Facile Synthesis of 3,6-Disubstituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles via Oxidative Cyclization of *n*-Heteroaryl-Substituted Hydrazones and Their Biological Activity

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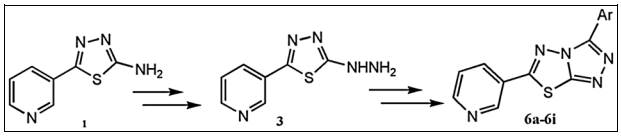
Received October 5, 2010
DOI 10.1002/jhet.870
View this article online at wileyonlinelibrary.com.



Fused 3,6-disubstituted triazolothiadiazoles were synthesized in good yield from a rapid and convenient oxidative cyclization of *N*-heteroaryl-substituted hydrazones promoted by chloramine-T trihydrate at ambient temperature. The structure of the synthesized compounds was confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. The synthesized compounds were evaluated for their antioxidant and antitubercular activities. All the compounds **5a**-**i** and **6a**-**i** showed good antitubercular activity. However, only compounds **5a**-**i** showed good antioxidant activity.

J. Heterocyclic Chem., **49**, 823 (2012).

INTRODUCTION

Nitrogen- and sulfur- containing five- membered heterocycles were known for their broad spectrum of biological activities and other applications [1–6]. The fused 3,6-disubstituted triazolothiadiazoles were reported with variety of biological activities like antibacterial [7,8], anti-inflammatory [9], herbicidal [10], anticancer [11], and anti-HIV-1 effects [12].

Several reports [13–15], revealed that the only possible route for the synthesis of 3,6-disubstituted [1,2,4]triazolo [3,4-b][1,3,4]thiadiazole derivatives involves the condensation of 4-amino-5-substituted-1,2,4-triazole-3-thiols with aromatic/heteroaromatic carboxylic acids in $POCl_3$ or PPA at higher temperatures.

Synthesis of various fused heterocycles from *N*-heteroaryl-substituted hydrazones using oxidizing agents such as $Pb(OAc)_4$, $FeCl_3$, trifluoroboronetharate, HNO_3 in DMF, and $PhI(OAc)_2$ was summarized by Shawali [16]. Although many fused five- membered bicyclic heterocycles were reported, the 3,6-disubstituted[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole derivatives were not yet reported by oxidative cyclization method. Oxidation by the above mentioned reagents involved higher temperatures and longer reaction times. However, the oxidative cyclization of *N*-heteroaryl-substituted hydrazones to the corresponding 3,6-disubstituted [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives was accomplished conveniently and rapidly (<5 min) at ambient temperature by using chloramine-T trihydrate as an oxidant.

RESULTS AND DISCUSSION

Chemistry. The starting compound **1** was prepared by direct cyclization of nicotinic acid and thiosemicarbazide in polyphosphoric acid, which was diazotized in hydrochloric acid and glacial acetic acid with Cu powder catalysis, to get the 2-chloro compound **2** [17]. Then the compound **2** was treated with hydrazine hydrate in ethanol under reflux condition to get 1-(5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl) hydrazine **3**, which served as a good precursor for new fused heterocycles (Scheme 1). The structure of the compound **3** was confirmed by its spectral data (see Supporting Information). The required aldehyde *N*-1-(5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl) hydrazones **5** were prepared in good yields by the condensation of the hydrazine derivative **3** with the appropriate aldehydes **4**.

All these hydrazones have not been reported hitherto. The structures of the hydrazones were confirmed by ^{1}H NMR, MS, and FTIR spectral data. For example, in each case, their ^{1}H NMR spectra in DMSO-d_6 revealed a characteristic signal in the region γ 8.0–8.4 represents the presence of —N═CH— proton. Their FTIR spectra showed the N-H stretch of the hydrazone as a broad band in the region 3512–3287 cm^{-1}. Further their MS spectra showed [M+1] peak in ES positive mode.

The reaction of hydrazone **5h** with different oxidizing reagents such as ceric ammonium nitrate (CAN) [18], $FeCl_3$ [19], for specified time and at specified temperatures gave oxidized product **6h** with low yields (Table 1). The formation of product **6h** was monitored by TLC, and

Scheme 1. Reaction sequence for the synthesis of aldehyde hydrazones (**5a-i**), and 3-substituted-6-(pyridine-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles (**6a-i**).

