Synthesis and antituberculosis activity of 2-(aryl/alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazoles and their Schiff bases

Nilufer Solak and Sevim Rollas*

Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34668, Istanbul-Turkey
E-mail: sevim@sevimrollas.com

Abstract
A series of new Schiff bases were synthesized through the condensation reaction of 1,3,4-thiadiazoles containing a aromatic primary amine and 3-hydroxybenzaldehyde, salicylaldehyde, 5-nitrofurfuraldehyde or 3-nitrobenzaldehyde. The synthesized compounds screened for antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using BACTEC 460 radiometric system. Among the tested compounds, 2-phenylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazol (**5a**) showed the highest inhibitory activity (51%). The activities of the synthesized Schiff bases were compared with those of the starting 2-(aryl/alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazoles (**4a-e**). Some Schiff bases showed higher growth inhibition than the starting amines.

Keywords: Schiff bases, 1,3,4-thiadiazoles, antituberculosis activity, synthesis

Introduction

Schiff bases which contain an azomethine group attract much interest due to their synthetic along with antibacterial,[1,2] antiinflammatory,[3] antitumor,[4] and antimycobacterial[5] properties. Furthermore, thiadiazoles possess antifungal,[6] antituberculosis,[7-9] anticancer,[10,11] antimicrobial,[12-15] anti-inflammatory,[16-18] antihypertensive,[19,20] local anesthetic,[21] anticonvulsant,[22-24] and antitrypanosomal[25] activities.

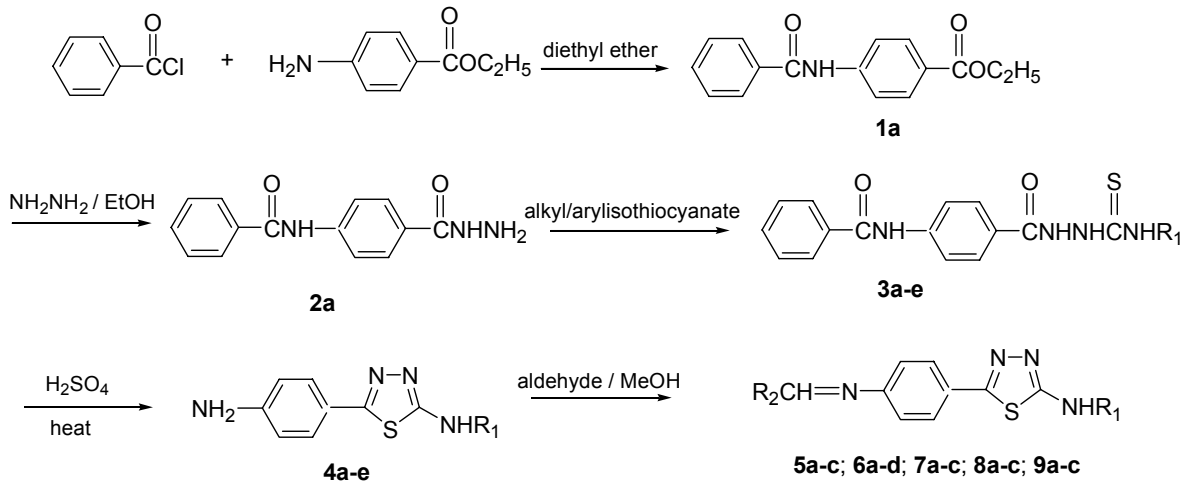
The treatment of mycobacterial infections, especially tuberculosis, has become an important problem to solve due to the emergence of multidrug resistance. As a contribution to the new antituberculosis drug development we have previously synthesized 1,3,4-thiadiazole derivatives.[9, 26] The aim of this study was to synthesize some Schiff bases having inhibition against *Mycobacterium tuberculosis* $H_{37}Rv$ using 1,3,4-thiadiazoles,[26] elucidate their structures and compare the inhibition effects of the 1,3,4-thiadiazoles and the Schiff bases.

Tuberculosis Activity Antimicrobial Acquisition and Coordinating Facility (TAACF) of Southern Research Institute screened the compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ at 6.25 μg/mL.

Results and Discussion

In this study, ethyl 4-(benzoylamino) benzoate (**1a**) was prepared by the reaction of benzocain and benzoylchloride in ether, followed by refluxing with hydrazine hydrate.[27] The resulting compound was treated with different aryl/alkylisothiocyanate to prepare 1-[4-(benzoylamino)benzoyl]-4-alky/arylthiosemicarbazide (**3a-e**), which were cyclized into 2-(aryl/alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazol (**4a-e**) in sulfuric acid.[26] Schiff bases (**5a,c, 6a-d, 7a-c, 8a-c, 9a-c**) were synthesized by the reaction of 1,3,4-thiadiazoles (**4a-e**) and 3-hydroxybenzaldehyde (only **6d**), salicylaldehyde, 5-nitrofurfuraldehyde or 3-nitrobenzaldehyde in methanol.

The syntheses of the Schiff bases are depicted in Scheme 1.

