1,3,4-Thiadiazole Derivatives. Synthesis, Structure Elucidation, and Structure−Antituberculosis Activity Relationship Investigation

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A series of 2,5-disubstituted-1,3,4-thiadiazoles were synthesized, the compounds structures were elucidated and screened for the antituberculosis activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system. Among the tested compounds, 2-phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole **22** showed the highest inhibitory activity. The relationships between the structures of compounds and their antituberculosis activity were investigated by the Electronic−Topological Method (ETM) and feed forward neural networks (FFNNs) trained with the back-propagation algorithm. As a result of the approach, a system of pharmacophores and anti-pharmacophores has been found that effectively separates compounds of the examination set into groups of active and inactive compounds. The system can be applied to the screening and design of new active compounds possessing skeletons similar to those used in the present study.

Introduction

During recent years there has been intense investigation of different classes of thiadiazole compounds, many of which known to possess interesting biological properties such as antimicrobial,[1−3] antituberculosis,[4] anti-inflammatory,[5−7] anticonvulsant,[8,9] antihypertensive,[10,11] local anesthetic,[12] anticancer,[13,14] and hypoglycemic activities.[15]

Increasingly, the treatment of mycobacterial infections, especially tuberculosis, has become an important problem due to the emergence of multidrug-resistance. The 1,3,4-thiadiazole derivatives were synthesized with the aim of new antituberculosis drugs development. In our previous study, one of thiadiazole derivatives, namely 2-(4-chlorophenylamino)-5-(4-aminophenyl)-1,3,4-thiadiazole, showed 57% inhibition against *Mycobacterium tuberculosis*.[16] This observation had an impact on our further work on the synthesis and search for some new thiadiazoles with the antituberculosis activity. The structures of the synthesized compounds were elucidated using UV, IR, ^{1}H NMR, mass spectroscopy, and elemental analysis. The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the Southern Research Institute screened the compounds for antituberculosis activity.

The present study that uses both the Electronic−Topological Method (ETM) and Neural Network (NN) methods aims also in finding new antituberculosis compounds. The better is the description of a molecule in terms of structural parameters representing its activity, the better are the results of pattern recognition and separation of the molecules into active and inactive ones. The ETM can be considered as belonging to the class of structure-based approaches[17−21] for the structure−activity relationship (SAR) study. As a consequence of the choice, the ETM is capable of taking into account any individual properties of separate atoms and bonds that is very important for revealing details of interactions between a biologic receptor and an active molecule.

Many enough published studies have used ETM to find SAR models involving a representative list of activities and thousands of compounds belonging to different chemical classes (some of them are given as an example[22−25]). The ETM software has also undergone considerable development, so as the method itself.[26−28] The new ETM-based system capable of unifying Web-based SAR resources and using Internet communications in the frameworks of a project named ETOSAR makes the ETM a valuable tool for SAR studies and provides rules for the synthesis of new potentially active compounds. For the analysis of data on the pharmacophores in this study we have used one of the most well-known NN—the feed forward neural networks (FFNNs) trained with the back-propagation algorithm.[29,30]

Results and Discussion

Chemistry. In our research, 4-substituted benzoic acid hydrazides **2a**−**d** being the starting materials were prepared by etherification of 4-substituted benzoyl chloride with phenol in sodium hydroxide, followed by refluxing with hydrazine hydrate in dry methanol (Scheme 1). After treatment with different arylisothiocyanates in ethanol or acetonitrile, compounds **2a**−**e** and isoniazid gave the thiosemicarbazides **3**, which were cyclized into the thiadiazoles as the result of a ring

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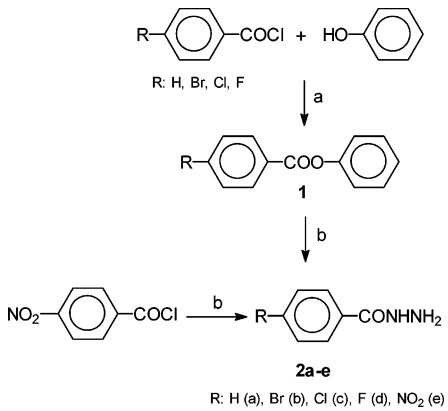
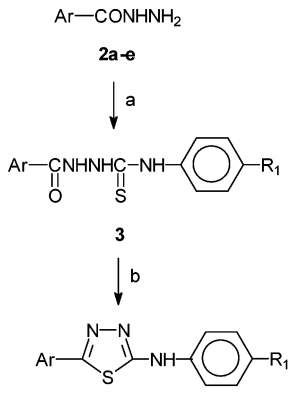
Scheme 1[a]



[a] Reagents and conditions: (a) NaOH; (b) NH_2NH_2, CH_3OH.

Scheme 2[a]



[a] Reagents and conditions: (a) 4-RC_6H_4NCS, C_2H_5OH or CH_3CN; (b) concentrated H_2SO_4.

closure reaction with concentrated sulfuric acid at room temperature. The synthetic pathway of the compounds is represented in Scheme 2.

The antituberculosis tests indicated that compound **22** exhibited the highest inhibitory activity (69% inhib) against in vitro growing *Mycobacterium tuberculosis* at a concentration >6.25 μL/mL (MIC). These compounds, while not active enough to be considered as therapeutics, are definitely lead compounds in the search for novel agents to combat resistance.

Structure−Activity Relationship (SAR) Investigation. Data Sets. Compounds under study (66 molecules) are shown in Table 1. Molecules under study were classified as high activity compounds (28 molecules with % inhib ≥ 35), low activity compounds (18 molecules with 35 > % inhib > 18) and inactive compounds (20 molecules with % inhib ≤ 18).

To identify activity features (or pharmacophores), ETM calculations were carried out twice: first, low activity compounds were considered as belonging to the active class, and then as belonging to the inactive class.