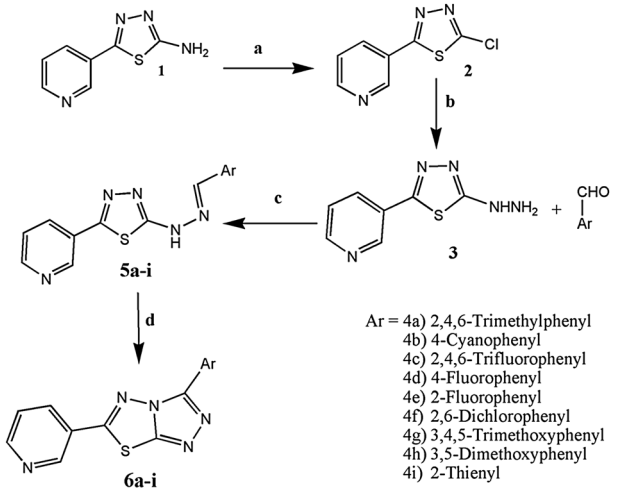


Table 1

Optimization of oxidative cyclization.[a]

Entry	Oxidant	Solvent	Time	Temp (°C)	Yields (%)[b]
1	CAN	DCM	20 h, 16 h[c]	RT	NR[d], 18[c]
2	$FeCl_3$	MeOH	16 h	Reflux	26
3	IBD	DCM	10 min	RT	47.2
4	Chloramine-T $3H_2O$	Ethanol	5 min	RT	78.47

[a]Reactions were carried out with 1 mmol of **5h**, 1 mmol of oxidant and 3 mL of solvent.
for the specified time period and temperature.
[b]Isolated yields.
[c]The reaction was carried out under reflux temperature.
[d]NR, no reaction.

purified by column chromatography after completion of the reaction. Further it was characterized by ^{1}H NMR, LC-MS, and FTIR analysis. To improve the yield we used iodobenzene diacetate [20], (47.2%) and chloramine-T trihydrate [21], (78.47%) as oxidizing agents (Table 1). Chloramine-T trihydrate in ethanol gave the product in good yield at room temperature. The advantage of this protocol is characterized shorter reaction time (<5min) using nontoxic oxidant.

The optimized chloramine-T trihydrate in ethanol reaction condition was applied to all the hydrazones **5a-i** to get the oxidized products **6a-i** in good yields (Table 2). The ^{1}H NMR spectra of **6** in $CDCl_3$ showed, in each case, the absence of —CH═N— and —NH—N═ protons and LC—MS spectra showed two digit less molecular mass than the respective hydrazone. FTIR spectra showed the absence of stretch due to -NH- in the region 3500–3250 cm^{-1}. This finding clearly assigned the structure of 3-substituted-6-(pyridine-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles **6a-i**. Further the ^{13}C NMR and DEPT-135 spectra of **6a** are supporting its structure. The conversion of **5** to **6** is similar to the other related oxidative cyclization of aldehyde *N*-heteroarylhydrazones with iron(III) chloride, which have been reported to proceed via generation of the respective nitrilimines, which undergo *in situ* 1,5-electrocyclization to give the respective fused heterocycles [18,22].

Biological activity. *Antioxidant activity.* All the newly synthesized compounds (**5a-i** and **6a-i**) were screened for their free radical scavenging activity. The free radical scavenging activity of the synthesized compounds was determined by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) method [23]. The samples were prepared at different concentrations (10, 20, 50, 75, 100 μg/mL). Butylated hydroxytoluene (BHT), which is a good antioxidant, is

Table 2

Physical data of the synthesized compounds **5a–i** and **6a–i**.

