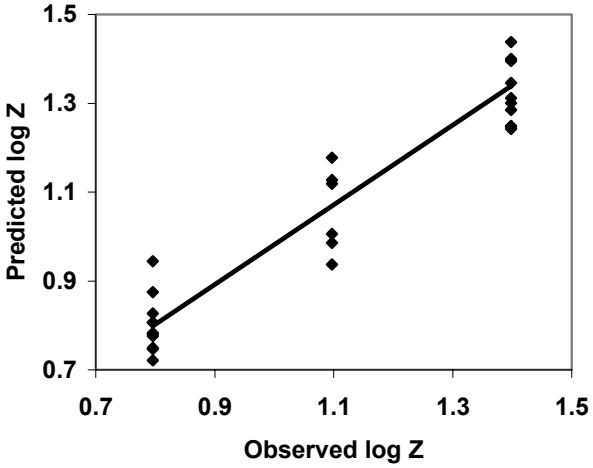

Figure 4. The plot of calculated vs. observed antimicrobial activity of 1,2,4-triazole derivatives in model C.

5. CONCLUSION

The research study reports the successful synthesis and antimicrobial activity of new 1,2,4-triazolothiadiazoles and 1,2,4-trazolothiadiazines carrying 4-methyl/ethyl thio and methyl sulphonyl phenoxy moieties at position 3. The antimicrobial activity study revealed that all the compounds tested showed moderate to good antimicrobial activity against pathogenic strains. Structure-biological activity relationship of title compounds showed that the presence 4-thioalkyl phenoxy groups at position 3 and biologically active groups like $-CH_3$, $-OCH_3$, $-NH_2$, and 2,3-dichloro groups at aryl moiety attached to position 6 of title compounds are responsible for increased antimicrobial activity in newly synthesized title compounds.

In this QSAR study, the proposed QSAR model, due to the high predictive ability, can therefore act as a useful aid to the costly and time consuming experiments for determining the maximal antimicrobial activities. We first tried to identify descriptors trends which lead to antimicrobial activity based on the proposed QSAR equation. We have obtained three mathematical models between descriptors and the antimicrobial activities with statistical analysis and the models (A, B and C) have shared six Edge adjacency indices, GETAWAY descriptors, 3D-MoRSE descriptors, Burden eigenvalues and Constitutional descriptors class descriptors (Table 2). As mentioned before, Eigenvalue 11 from edge adj. matrix weighted by resonance integrals (eeig11r) is the most important variable for predicting antimicrobial activity. The remaining five descriptors involve the

summations of different functions corresponding to the different fragment lengths and with resonance integrals, atomic polarizabilities, atomic Sanderson electronegativities, atomic van der Waals volumes and atomic masses as the weighting parameter (Table 2). Since these molecular descriptors are the main factors which influence the antimicrobial activities of 1,2,4-triazole derivatives, it is necessary to explore such descriptors. Meanwhile, studying their applicability could lead to a vital improvement in QSAR studies.

Therefore, obtained data by adequate designed QSAR studies allow observing aspects and essential molecular characteristics to have an increased biological activity, suggesting certain structural requirements for an increased antimicrobial potential. Our results open very interesting perspectives regarding 1,2,4-triazole derivatives. Finally the QSAR model could be helpful to predict the antimicrobial activities of compounds by calculating the descriptors involved in the QSAR equation.

Table 6. The statistics of various combinations of the descriptors.

descriptor	R	R Square	Adjusted R Square	Std. Error of the Estimate
	Model A			
eeig11r	0.473	0.223	0.191	0.240
eeig11r, mor20e	0.670	0.449	0.401	0.207
eeig11r, mor20e, belm2	0.801	0.641	0.592	0.171
eeig11r, mor20e, belm2, eeig12r	0.864	0.746	0.698	0.147
eeig11r, mor20e, belm2, eeig12r, mor18v	0.919	0.845	0.806	0.118
eeig11r, mor20e, belm2, eeig12r, mor18v, ish	0.949	0.900	0.868	0.097
	Model B			
eeig11r	0.550	0.303	0.274	0.258
eeig11r, mor20p	0.689	0.475	0.429	0.228
eeig11r, mor20p, belm2	0.788	0.621	0.570	0.198
eeig11r, mor20p, belm2, rtm+	0.856	0.733	0.682	0.170
eeig11r, mor20p, belm2, rtm+, behp5	0.917	0.841	0.801	0.135
eeig11r, mor20p, belm2, rtm+, behp5, Mv	0.946	0.896	0.863	0.112
	Model C			
eeig11r	0.568	0.323	0.297	0.227
eeig11r, eeig09x	0.726	0.527	0.489	0.194
eeig11r, eeig09x, r6m+	0.791	0.626	0.579	0.176
eeig11r, eeig09x, r6m+, r3u+	0.832	0.692	0.638	0.163
eeig11r, eeig09x, r6m+, r3u+, belm2	0.878	0.771	0.719	0.144
eeig11r, eeig09x, r6m+, r3u+, belm2, eeig12r	0.949	0.901	0.873	0.097

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