The Search for Pharmacophores (Ph) and Antipharmacophores (APh) by Using ETM. Conformational analysis and quantum chemistry calculations were carried out by means of molecular mechanics method (MMP2) and semiempirical quantum chemistry method (AM1), respectively. Diagonal elements of the matrixes called Electronic−Topological Matrixes of Contiguity (ETMC, for short) reflect one or more atomic properties (represented by a separate value or by a vector of characteristics). Off-diagonal elements characterize bonds between pairs of atoms, if they exist, or distances, otherwise. (Usually, only the upper triangle of each matrix is used in calculations because of the symmetry of bonds.) Also, more that one property can be taken for bonds. However, the ETM calculations use only by one property for atoms and bonds, for simplicity. If there are more than one property for atoms and bonds, the ETM calculations can be repeated for each property but separately. In our case, effective charges on atoms are taken as diagonal elements, and the values of Wiberg's index represent off-diagonal elements corresponding to bonds; if no bond, then off-diagonal elements are distances for corresponding pairs of atoms.

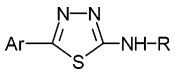
Computational part of the ETM is a sequence of the following steps:

- Conformational analysis
- Quantum-chemistry calculations
- The ETMCs formation
- The search for structural features, responsible for the compounds activity/inactivity (the features are referenced as pharmacophores/anti-pharmacophores, correspondingly). To find pharmacophores, a template *active* compound and the rest of compounds are compared as weighted graphs; to find anti-pharmacophores, an *inactive* compound is used as a template for the comparison. At the same time, the molecules flexibility is taken into account by the comparison of atomic and bond weights.

The last two steps represent the essential part of the ETM. The main advantages of the ETM are that its molecular descriptions reflect the molecules electronic and 3D conformational properties of compounds and do not depend on the atoms numeration and sorts.

Thus, for each template compound (active or inactive), its ETMC was compared with the ETMCs of the rest of compounds in both classes of the series taken for this study. The comparison resulted in a few common structural fragments for the two cases. The fragments were found as submatrixes of the ETMCs corresponding to templates (they will be referenced as electron-topological submatrixes of contiguity, or ETSCs, for short). Consequently, all pharmacophores and anti-pharmacophores found from the ETM calculations form a system for the activity identification. For the series studied the system includes 15 pharmacophores and 10 anti-pharmacophores. From the compound **22** taken as the template active compound, an activity feature 1 (or the **Ph1** pharmacophore) was found. It is given in Figure 1 with the corresponding ETSC, which describes electronic−topological characteristics of the fragment (see Figure 1a). As seen from the **Ph1** pharmacophore structure, it consists of five atoms (C_1, C_{10}, C_{16}, H_{19}, F_{20}), which relate structurally to the substituents attached to the thiadiazole ring. Charges on C_{10} and C_{16} atoms, which are situated at a distance of 11.16 Å, are $-0.15\bar{e}$ and $-0.16\bar{e}$, respectively. The charge on the atom H_{19}, which is chemically bonded to the nitrogen atom, is $0.24\bar{e}$.

Table 1. Antituberculosis Activity of Compounds (**4**−**69**)[a]



no.	Ar	R	% inhib	no.	Ar	R	% inhib
4	C_6H_5	C_6H_5	65	**37**	4-C_6H_4N	4-FC_6H_4	49
5	C_6H_5	4-BrC_6H_4	36	**38**	4-C_6H_4N	4-$CH_3C_6H_4$	38
6	C_6H_5	4-ClC_6H_4	30	**39**	4-C_6H_4N	4-$NO_2C_6H_4$	18
7	C_6H_5	4-FC_6H_4	50	**40**	4-$NH_2C_6H_4$	CH_3	29
8	C_6H_5	4-$CH_3C_6H_4$	21	**41**	4-$NH_2C_6H_4$	C_2H_5	34
9	C_6H_5	4-$NO_2C_6H_4$	18	**42**	4-$NH_2C_6H_4$	C_3H_7	0
10	4-BrC_6H_4	C_6H_5	0	**43**	4-$NH_2C_6H_4$	C_6H_{11}	41
11	4-BrC_6H_5	4-BrC_6H_4	33	**44**	4-$NH_2C_6H_4$	$CH_2C_6H_5$	37
12	4-BrC_6H_4	4-ClC_6H_4	33	**45**	4-$NH_2C_6H_4$	C_6H_5	16
13	4-BrC_6H_4	4-FC_6H_4	42	**46**	4-$NH_2C_6H_4$	4-ClC_6H_4	57
14	4-BrC_6H_4	4-$CH_3C_6H_4$	31	**47**	4-$NH_2C_6H_4$	4-FC_6H_4	3
15	4-BrC_6H_4	4-$NO_2C_6H_4$	43	**48**	4-$NH_2C_6H_4$	4-$CH_3OC_6H_4$	7
16	4-ClC_6H_4	C_6H_5	50	**49**	4-$NH_2C_6H_4$	2-$CH_3C_6H_4$	0
17	4-ClC_6H_4	4-BrC_6H_4	34	**50**	4-$NH_2C_6H_4$	4-$CH_3C_6H_4$	22
18	4-ClC_6H_4	4-ClC_6H_4	32	**51**	4-$NH_2C_6H_4$	4-$NO_2C_6H_4$	37
19	4-ClC_6H_4	4-FC_6H_4	54	**52**	4-$C_6H_5NH\,CSNHC_6H_4$	C_3H_7	6
20	4-ClC_6H_4	4-$CH_3C_6H_4$	42	**53**	4-$C_6H_5NH\,CSNHC_6H_4$	C_6H_{11}	67
21	4-ClC_6H_4	4-$NO_2C_6H_4$	44	**54**	4-$C_6H_5NH\,CSNHC_6H_4$	$CH_2C_6H_5$	16
22	4-FC_6H_4	C_6H_5	69	**55**	4-$C_6H_5NH\,CSNHC_6H_4$	C_6H_5	1
23	4-FC_6H_4	4-BrC_6H_4	40	**56**	4-$C_6H_5NH\,CSNHC_6H_4$	4-ClC_6H_4	32
24	4-FC_6H_4	4-ClC_6H_4	42	**57**	4-$C_6H_5NH\,CSNHC_6H_4$	4-FC_6H_4	0
25	4-FC_6H_4	4-FC_6H_4	52	**58**	4-$C_6H_5NH\,CSNHC_6H_4$	4-$CH_3OC_6H_4$	5
26	4-FC_6H_4	4-$CH_3C_6H_4$	39	**59**	4-$C_6H_5NH\,CSNHC_6H_4$	2-$CH_3C_6H_4F$	3
27	4-FC_6H_4	4-$NO_2C_6H_4$	8	**60**	$(CH_3CO)_2C{=}NNHC_6H_4$	CH_3	46
28	4-$NO_2C_6H_4$	C_6H_5	39	**61**	$(CH_3CO)_2C{=}NNHC_6H_4$	C_2H_5	39
29	4-$NO_2C_6H_4$	4-BrC_6H_4	26	**62**	$(CH_3CO)_2C{=}NNHC_6H_4$	C_3H_7	36
30	4-$NO_2C_6H_4$	4-ClC_6H_4	29	**63**	$(CH_3CO)_2C{=}NNHC_6H_4$	C_6H_{11}	11
31	4-$NO_2C_6H_4$	4-FC_6H_4	30	**64**	$(CH_3CO)_2C{=}NNHC_6H_4$	$CH_2C_6H_5$	0
32	4-$NO_2C_6H_4$	4-$CH_3C_6H_4$	33	**65**	$(CH_3CO)_2C{=}NNHC_6H_4$	C_6H_5	0
33	4-$NO_2C_6H_4$	4-$NO_2C_6H_4$	20	**66**	$(CH_3CO)_2C{=}NNHC_6H_4$	4-ClC_6H_4	15
34	4-C_6H_4N	C_6H_5	38	**67**	$(CH_3CO)_2C{=}NNHC_6H_4$	4-FC_6H_4	0
35	4-C_6H_4N	4-BrC_6H_4	53	**68**	$(CH_3CO)_2C{=}NNHC_6H_4$	2-$CH_3C_6H_4$	5
36	4-C_6H_4N	4-ClC_6H_4	59	**69**	$(CH_3CO)_2C{=}NNHC_6H_4$	4-$CH_3C_6H_4$	0