Ar	Compound	m.p. (°C)	Yield (%)	*m/z*
2,4,6-trimethylphenyl	**5a**	246–247	92.10	323
	6a	154–155	72.58	321
4-cyanophenyl	**5b**	242–244	91.13	306
	6b	241–242	68.54	304
2,4,6-trifluorophenyl	**5c**	238–239	92.75	335
	6c	183–184	70.52	333
4-fluorophenyl	**5d**	220–222	93.02	299
	6d	209–210	69.26	297
2-fluorophenyl	**5e**	224–225	89.14	299
	6e	206–207	71.70	297
2,6-dichlorophenyl	**5f**	245–246	91.16	349
	6f	249–250	68.81	347
3,4,5-trimethoxyphenyl	**5g**	229–230	94.69	371
	6g	211–214	62.37	369
3,5-dimethoxyphenyl	**5h**	221–222	91.73	341
	6h	199–201	78.47	339
2-thienyl	**5i**	213–214	91.52	287
	6i	200–201	66.49	285

taken as a standard in this study. The hydrazone derivatives **5a-i** showed good free radical scavenging activity at all different five concentrations studied, where as the cyclized compounds **6a-i** showed very less radical scavenging effect. The results of the antioxidant activity were summarized in Table 3.

Antitubercular activity. The antimycobacterial activity of compounds **5a-i** and **6a-i** were assessed against *M. tuberculosis* H_{37}Rv (ATCC 27294) using microplate Alamar Blue assay (MABA) [24]. This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods [25,26], and the activity is expressed as the minimum inhibitory concentration (MIC) in μg/mL. The MIC is defined as the lowest drug concentration required to complete inhibition of bacterial growth. Pyrazinamide was used as standard. All the tested compounds **5a-i** and **6a-i** showed better *in vitro* activity (MIC 25–50 μg/mL) than pyrazinamide (MIC of 100 μg/mL) which is a first line antitubercular drug. The MICs of the compounds were reported in Table 4.

CONCLUSION

In conclusion, we have synthesized the 3,6-disubstituted [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives from a rapid oxidative cyclization of aldehyde *N*-heteroaryl hydrazones promoted by chloramine- T. trihydrate in ethanol at room temperature. The antioxidant activity revealed that the compounds **5a-i** showed very good antioxidant property than **6a-i**. At all concentrations tested the compound **5c** showed very good free radical scavenging activity than all the compounds including standard BHT. This potent activity is attributed to the presence of two labile hydrogens in the form of —CH═N—NH— skeleton and 2,4,6-trifluorophenyl ring attached to it. Because of the strong electron withdrawing nature of 2,4,6-trifluorophenyl ring, the labile hydrogens of —CH═N—NH— will become

Table 3

Antioxidant activity of the synthesized compounds **5a–i** and **6a–i**.

	% inhibition at different concentrations				
Compound	10 (μg/mL)	20 (μg/mL)	50 (μg/mL)	75 (μg/mL)	100 (μg/mL)
5a	10	22	62	76	83
5b	10	21	58	73	84
5c	74	89	96	98	99
5d	54	69	86	91	93
5e	46	65	78	87	92
5f	31	48	72	81	85
5g	11	21	60	77	88
5h	10	19	54	71	83
5i	14	26	61	82	89
6a	3	4	6	7	8
6b	3	7	13	20	23
6c	3	5	8	9	10
6d	2	5	10	14	17
6e	3	5	11	16	18
6f	5	9	16	20	22
6g	2	5	12	14	16
6h	3	7	10	13	17
6i	2	6	11	14	16
BHT	43	59	82	95	97

Table 4

Antitubercular activity of hydrazones **5a–i** and fused triazolothiadiazoles **6a–i** against *M. tuberculosis* H37Rv strain (ATCC 27294).

	MIC (μg/mL)
Compound	*M. tuberculosis* H37Rv
5a	50
5b	50
5c	50
5d	50
5e	50
5f	25
5g	50
5h	50
5i	50
6a	50
6b	50
6c	50
6d	50
6e	50
6f	25
6g	50
6h	50
6i	50
Pyrazinamide	100

more labile. Hence **5c** can lose the hydrogens more easily to neutralize the free radical, as a result **5c** shows better free radical scavenging than all other compounds. The poor antioxidant activity of **6a-i** is due to the lack of labile hydrogens. In relation to the antimycobacterial activity, it was found that all the compounds **5a-i** and **6a-i** (25–50 μg/mL) exhibited activities better than pyrazinamide (100 μg/mL), a first line antitubercular drug. The structure and biological activity relationship of title compounds showed that the presence of 1,3,4-thiadiazole and triazolothiadiazole nuclei, as well as biologically active 2,4,6-trifluoro, 2,4,6-trimethyl, 4-cyano, 2-fluoro,4-fluoro, 2,6-dichloro groups attached to the phenyl ring of triazole nucleus are responsible for good antitubercular activity.

EXPERIMENTAL

General. Analytical grade solvents and commercially available reagents were used without further purification. The column chromatography was carried out over silica gel (60–120 mesh), purchased from Sisco Research Laboratories. Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on 400 and 500-MHz Bruker spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in γ relative to TMS, the coupling constants are given in Hz. IR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Avatar 330 FTIR spectrometer equipped with DTGS detector. Mass spectra were recorded using Agilent 1100 MSD spectrometer in electro spray mode. The starting compound **1** was prepared by previously reported direct cyclization of nicotinic acid and thiosemicarbazide in polyphosphoric acid followed by the diazotization [17], to get the chloro compound **2**.

Synthesis of 1-(5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl)hydrazine (3). To 3-(5-chloro-1,3,4-thiadiazol-2-yl)pyridine **2** (2.0 g, 10.15 mmol) in ethanol (25 mL) was added hydrazine hydrate (98%, 1.23 mL) and the mixture was refluxed under stirring for 1 h. The solid that precipitated was filtered, washed with little water and ethanol to give hydrazine derivative **3** as pale yellow solid.

Yield 1.71 g (87.28%). m.p. 193–194°C; IR υ (KBr) 3446, 3284 (-NH_2 symmetric and asymmetric str), 3191 (Ar-H) cm^{-1}; ^{1}H NMR (DMSO-d_6): γ 5.28 (s, 2H, NH_2), 7.48–7.51 (m, 1H, pyridine-C_5H), 8.13–8.16 (m, 1H, pyridine-C_4H), 8.60 (dd, 1H, J_1 = 3.2 Hz, J_2 = 1.2 Hz, pyridine-C_6H), 8.96 (d, 1H, J = 1.6 Hz, -NH), 9.11 (s, 1H, pyridine-C_2H); MS: ES+ 194.05, ES- 192.10.

General procedure for the preparation of aldehyde N-(1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazones (5a–i). The mixture of 1-(5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl)hydrazine **3** (0.5 g, 2.59 mmol) and the appropriate aldehyde **4** (2.59 mmol) in methanol (5 mL) was stirred at room temperature for 20–30 min. The completion of the reaction was indicated by TLC and the precipitate formed was filtered off, washed with water and then with ethanol and finally recrystallized from IPA to give the corresponding hydrazone derivative **5**.

2,4,6-Trimethylbenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5a). Pale yellow solid (yield 0.77 g, 92.10%), m.p. 246-247°C; IR υ (KBr) 3432 (N-H), 3036 (Ar-H), 2923 (CH_3-H), 1624 (C═N) cm^{-1}; ^{1}H NMR (400 MHz) (DMSO-d_6): γ 2.25 (s, 3H, phenyl-4-methyl), 2.42 (s, 6H, phenyl-2,6-dimethyl), 6.95 (d, 2H, J = 6.8 Hz, phenyl), 7.52–7.55 (m, 1H, pyridine-C_5H), 8.22 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.40 (brs, 1H, —CH═N—), 8.65 (br, 1H, pyridine-C_6H), 9.03 (br, 1H, pyridine -C_2H), 12.50 (br, 1H, —NH—); LC-MS : *m/z* 324.1 (M+1). Anal. Calcd. for $C_{17}H_{17}N_5S$: C, 63.13; H, 5.30; N, 21.65. Found: C, 63.01; H, 5.46; N, 21.58%.

4-Cyanobenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5b). Pale yellow solid (yield 0.72 g, 91.13%), m. p. 242–244°C; IR υ (KBr) 3435 (N-H), 3052 (Ar—H), 2223 (CN), 1619 (C═N) cm^{-1}; ^{1}H NMR(400 MHz) (DMSO-d_6): γ 7.5 (m, 1H, pyridine-C_5H), 7.85–7.92 (dd, 4H, J_1 = 8.4 Hz, J_2 = 12 Hz, phenyl), 8.19 (s, 1H, —CH═N—), 8.23 (br, 1H, pyridine-C_4H), 8.63 (br, 1H, pyridine-C_6H), 9.30 (br,1H, pyridine-C_2H); LC-MS : *m/z* 307 (M+1). Anal. Calcd. for $C_{15}H_{10}N_6S$: C, 58.81; H, 3.29; N, 27.43. Found: C, 58.92; H, 3.38; N, 27.26%.