[a] The data on the antituberculosis activity of **40**−**69** are taken from studies.[16,52]

Submatrix given in Fig. **1** is found after setting some allowable limits for finding equivalent matrix elements. The limits are $\delta_1 = \pm 0.05$ for its diagonal elements and $\delta_2 = \pm 0.15$ for the off-diagonal ones. The pharmacophore found from the ETM-calculations is realized in 34 active compounds, and the probability P_A of its realization in this class is about 0.95.

The pharmacophore Ph2 (see Figure 1b) was calculated from template compound **36**. Ph2 includes C_8, C_9, C_{14}, N_{17}, and C_{18} atoms. Ph2 is found in 34 active and two inactive compounds. Thus, the probability of its realization is 0.92. One common use for the structural methods criterion (C_A) that evaluates the *probability* of a pharmacophore (Ph) occurrence in the series under study is given by the following formula[31]:

$$C_A(\mathrm{Ph}) = (n_A + 1)/(n_A + n_{IA} + 2) \qquad (1)$$

where n_A, n_{IA} are numbers of active/inactive compounds, respectively, which contain the Ph. For an anti-pharmacophore (APh), (n_{IA}+1) is to be used at place of (n_A+1) in the formula (1). The rest of pharmacophores, Ph3−Ph15, were found analogously, and the probabilities of their realization in the class of active compounds varied in the limits of 0.86−0.95.

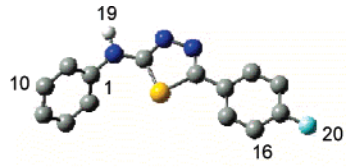
To determine anti-pharmacophores, ETMCs of some inactive compounds were taken as templates. Ten pharmacophores, APh1−APh10, were found, in the total. ETSCs that correspond to APh1 and APh2 are given in Figure 2 along with structures of the corresponding templates after which the anti-pharmacophores are found.

As seen from Figure 2a, APh1 (after template inactive compound **49**) consists of atoms N_5, N_6, C_{16}, and H_{21}. APh1 is present in 17 inactive molecules and two active molecules, and probability of its realization is 0.84. For the anti-pharmacophore APh2, inactive compound **69** was selected as template compound, and the probability of APh2 realization is 0.89, respectively.

When comparing the structures of the pharmacophores and anti-pharmacophores, one can pay attention to the differences in their spatial and electron characteristics. Thus, the pharmacophores and anti-pharmacophores, used together, play an important role in the activity prediction in the process of a new drug search. In this way, the set of activity/inactivity fragments, found as the result of this study, forms a basis for the development of a system for the antituberculosis activity prediction.

Neural Network Application. Artificial Neural Networks (ANNs) is a group of methods that are increasingly being used in drug design to study quantitative/qualitative SAR (QSAR). This method is able to elucidate SARs and take into account any nonlinear character of these relationships. Thus, this method can be of significant interest in three-dimensional (3D) QSAR studies.

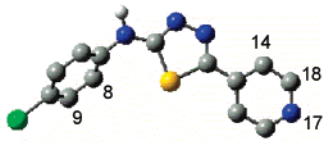
The architecture of the artificial supervised NN applied to our study consists of three layers, with five



N22

C1	C10	C16	H19	F20
0.07	2.81	8.43	2.05	10.46
	-0.15	11.12	4.71	13.03
		-0.16	7.24	2.39
			0.24	9.47
L_A/L_{NA}=34/1, C_A= *0.95*				-0.10

a)

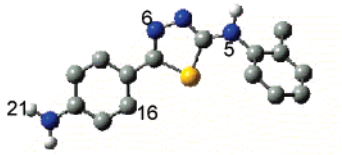


N36

C8	C9	C14	N17	C18
-0.17	1.44	8.53	10.34	9.87
	-0.10	9.63	11.57	11.00
		-0.14	2.43	1.38
			-0.13	1.44
L_A/L_{NA}=34/2, C_A= *0.92*				-0.07

b)

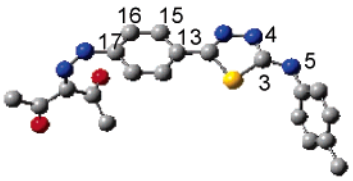
Figure 1. The Ph1 (a) and Ph2 (b) pharmacophores found relative to active molecules **22** and **36**, respectively.