2,4,6-Trifluorobenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5c). Off white solid (yield 0.64 g, 92.75%), m.p. 238-239°C; IR υ (KBr) 3461 (N-H), 3025 (Ar-H), 1619 (C═N) cm^{-1}; ^{1}H NMR (300 MHz) (DMSO-d_6): γ 7.30 (t, 2H, ^3J = 18.3 Hz, phenyl), 7.54 (m, 1H, pyridine-C_5H), 8.15 (s, 1H, —CH═N—), 8.24 (d, 1H, J = 8.1 Hz, pyridine-C_4H), 8.66 (m, 1H, pyridine-C_6H), 9.04 (brs, 1H, pyridine-C_2H), 12.90 (br, 1H, —NH—, D_2O Exchanged); MS: CI+ 336.2, CI-334.2.

4-Fluorobenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5d). Pale yellow solid (yield 0.72 g, 93.02%), m. p. 220–222°C; IR υ (KBr) 3430 (-N-H), 3041 (Ar-H), 1616 (—C═N) cm^{-1}; ^{1}H NMR (400 MHz) (DMSO-d_6): γ 7.30 (t, 2H, ^3J = 17.6 Hz, phenyl-C_3, C_5H), 7.53–7.57 (m, 1H, pyridine-C_5H), 7.73–7.77 (m, 2H, phenyl-C_2, C_6H), 8.14 (s, 1H, —CH═N—), 8.25 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.67 (d, 1H, J = 4 Hz, pyridine-C_6H), 9.05 (s, 1H, pyridine-C_2H), 12.65 (br, 1H, -NH-); LC-MS: *m/z* 300.1 (M+1).

2-Fluorobenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5e). Pale yellow solid (yield 0.69 g, 89.14%), m. p. 224–225°C; IR υ (KBr) 3425 (N-H), 3042 (Ar—H), 1616 (C═N) cm^{-1}; ^{1}H NMR (400 MHz) (DMSO-d_6): γ 7.27–7.35 (m, 2H, phenyl-C_3,C_5H), 7.45–7.48 (m, 1H, phenyl-C_4H), 7.50-7.56 (m, 1H, pyridine-C_5H), 7.86 (dd, 1H, J_1 = 8 Hz, J_2 = 7.2 Hz, phenyl-C_6H), 8.25 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.30 (s, 1H, -CH═N-), 8.67 (d, 1H, J = 4 Hz, pyridine-C_6H), 9.05 (s, 1H, pyridine-C_2H), 12.81(br, 1H, -NH-); LC-MS: *m/z* 300.1 (M+1).

2,6-Dichlorobenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5f). Pale yellow solid (yield 0.83 g, 91.61%), m.p. 245-246°C; IR υ (KBr) 3426 (N—H), 3037 (Ar-H), 1623 (C═N) cm^{-1}; ^{1}H NMR (400 MHz) (DMSO-d_6): γ 7.43 (t, 1H, J_1 = 7.6 Hz, J_2 = 8.4 Hz, phenyl-C_4H), 7.52-7.59 (m, 3H, pyridine-C_5H and phenyl-C_3,C_5H), 8.24 (d, 1H, J = 7.6 Hz, pyridine-C_4H), 8.35 (s, 1H, -CH═N), 8.67 (d, 1H, J = 3.6 Hz, pyridine-C_6H), 9.04 (s, 1H, pyridine-C_2H), 12.95 (br, 1H, -NH-). Anal. Calcd. For $C_{14}H_9Cl_2N_5S$: C, 48.01; H, 2.59; N, 20.00. Found: C, 47.85; H, 2.67; N, 19.93%.

3,4,5-Trimethoxybenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5g). Pale yellow solid (yield 0.91 g, 94.69%), m.p. 229–230°C; IR υ (KBr) 3437 (N-H), 3021 (Ar-H), 2933 (CH_3-H), 1624 (C═N) cm^{-1}; ^{1}H NMR(400 MHz) (DMSO-d_6): γ 3.7 (s, 3H, phenyl-4-methoxy), 3.84 (s, 6H, phenyl-3,5-dimethoxy), 7.01 (s, 2H, phenyl-C_2,C_6H), 7.54 (m,1H, pyridine-C_5H), 8.06 (s, 1H, —CH═N—), 8.24 (d, 1H, J = 7.6 Hz, pyridine-C_4H), 8.65 (d, 1H, J = 4 Hz, pyridine-C_6H), 9.05 (s, 1H, pyridine-C_2H), 12.67 (br, 1H, —NH—); MS : ES+ 372.17 (M+1). Anal. Calcd. For $C_{17}H_{17}N_5O_3S$: C, 54.97; H, 4.61; N, 18.86. Found: C, 54.82; H, 4.71; N, 19.05%.

3,5-Dimethoxybenzaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)hydrazone (5h). Pale yellow solid (yield 0.81 g, 91.73%), m.p. 221–222°C; IR υ (KBr) 3420 (N-H), 3019 (Ar-H), 2925 (CH_3-H), 1613 (C═N) cm^{-1}; ^1H NMR (400 MHz) (DMSO-d_6): γ 3.8 (s, 6H, phenyl-3,5-dimethoxy), 6.56 (s, 1H, phenyl-C_4H), 6.86 (s, 2H, phenyl-C_2,C_6H), 7.56 (m, 1H, pyridine-C_5H), 8.05 (s, 1H, —CH═N—), 8.23 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.65 (br, 1H, pyridine-C_6H), 9.05 (s, 1H, pyridine-C_2H), 12.56 (br, 1H, —NH—). Anal. Calcd. For $C_{16}H_{15}N_5O_2S$: C, 56.29; H, 4.43; N, 20.51. Found: C, 50.14; H, 4.49; N, 20.78%.

2-Thiophenaldehyde 1-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl) hydrazone (5i). Pale yellow solid (yield 0.68 g, 91.52%), m.p. 213-214°C; IR υ (KBr) 3425, 3031, 1617 cm^{-1}; ^1H NMR (400 MHz) (DMSO-d_6): γ 7.13 (dd, 1H, J_1 = 4 Hz, J_2 = 3.6 Hz, thiophene-C_4H), 7.43 (d, 1H, J = 3.2 Hz, thiophene-C_3H), 7.52-7.55 (m, 1H, pyridine-C_5H), 7.64 (dd, 1H, J_1 = 5.2 Hz, J_2 = 3.2 Hz, thiophene-C_5H), 8.24 (d, 1H, J = 7.6 Hz, pyridine-C_4H), 8.33 (s, 1H, -CH═N-), 8.65 (d, 1H, J = 3.6 Hz, pyridine-C_6H), 9.05 (s, 1H, pyridine-C_2H), 12.55 (br, 1H, —NH—); LC-MS: *m/z* 288.1 (M+1). Anal. Calcd. For $C_{12}H_9N_5S_2$: C, 50.16; H, 3.16; N, 24.37. Found: C, 50.29; H, 3.04; N, 24.56%.

General procedure for the synthesis of 3-substituted-6-(pyridine-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (6a-i). To a heterogeneous solution of the appropriate hydrazone 5 (1.54 mmol) in ethanol (10 mL) was added chloramine-T trihydrate (1.54 mmol) in 2–3 portions over 2–3 min at room temperature and the mixture was stirred at the same temperature for 5–6 min. The precipitated solid was filtered off, washed with water and then with ethanol and purified by column chromatography (40% ethyl acetate-chloroform) to give the respective 3-[pyridine-3-yl]-6-(substituted)-[1,2,4]triazolo [3,4b][1,3,4]thiadiazole **6**.

3-Mesityl-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6a). White solid (yield 0.36 g, 72.58%), m.p. 154-155°C; IR υ (KBr) 3041 (Ar-H), 2923 (CH_3-H), 1616 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 2.19 (s, 6H, phenyl-2,6-dimethyl), 2.38 (s,3H, phenyl-4-methyl), 7.03 (s, 2H, phenyl-C_3, C_5H), 7.45-7.48 (m, 1H, pyridine-C_5H), 8.14-8.17 (m, pyridine-C_4H), 8.80 (d, J = 3.6 Hz, pyridine-C_6H), 9.08 (d, 1H, J = 2Hz, pyridine-C_2H); ^{13}C NMR (125 MHz) ($CDCl_3$): γ 20.13, 21.34, 121.79, 123.99, 125.92, 128.66, 134.35, 138.83, 140.71, 146.89, 148.0, 152.89, 153.29, 163.55; LC-MS: *m/z* 322.1 (M+1). Anal. Calcd. for $C_{17}H_{15}N_5S$: C, 63.53; H, 4.70; N, 21.79. Found: C, 63.50; H, 4.50; N, 21.53%.