N49

N5	N6	C16	H21
-0.19	3.58	6.22	9.92
	-0.06	2.96	6.92
L_A/L_{NA}=2/17		-0.04	3.98
C_A= *0.84*			0.24

a)



N69

C3	N4	N5	C13	C15	C16	C17
-0.20	1.42	1.08	3.83	4.83	6.15	6.63
	-0.11	2.44	3.61	4.24	5.63	6.38
		-0.20	5.19	6.22	7.53	7.97
			-0.03	1.36	2.43	2.82
L_A/L_{NA}=1/15				-0.06	1.44	2.42
C_A= *0.89*					-0.17	1.35
						0.09

b)

Figure 2. The APh1 (a) and APh2 (b) anti-pharmacophore found relative to inactive molecules **49** and **69**, respectively.

neurons in one hidden layer. A single output node was used to code activities of inhibitors. The bias neuron was presented on the input and hidden layers. At least M = 200 independent FFNNs were trained to analyze each set of variables. The values predicted for each analyzed case were averaged over all M networks predictions, and the means were used to calculate statistical coefficients with targets. The other details of the algorithm can be found elsewhere.[32,33]

The avoidance of overfitting/overtraining has been shown to be an important factor for improving the predictive ability and correct selection of variables in the FFNNs. The Early Stopping over Ensemble (ESE) technique was used in the current study to accomplish this. We used a subdivision of the initial training set into two equal learning/validation subsets. The first set was used to train the neural network, while the second one was used to monitor the training process measured by root-mean-square error. An *early stopping point* determined as a best fit of a network to the validation set was used to stop the neural network learning. Thus, statistical parameters calculated at the early stopping point were used. The training was terminated by limiting the network run to 10 000 epochs (*total* number of epochs) or after 2000 epochs (*local* number of epochs) following the last improvement of root-mean-square error in the early stopping point. The root-mean-square error E was computed as a criterion for network learning to determine the stop points of a training procedure. The quality of the model was tested by the leave-one-out (LOO) cross-validation procedure carried out on the training set with q^2 value (introduced by Cramer et al.[34]) defined as

$$q^2 = (\mathrm{SD} - \mathrm{press})/\mathrm{SD}$$

In the equation, SD represents the variance of a target value relative to its mean and 'press' is the average squared errors of predicted values obtained from the LOO procedure.

The LOO cross-validation procedure, which was used to supervise the predictive performance of the ANN, has shown that pruning algorithms[35,36] can be used to optimize the number of input parameters for the ANNs training and to select the most significant ones. These algorithms operate in a manner similar to stepwise multiple regression analysis and, at each step, exclude by one input parameter that has been estimated as a nonsignificant one. The pruning algorithms were used in the current study to determine significant parameters of input data points of the analyzed molecules as described in references.

As the second stage, we decided to examine if all 25 molecular fragments (pharmacophores and anti-pharmacophores) are relevant for the antituberculosis activity prediction. For these 25 fragments and 85 compounds in the series studied, a table of weights was formed as consisting of 85 rows of 25 elements being 1 (the fragment is present in the corresponding molecular structure) or 0 (the fragment is absent).

Application of pruning methods allowed for the selection of only five the most appropriate parameters responsible for the antituberculosis inhibitory activity.

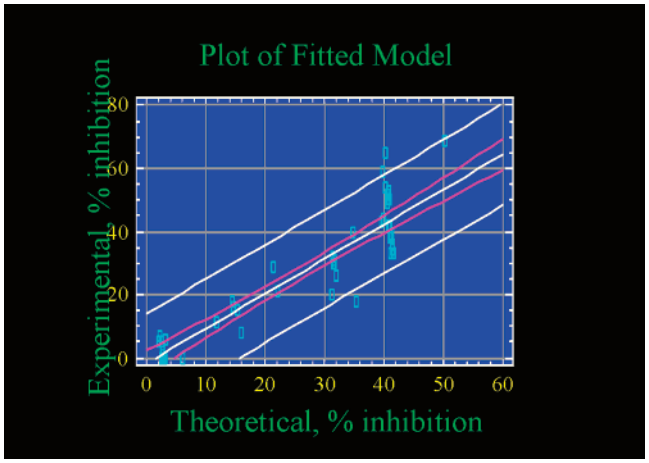


Figure 3. Neural network leave-one-out cross validation inhibitory activity against *M. tuberculosis* H37Rv results.

The calculated result shows that the reduction of the initially obtained seven parameters to five of them did not decrease the cross-validated q^2 coefficient (0.86 ± 0.01). As illustrated in Figure 3, there was a good concordance between the predicted and experimental values of the antituberculosis activities. This result confirms our hypothesis on that only both pharmacophores and anti-pharmacophores taken as parameters can be used for identifying active molecules and building the system for the antituberculosis activity prediction.

Conclusion

A series of 2,5-disubstituted-1,3,4-thiadiazoles were synthesized, their structures were elucidated and screened for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv.

The systematic SAR study was carried out by the ETM application to the synthesized series of compounds capable of demonstrating antituberculosis activity. Data obtained from conformation and quantum-chemistry calculations were used to form electronic−topological matrixes. These matrixes were effectively used in the search for a system of pharmacophores and anti-pharmacophores capable of effective separation of compounds from the examination set into groups of active and inactive compounds. Low activity molecules are badly responsive to the activity prognostication, because they form a buffer zone consisting of compounds that can include both pharmacophores and anti-pharmacophores. The system mentioned is supposed to be applied to screening and design of new active compounds possessing skeletons similar to those used in the present study.

Experimental Section

All chemicals and solvents were purchased from Merck, Aldrich, and Fluka. Melting points were taken on apparatus Buchi 530 in open capillaries and were uncorrected. The purity of the synthesized compounds was checked on HPLC using acetonitrile-water (65:35, v/v) as the mobile phase (Agilent 1100 series-Diode Array Dedector). UV spectra were recorded on a Beckman DU 530 spectrophotometer. IR spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded in DMSO on a Bruker AVANC-DPX-400 spectrometer and chemical shifts were given in δ ppm with tetramethylsilane (TMS). Mass spectra were run on a Fisons Instruments VG, Platform LC-MS at 70 eV. All new compounds were analyzed for C, H, N and the results were in an acceptable range (^{1}H NMR, mass spectra, and elemental analysis were provided by the Scientific and Technical Research Council of Turkey, Tübitak).