3-(4-cyanophenyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6b). Light brown solid (yield 0.34 g, 68.54%), m.p. 241-241°C; IR υ (KBr) 3052 (Ar-H), 2225 (CN), 1620 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 7.57 (m, 1H, pyridine-C_5H), 7.86 (d, 2H, J = 8.4 Hz, phenyl-C_3,C_5H), 8.27 (d, J = 7.6 Hz, pyridine-C_4H), 8.45 (d, 2H, J = 8 Hz, phenyl-C_2, C_6H), 8.88 (br, pyridine-C_6H), 9.21 (br, 1H, pyridine-C_2H); ^{13}C NMR (125 MHz) ($CDCl_3$): γ 113.95, 118.23, 124.23, 125.52, 126.69, 129.42, 132.84, 134.48, 145.24, 148.09, 153.80, 154.79, 164.91; LC-MS: *m/z* 304.9 (M+1). Anal. Calcd. for $C_{15}H_8N_6S$: C, 59.20; H, 2.65; N, 27.61. Found: C, 59.32; H, 2.71; N, 27.69%.

3-(2,4,6-trifluorophenyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c). White solid (yield 0.28 g, 70.52%), m. p. 183-184°C; IR υ (KBr) 3093 (Ar-H), 1642 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 6.86 (t, 2H, ^3J = 18.5 Hz, phenyl), 7.51 (br, 1H, pyridine-C_5H), 8.20 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.83 (br, 1H, pyridine-C_6H), 9.12 (br, 1H, pyridine-C_2H); ^{13}C NMR (125 MHz) ($CDCl_3$): γ 101.15 (t, J = 25 Hz), 101.53 (t, J = 26.25 Hz), 124.1, 125.67, 134.46, 135.61, 148.03, 153.50, 154.32, 161.34 (dq, J = 255.0 Hz), 164.28, 164.68 (dt, J = 253.75); LC-MS: *m/z* 333.9 (M+1).

3-(4-fluorophenyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6d). Off white solid (yield 0.275 g, 69.26%), m.p. 209-210°C; IR υ (KBr) 3061 (Ar-H), 1626 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 7.27 (t, 2H, ^3J = 17.2 Hz, phenyl-C_3,C_5H), 7.56 (m, 1H, pyridine-C_5H), 8.25 (d, J = 7.6 Hz, pyridine-C_4H), 8.38-8.42 (m, 2H, phenyl-C_2,C_6H), 8.85 (br, pyridine-C_6H), 9.19 (br, 1H, pyridine-C_2H); LC-MS: *m/z* 297.9 (M+1).

3-(2-fluorophenyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6e). Light brown solid (yield 0.28 g, 71.7%), m.p. 206–207°C; IR υ (KBr) 3056 (Ar—H), 1632 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 7.29–1.38 (m, 2H, phenyl-C_3,C_5H), 7.49–7.59 (m, 2H, phenyl-C_4H and pyridine-C_5H), 8.06 (m, 1H, phenyl-C_6H), 8.22 (d, 1H, J = 7.6 Hz, pyridine-C_4H), 8.81 (m, 1H, pyridine-C_6H), 9.15 (s, 1H, pyridine-C_2H); LC-MS: *m/z* 298.1 (M+1).

3-(2,6-dichlorophenyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole (6f). Light brown solid (yield 0.342 g, 68.81%), m.p. 249–250°C; IR υ (KBr) 3061 (Ar—H), 1621 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 7.40–7.54 (m, 4H, phenyl and pyridine-C_5H), 8.17 (m, 1H, pyridine-C_4H), 8.82 (m, 1H, pyridine-C_6H), 9.10 (d, 1H, J = 2 Hz, pyridine-C_2H); LC-MS: *m/z* 348.0 (M+1), (Cl^{35}). Anal. Calcd. For $C_{14}H_7Cl_2N_5S$: C, 48.29; H, 2.03; N, 20.11. Found: C, 48.12; H, 2.23; N, 20.02%.