General Procedure for the Synthesis of 4-Substituted Phenyl Benzoate (1). To the solution of 0.1 mol of phenol in 100 mL of 10% of sodium hydroxide, 0.1 mol of 4-substituted benzoyl chloride was added and stirred for 30 min. The solid product was washed with distilled water and crystallized from ethanol.[37]

General Procedure for the Synthesis of 4-Substituted Benzoic Acid Hydrazides (2a−d). To the solution of 0.05 mol of **1** in 3 mL of methanol was added 0.1 mol of 99% hydrazine hydrate. The mixture was refluxed on a water bath for 30 min. After cooling, the precipitate was collected, washed with distilled water, and recrystallized from ethanol.[37]

General Procedure for the Synthesis of 4-Nitrobenzoic Acid Hydrazide (2e). To the solution of 0.05 mol of 4-nitrobenzoyl chloride in 75 mL of methanol was added 0.1 mol of 99% hydrazine hydrate. The mixture was refluxed on a water bath for 6 h. After cooling, the precipitate was collected, washed with distilled water, and recrystallized from ethanol.[38]

General Procedure for the Synthesis of 1-Aroyl-4-arylthiosemicarbazides (3). To the solution of 0.0075 mol of 4-substituted benzoic acid hydrazides **2a**−**e** or isoniazid in 30 mL of ethanol or acetonitrile was added 0.0075 mol of the appropriate arylisothiocyanate. The mixture was refluxed on a water bath for 3 h. The solid product, obtained on cooling, was washed with distilled water and recrystallized from ethanol.[38−40]

General Procedure for the Synthesis of 2,5-Disubstituted-1,3,4-thiadiazoles (4−39). To 0.001 mol of appropriate 1-aroyl-4-arylthiosemicarbazides **3** was added concentrated sulfuric acid (1 mL) dropwise. The mixture was stirred at room temperature for 30 min. The reaction content was poured into ice−water mixture. The precipitated solid was washed with sodium carbonate solution followed by water and recrystallized from ethanol.[39−41]

2-Phenylamino-5-phenyl-1,3,4-thiadiazole (4): 91% yield, mp 176−180 °C (lit..[42] mp 199−200 °C).Retention time (t_R) 2.47 min. IR (KBr) 3252, 3198 (NH), 1620 (C=N), 1031 (N−N); 689 (C−S−C) cm^{-1}.

2-(4-Bromophenylamino)-5-phenyl-1,3,4-thiadiazole (5): 63% yield, mp 178−182 °C. t_R 2.63 min. IR (KBr) 3240 (NH), 1620 (C=N), 1070 (Ar−Br), 1050 (N−N), 680 (C−S−C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 7.52−7.88 (m, 9H, Ar-protons), 10.95 (s, 1H, NH). MS (EIMS) *m/z* 331 (M^+).

2-(4-Chlorophenylamino)-5-phenyl-1,3,4-thiadiazole (6): 85% yield, mp 222−224 °C.[43] t_R 3.42 min. IR (KBr) 3260, 3200 (NH), 1620 (C=N), 1090 (Ar−Cl), 685 (C−S−C) cm^{-1}.

2-(4-Fluorophenylamino)-5-phenyl-1,3,4-thiadiazole (7): 80% yield, mp 200−202 °C. t_R 2.52 min. IR (KBr) 3250, 3210 (NH), 1620 (C=N), 1225, 1160 (Ar−F), 685 (C−S−C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 7.22 (t, 2H, meta-protons to F), 7.50−7.55 (m, 3H, meta- and para-protons to thiadiazole), 7.67−7.71 (m, 2H, ortho-protons to F), 7.85−7.87 (m, 2H, ortho-protons to thiadiazole), 10.57 (s, 1H, NH); MS (EIMS) *m/z* 271 (M^+). Anal. ($C_{14}H_{10}FN_3S$) C, H, N, S.

2-(4-Methylphenylamino)-5-phenyl-1,3,4-thiadiazole (8): 59% yield, mp 176−180 °C.[37] t_R 3.15 min. IR (KBr) 3260, 3200 (NH), 1620 (C=N), 690 (C−S−C) cm^{-1}.

2-(4-Nitrophenylamino)-5-phenyl-1,3,4-thiadiazole (9): 53% yield, mp 216−220 °C. t_R 2.63 min. IR (KBr) 3260, 3220 (NH), 1630 (C=N), 1580, 1325 (Ar−NO_2), 685 (C−S−C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 7.54−7.55 (m, 3H, meta-protons to NO_2 and para-proton to thiadiazole), 7.87−7.92 (m, 4H, ortho- and meta-protons to thiadiazole), 8.28 (d, 2H, ortho-protons to NO_2), 11.25 (s, 1H, NH). MS (EIMS) *m/z* 298 (M^+). Anal. ($C_{14}H_{10}N_4O_2S\cdot H_2O$) C, H, S.

2-Phenylamino-5-(4-bromophenyl)-1,3,4-thiadiazole (10): 86% yield, mp 338−339 °C.[45] t_R 3.74 min. IR (KBr) 3347, 3243 (NH), 1613 (C=N), 1070 (Ar−Br), 1047 (N−N), 683 (C−S−C) cm^{-1}.

2-(4-Bromophenylamino)-5-(4-bromophenyl)-1,3,4-thiadiazole (11): 73% yield, mp 244−247 °C. t_R 6.28 min. IR (KBr) 3250 (NH), 1620 (C=N), 1070 (Ar−Br), 660 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.49 (d, 2H, J = 8.9 Hz, ortho-protons to sec. amine), 7.59 (d, 2H, J = 8.9 Hz, meta-protons to sec. amine), 7.66 (d, 2H, J = 8.5 Hz, meta-protons to thiadiazole), 7.76 (d, 2H, J = 8.5 Hz, ortho-protons to thiadiazole), 10.67 (s,1H, NH). MS (EIMS) *m/z* 411 (M^+). Anal. ($C_{14}H_9Br_2N_3S$) C, H, N, S.

2-(4-Chlorophenylamino)-5-(4-bromophenyl)-1,3,4-thiadiazole (12): 76% yield, mp 248−250 °C. t_R 5.59 min. IR (KBr) 3260 (NH), 1620 (C=N), 1090 (Ar−Br), 660 (C−S−C) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.37 (d, 2H, J = 8.9 Hz, meta-protons to Cl), 7.63−7.67 (m, 4H, ortho-protons to Cl and Br), 7.76 (d, 2H, J = 8.5 Hz, meta-protons to Br), 10.67 (s, 1H, NH). MS (EIMS): *m/z* 367 (M^+). Anal. ($C_{14}H_9BrClN_3S$) C, H, N, S.

2-(4-Fluorophenylamino)-5-(4-bromophenyl)-1,3,4-thiadiazole (13): 63% yield; mp 235−237 °C. t_R 3.86 min. IR (KBr) 3280, 3220 (NH), 1630 (C=N), 1160, 1120 (Ar−F), 1090 (Ar−Br), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.22 (t, 2H, meta-protons to F), 7.67−7.72 (m, 4H, ortho-protons to F and Br), 7.81 (d, 2H, J = 8.5 Hz, meta-protons to Br), 10.60 (s, 1H, NH). MS (EIMS) *m/z* 349 (M^+). Anal. ($C_{14}H_9BrFN_3S$) C, H, N, S.

2-(Methylphenylamino)-5-(4-bromophenyl)-1,3,4-thiadiazole (14): 68% yield, mp 218−219 °C.[46] t_R 5.03 min. IR (KBr) 3240 (NH), 1610 (C=N), 1070 (Ar−Br), 635 (C−S−C) cm^{-1}.

2-(4-Nitrophenylamino)-5-(4-bromophenyl)-1,3,4-thiadiazole (15): 68% yield, mp 293−294 °C. t_R 3.93 min. IR (KBr) 3270, 3220 (NH), 1625 (C=N), 1580, 1330 (Ar−NO_2), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.74−7.76 (m, 2H, meta-protons to NO_2), 7.85−7.90 (m, 4H, ortho- and meta-protons to Br), 8.28−8.30 (m, 2H, ortho-protons to NO_2), 11.31 (s, 1H, NH). MS (EIMS) *m/z* 376 (M^+). Anal. ($C_{14}H_9BrN_4O_2S$) C, H, N, S.

2-Phenylamino-5-(4-chlorophenyl)-1,3,4-thiadiazole (16): 50% yield, mp 216−217 °C (lit..[43,46,47] mp 220−222 °C)· t_R 3.52 min. IR (KBr) 3241, 3192 (NH), 1614 (C=N), 1092 (Ar−Cl), 1034 (N−N), 683 (C−S−C) cm^{-1}.

2-(4-Bromophenylamino)-5-(4-chlorophenyl)-1,3,4-thiadiazole (17): 68% yield, mp 248−250 °C. t_R 5.61 min. IR (KBr) 3250 (NH), 1620 (C=N), 1090 (Ar−Cl), 1075 (Ar−Br), 660 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.53 (d, 2H, J = 8.9 Hz, meta-protons to Br), 7.57 (d, 2H, J = 8.6 Hz, ortho-protons to Cl), 7.64 (d, 2H, J = 8.9 Hz, ortho-protons to Br), 7.87 (d, 2H, J = 8.6 Hz, meta-protons to Cl), 10.54 (br.s, 1H, NH). MS (EIMS) *m/z* 367 (M^+). Anal. ($C_{14}H_9BrClN_3S$) C, H, N, S.

2-(4-Chlorophenylamino)-5-(4-chlorophenyl)-1,3,4-thiadiazole (18): 65% yield, mp 236−240 °C (lit.[46] mp 242 °C)· t_R 4.99 min. IR (KBr) 3260, 3200 (NH), 1620 (C=N), 1090 (Ar−Cl), 1020 (N−N), 660 (C−S−C) cm^{-1}.

2-(4-Fluorophenylamino)-5-(4-chlorophenyl)-1,3,4-thiadiazole (19): 70% yield, mp 232−234 °C. t_R 3.60 min. IR (KBr) 3260, 3220 (NH), 1625 (C=N), 1230, 1160 (Ar−F), 1090 (Ar−Br), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.19 (t, 2H, meta-protons to F), 7.57 (d, 2H, J = 8.5 Hz, ortho-protons to Cl), 7.65−7.67 (m, 2H, ortho-protons of phenyl to F), 7.87 (d, 2H, J = 8.5 Hz, meta-protons to Cl), 10.50 (br.s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 116.46, 116.69 (meta-carbons to F), 120.12, 120.21 (ortho-carbons to F), 129.25 (ortho-carbons to Cl), 130.18 (meta-carbons to Cl), 135.56 (para-carbon to F), 137.83 (para-carbon to Cl), 157.06 (ipso-carbon to Cl), 157.22 (thiadiazole C_5); 159.43 (ipso-carbon to F), 165.33 (thiadiazole C_2). MS (EIMS) *m/z* 305 (M^+). Anal. ($C_{14}H_9ClFN_3S$) C, H, N, S.

2-(4-Methylphenylamino)-5-(4-chlorophenyl)-1,3,4-thiadiazole (20): 57% yield, mp 213−214 °C (lit.[46] mp 223 °C)· t_R 4.53 min. IR (KBr) 3250 (N−H), 1615 (C=N), 1090 (Ar−Cl), 670 (C−S−C) cm^{-1}.

2-(4-Nitrophenylamino)-5-(4-chlorophenyl)-1,3,4-thiadiazole (21): 61% yield, mp 300−302 °C. t_R 3.58 min. IR (KBr) 3220 (NH), 1625 (C=N), 1585, 1330 (Ar−NO_2), 1090 (Ar−Cl), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.53 (d, 2H, J = 8.5 Hz, ortho-protons to Cl), 7.81 (d, 2H, J = 9.2 Hz, meta-protons to NO_2), 7.86 (d, 2H, J = 8.5 Hz, meta-protons to Cl), 8.21 (d, 2H, J = 9.2 Hz, ortho-protons to NO_2), 11.13 (s, 1H, NH). MS (EIMS) *m/z* 332 (M^+). Anal. ($C_{14}H_9ClN_4O_2S$) C, H, N, S.