3-(3,4,5-trimethoxy)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole (6g). Light brown solid (yield 0.31 g, 62.37%), m.p. 211-214°C; IR υ (KBr) 3046 (Ar—H), 2929 (CH_3-H), 1635 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 3.94 (s, 3H, phenyl-4-methoxy), 3.98 (s, 6H, phenyl-3, 5-dimethoxy), 7.54 (m, 1H, pyridine-C_5H), 8.20 (d, 1H, J = 7.4 Hz, pyridine-C_4H), 8.84 (d, 1H, J = 4 Hz, pyridine-C_6H), 9.21 (s, 1H, pyridine-C_2H); LC-MS: *m/z* 370.0 (M+1). Anal. Calcd. For $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96. Found: C, 55.38; H, 3.92; N, 18.85%.

3-(3,5-dimethoxy)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6h). Off white solid (yield 0.39 g, 78.47%), mp 199–201°C; IR υ (KBr) 3039 (Ar-H), 2932 (CH_3-H) , 1626 (C═N) cm^{-1}; ^1H NMR (400 MHz) ($CDCl_3$): γ 3.86 (s, 6H, phenyl-3,5-dimethoxy), 6.61 (s, 1H, phenyl-C_4H), 7.51–7.60 (m, 3H, phenyl-C_2,C_6H and pyridine-C_5H), 8.25 (d, 1H, J = 8 Hz, pyridine-C_4H), 8.85(br, 1H, pyridine-C_6H), 9.21 (s, 1H, pyridine-C_2H); MS: ES+ 339.89 (M+1), 361.88 (M+Na) $+^{\cdot}$ Anal. Calcd. For $C_{16}H_{13}N_5O_2S$: C, 56.63; H, 3.86; N, 20.64. Found: C, 56.41; H, 3.91; N, 20.89%.

3-(2-theinyl)-6-(pyridine-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (6i). Light brown solid (yield 0.264 g, 66.49%), m. p. 200-201°C; IR υ (KBr) 3068 (Ar-H), 1642 (C═N) cm^{-1}; ^1H NMR (400 MHz) (DMSO-d_6): γ 7.23 (dd, 1H, J_1 = 4 Hz, J_2 = 1.2 Hz, thiophene-C_4H), 7.55 (m, 2H, thiophene-C_3H and pyridine-C_5H), 8.07 (dd, 1H, J_1 = 0.8 Hz, J_2 = 2.8 Hz, thiophene-C_5H), 8.23–8.26 (m, 1H, pyridine-C_4H), 8.85 (dd, 1H, J_1 = 3.6 Hz, J_2 = 1.2 Hz, pyridine-C_6H), 9.2 (d, 1H, J = 1.6 Hz, pyridine-C_2H); LC-MS: *m/z* 286.0 (M+1). Anal. Calcd. For $C_{12}H_7N_5S_2$: C, 50.51; H, 2.47; N, 24.54. Found: C, 50.74; H, 2.41; N, 24.72%.

Antioxidant activity. Briefly, 1 mL of 0.1 mM methanolic solution of DPPH was added to 3 mL of the synthesized samples **5a-i** and **6a-i**, at different concentrations in methanol (10, 20, 50, 75, 100 μg/mL). The samples were kept in the dark

for 30 min after which the absorbance was measured at 517 nm in UV spectrophotometer (Systronics 2202). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Butylated hydroxy toluene (BHT), which is a good antioxidant, is taken as a standard in this study.

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH} \cdot \text{Scavenging effect}(\%) = [(A_c - A_s)/A_o)100]$$

where A_c is the absorbance of the control reaction and A_s is the absorbance in the presence of sample.

Antitubercular activity. Briefly, 200 μL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 μL of the Middlebrook 7H9 broth and a serial dilution of compounds was made directly on plate. The final drug concentrations tested were 0.2 to 100 μg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After this time, 25 μL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Acknowledgments. The authors acknowledge the Head, SAIF, IIT, Chennai for NMR spectra. They are thankful to IICT, Hyderabad for providing MS and LC-MS facility. They also acknowledge SAIF, CDRI, Lucknow for providing elemental analysis data. They are very much pleased to the Head, Dept. of microbiology, Maratha Mandal Dental College, Belgaum, Karnataka for antitubercular activity studies.

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