2-Phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole (22): 63% yield, mp 258−262 °C. t_R 2.53 min. IR (KBr) 3240, 3180 (NH), 1615 (C=N), 1170 (Ar−F), 690 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.03 (t, 1H, para-proton to sec. amine), 7.34−7.39 (m, 4H, ortho-protons to sec. amine and meta-protons to F), 7.65 (d, 2H, J = 7.8 Hz, meta-protons to sec. amine), 7.90−7.94 (m, 2H, ortho-protons to F), 10.49 (br.s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 117.09, 117.31 (ortho-carbons to F), 118.38 (meta-carbons to sec. amine), 122.96 (ipso-carbon to sec. amine), 127.69 (para-carbons to F), 129.85, 129.93 (meta-carbons to F), 130.02(o- carbons to sec. amine), 141.36 (para-carbon to sec. amine), 157.29 (thiadiazole C_5), 162.69 (ipso-carbon to F), 165.05 (thiadiazole C_2); MS (EIMS) *m/z* 271 (M^+). Anal. ($C_{14}H_{10}FN_3S$. $^1/_2$ H_2O) C, H, N, S.

2-(4-Bromophenylamino)-5-(4-fluorophenyl)-1,3,4-thiadiazole (23): 76% yield, mp 230−236 °C. t_R 3.92 min. IR (KBr) 3244 (NH), 1619 (C=N), 1157 (Ar−F), 1078 (Ar−Br), 696 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.37−7.41 (m, 2H, meta-protons to F), 7.55−7.58 (m, 2H, meta-protons to Br), 7.65−7.68 (m, 2H, ortho-protons to Br), 7.93−7.97 (m, 2H, ortho-protons to F), 10.70 (s, 1H, NH). MS (EIMS) *m/z* 349 (M^+). Anal. ($C_{14}H_9BrFN_3S$) C, H, N, S.

2-(4-Chlorophenylamino)-5-(4-fluorophenyl)-1,3,4-thiadiazole (24): 53% yield, mp 238−240 °C. t_R 3.47 min. IR (KBr) 3250, 3190 (NH), 1620 (C=N), 1230, 1155 (Ar−F), 1090 (Ar−Cl), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.34−7.43 (m, 4H, meta-protons to Cl and F), 7.70 (d, 2H,J = 8.8 Hz, ortho-protons to Cl), 7.91−7.94 (2d, 2H, ortho-protons to F), 10.68 (s, 1H, NH). MS (EIMS) *m/z* 305 (M^+). Anal. ($C_{14}H_9ClFN_3S$) C, H, N, S.

2-(4-Fluorophenylamino)-5-(4-fluorophenyl)-1,3,4-thiadiazole (25): 74% yield, mp 226−228 °C. t_R 2.60 min. IR (KBr) 3210 (NH), 1626 (C=N), 1231, 1155 (Ar−F), 670(C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.23 (t, 2H, ortho-protons to sec. amine), 7.36−7.40 (m, 2H, meta-protons to F), 7.69−7.72 (m, 2H, meta-protons of phenyl to sec. amine), 7.92−7.95 (m, 2H, ortho-protons to F), 10.53 (s, 1H, NH). MS (EI MS) *m/z* 289 (M^+). Anal. ($C_{14}H_9F_2N_3S$) C, H, N, S.

2-(4-Methylphenylamino)-5-(4-fluorophenyl)-1,3,4-thiadiazole (26): 71% yield, mp 210−214 °C. t_R 3.20 min. IR (KBr) 3245 (NH), 1617 (C=N), 1229 (Ar−F), 673 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 7.20 (d, 2H, J = 8.1 Hz, ortho-protons to sec. amine), 7.35−7.40 (m, 2H, meta-protons to F), 7.55 (d, 2H, J = 8.3 Hz, meta-protons to sec. amine), 7.91−7.95 (m, 2H, ortho-protons to F), 10.41 (s, 1H, NH). MS (EI MS) *m/z* 285 (M^+). Anal. ($C_{15}H_{12}FN_3S$) C, H, N, S.

2-(4-Nitrophenylamino)-5-(4-fluorophenyl)-1,3,4-thiadiazole (27): 92% yield, mp 296−298 °C. t_R 2.61 min. IR (KBr) 3212 (NH), 1627 (C=N), 1587, 1336 (Ar−NO_2), 1235 (Ar−F), 676 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.39 (t, 2H, meta-protons to F), 7.87 (d, 2H, J = 9.1 Hz, meta-protons to NO_2),7.95−7.98 (2d, 2H, ortho-protons to F), 8.27 (d, 2H, J = 9.1 Hz, ortho-protons to NO_2), 11.10 (br.s, 1H, NH). MS (EIMS) *m/z* 316 (M^+). Anal. ($C_{14}H_9FN_4O_2S$) C, H, N, S.

2-Phenylamino-5-(4-nitrophenyl)-1,3,4-thiadiazole (28): 67% yield, mp 275−277 °C (lit.[43,47] mp 272−273 °C)·t_R 2.57 min. IR (KBr) 3196 (NH), 1621 (C=N), 1573, 1331 (Ar−NO_2), 680 (C−S−C) cm^{-1}.

2-(4-Bromophenylamino)-5-(4-nitrophenyl)-1,3,4-thiadiazole (29): 68% yield, mp 322−324 °C. t_R 1.23 min. IR (KBr) 3253 (NH), 1623 (C=N), 1565, 1376 (Ar−NO_2), 1075 (Ar−Br), 687 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.46 (d, 2H, J = 8.9 Hz, meta-protons to Br), 7.56 (d, 2H, J = 8.9 Hz, ortho-protons to Br), 8.04 (2d, 2H, J = 8.8 Hz, meta-protons to NO_2), 8.25 (d, 2H, J = 8.8 Hz, ortho-protons to NO_2), 10.75 (br.s, 1H, NH). MS (EIMS) *m/z* 376 (M^+). Anal.($C_{14}H_9BrN_4O_2S.^1/_2H_2O$) C, H, N, S.

2-(4-Chlorophenylamino)-5-(4-nitrophenyl)-1,3,4-thiadiazole (30): 53% yield, mp 334−335 °C. t_R 1.20 min. IR (KBr) 3260, 3200 (NH), 1625 (C=N), 1565, 1335 (Ar−NO_2), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.49 (d, 2H, J = 8.9 Hz, meta-protons to Cl), 7.77 (d, 2H, J = 8.9 Hz, ortho-protons to Cl), 8.19 (d, 2H, J = 8.8 Hz, meta-protons to NO_2), 8.40 (d, 2H, J = 8.8 Hz, ortho-protons to NO_2), 10.91 (s, 1H, NH). MS (EIMS) *m/z* 332 (M^+). Anal. ($C_{14}H_9ClN_4O_2S.^1/_2H_2O$) C, H, N.

2-(4-Fluorophenylamino)-5-(4-nitrophenyl)-1,3,4-thiadiazole (31): 38% yield, mp 322−324 °C. t_R 1.19 min. IR (KBr) 3270, 3220 (NH), 1630 (C=N), 1510, 1345 (Ar−NO_2), 670 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.26−7.30 (m, 2H, meta-protons to F), 7.73−7.77 (m, 2H, ortho-protons to F), 8.18 (d, 2H, J = 8.9 Hz, meta-protons to NO_2), 8.39 (d, 2H, J = 8.9 Hz, ortho-protons to NO_2), 10.76 (s, 1H, NH). MS (EIMS) *m/z* 316 (M^+). Anal. ($C_{14}H_9FN_4O_2S\cdot H_2O$) C, S.

2-(4-Methylphenylamino)-5-(4-nitrophenyl)-1,3,4-thiadiazole (32): 63% yield, mp 280−284 °C (lit.[44] mp 243 °C)·t_R 3.16 min. IR (KBr) 3244 (NH), 1616 (C=N), 1568, 1368 (Ar−NO_2), 683 (C−S−C) cm^{-1}.

2-(4-Nitrophenylamino)-5-(4-nitrophenyl)-1,3,4-thiadiazole (33): 64% yield, mp 368 °C. t_R 2.72 min. IR (KBr) 3362 (NH), 1595 (C=N), 1563, 1330 (Ar−NO_2), 687 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.79 (d, 2H, J = 9.0 Hz, ortho-protons to sec. amine), 8.09 (d, 2H, J = 8.8 Hz, meta-protons to NO_2), 8.19 (d, 2H, J = 9.0 Hz, meta-protons to sec. amine), 8.27 (d, 2H, J = 8.8 Hz, ortho-protons to NO_2), 11.40 (br.s, 1H, NH). MS (EIMS) *m/z* 343 (M^+). Anal. ($C_{14}H_9N_5O_4S$) C, H, N, S.

2-Phenylamino-5-(4-pyridyl)-1,3,4-thiadiazole (34): 22% yield, mp 238−240 °C.[48] t_R 2.83 min. IR (KBr) 3199 (NH), 1624 (C=N), 689 (C−S−C) cm^{-1}.

2-(4-Bromophenylamino)-5-(4-pyridyl)-1,3,4-thiadiazole (35): 82% yield, mp 278−280 °C.[48,49] t_R 3.77 min. IR (KBr) 3241 (NH), 1616 (C=N), 1073 (Ar−Br), 691 (C−S−C) cm^{-1}.

2-(4-Chlorophenylamino)-5-(4-pyridyl)-1,3,4-thiadiazole (36): 74% yield, mp 238−240 °C.[48,49] t_R 3.53 min. IR (KBr) 3174 (NH), 1597 (C=N), 1091 (Ar−Cl), 1047 (N−N), 695 (C−S−C) cm^{-1}.

2-(4-Fluorophenylamino)-5-(4-pyridyl)-1,3,4-thiadiazole (37): 81% yield, mp 246−248 °C.[49] t_R 2.75 min. IR (KBr) 3392 (NH), 1626 (C=N), 1224, 1168 (Ar−F), 642 (C−S−C) cm^{-1}.

2-(4-Methylphenylamino)-5-(4-pyridyl)-1,3,4-thiadiazole (38): 78% yield, mp 238−240 °C.[50] t_R 3.34 min. IR (KBr) 3245 (NH), 1631 (C=N), 670 (C−S−C) cm^{-1}, ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 7.20 (d, 2H, J = 8.3 Hz, ortho-protons to sec. amine), 7.54 (d, 2H, J = 8.3 Hz, meta- protons to sec. amine), 8.04 (d, 2H, J = 6.0 Hz, ortho-protons of pyridyl to thiadiazole), 8.79 (d, 2H, J = 6.0 Hz, meta-protons of pyridyl to thiadiazole),10.67 (br.s, 1H, NH), MS (EIMS) *m/z* 268 (M^+).

2-(4-Nitrophenylamino)-5-(4-pyridil)-1,3,4-thiadiazole (39): 86% yield, mp 302−304 °C.t_R 2.37 min. IR (KBr) 3267 (NH), 1596 (C=N), 1512, 1328 (Ar−NO_2), 701 (C−S−C) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.78 (d, 2H, J = 6.0 Hz, ortho-protons of pyridyl to thiadiazole), 7.81 (d, 2H, J = 9.0 Hz, meta-protons to NO_2), 8.20 (d, 2H, J = 9.0 Hz, ortho-protons to NO_2), 8.65 (d, 2H, J = 6.0 Hz, meta-protons of pyridyl to thiadiazole), 11.31 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 122.90 (para-carbon to NO_2), 126.34, 126.52 (ortho-carbons of pyridyl to thiadiazole), 131.14 (ipso-carbon to thiadiazole), 142.44 (meta-carbons to NO_2), 146.73 (ortho-carbons to NO_2), 151.67 (ipso-carbon to NO_2), 156.36 (thiadiazole C_5), 163.05 (thiadiazole C_2), 170.01 (meta-carbons of pyridyl to thiadiazole). MS (EIMS) *m/z* 299 (M^+). Anal. ($C_{13}H_9N_5O_2S$) C, H, N, S.

Antituberculosis Activity. All of compounds were evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis* by Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Southern Research Institute. Primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar Blue Assay (MABA).[51] Compounds effecting <90% inhibition in the primary screening (MIC > 6.25 μg/mL) were not generally evaluated further.

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Supporting Information Available: Elemental analyses of compounds; NMR and MS spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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