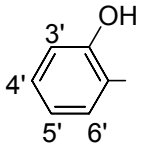
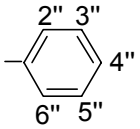
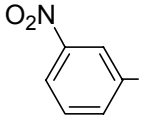
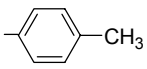
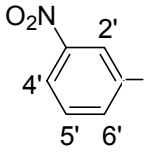
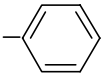
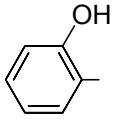
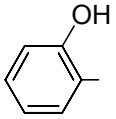
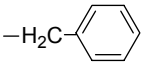
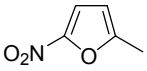
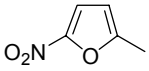
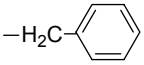
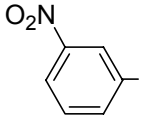
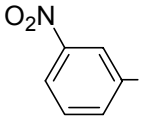
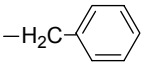
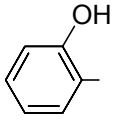
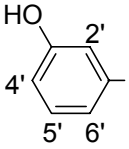
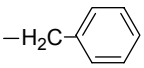
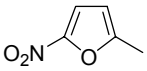
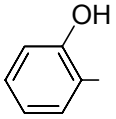
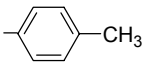
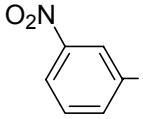
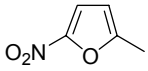
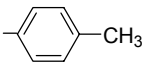


Scheme1. 5a,c: R_1= phenyl; **6a-d**: R_1= benzyl; **7a-c**: R_1= 4-methylphenyl; **8a-c**: R_1= ethyl; **9a-c**: R_1= methyl. **5a, 6a,7a, 8a, 9a**: R_2= 2-hydroxyphenyl; **6b, 7b, 8b, 9b**: R_2= 5-nitrofurfuryl; **5c, 6c, 7c, 8c, 9c**: R_2= 3-nitrophenyl; **6d**: R_2=3-hydroxyphenyl

The presence of the imine group (C=N) in the compounds was indicated in the IR spectra between 1612-1676 cm^{-1}. The imine group proton was observed between 10.29-8.73 ppm in the ^{1}H-NMR spectra. The existence of syn-anti conformation was suggested for **7a** and **9a** since the imine group proton of **7a** and **9a** showed two singlets at 8.92, 9.03 and 9.05, 10.29 respectively. The total area under the peaks was also indicative of a single proton. In addition, the methyl protons of **7a** showed chemical shifts at 2.29 ppm and 2.38 ppm and similarly methyl protons of **9a** had two doublets at 2.93, 2.99 ppm due to syn-anti conformation. Hydroxyl protons of **5a**, **6a**

and **8a** synthesized from salicylaldehyde showed various resonances around 12.84-12.93 ppm due to intramolecular hydrogen bond between the hydroxyl proton and the imine group nitrogen (OH·····N). [32] The ^{1}H-NMR data were consistent with the literature.[2, 33] Besides, in the mass spectra of compounds **5a**, **6b, 6c, 7a, 7b, 8a** and **8b**, molecular ion peaks (M^+ or MH^+) were observed at m/z 373, 405, 416, 387, 406, 324 and 344, respectively.

Table 1. Antituberculosis activity screening data of the synthesized compounds

Comp.	R_2	R_1	% [#] Inhibition	Comp.	R_2	R_1	% [#] Inhibition
5a			51	7c			36
5c			29	8a		$-CH_2CH_3$	0
6a			1	8b		$-CH_2CH_3$	0
6b			0	8c		$-CH_2CH_3$	0
6c			0	9a		$-CH_3$	13
6d			6	9b		$-CH_3$	0
7a			34	9c		$-CH_3$	0
7b			22				

[#] MIC (μg/mL) value > 6.25

The antituberculosis tests indicated that compound **5a** (containing a hydroxyl group) showed the highest inhibition (51%) against *Mycobacterium tuberculosis H37Rv* at a concentration of > 6.25 μg/mL. Patole et al.[5] indicate that conjugation of hydroxyl-rich ligands is effective in synergistically enhancing the antimycobacterial activity.

The activities of all the synthesized Schiff bases against *Mycobacterium tuberculosis H37Rv* were compared with 5-(4-aminophenyl)-1,3,4-thiadiazoles (**4a-e**).[26] The results of antituberculosis activity of the Schiff bases are given in Table 1. Compound **4a** exhibited 16% inhibition whereas compound **5a** and **5c** which were synthesized from **4a** exhibited 51%, and 34% inhibition, respectively. In addition, while **4c** showed 22% inhibitory activity, compounds **7a**, **7b** and **7c**, synthesized from **4c**, showed 34%, 22% and 36% inhibition, respectively.

As a result, the inhibition of the compounds, synthesized from salicylaldehyde (**5a, 7a**) and 3-nitrobenzaldehyde (**5c, 7c**), are higher than those of **4a** and **4c.** However**,** the inhibitions did not change for the compound synthesized from 5-nitrofurfuraldehyde (**7b**) and dramatic decreases were observed for compounds **6a-c**, **8a-c** and **9a-c** synthesized from **4b**, **4d** and **4e**, respectively. The Schiff bases (**5a**, **5c**, **7a**, **7c**), synthesized through the reaction of 1,3,4-thiadiazoles (**4a**, **4c**) with salicylaldehyde or 3-nitrobenzaldehyde, exhibited higher inhibitions compared to the corresponding 1,3,4-thiadiazoles (**4a**, **4c**). On the other hand, the inhibitions did not change for the compound **7b** obtained by the treatment of compound **4c** with 5-nitrofurfuraldehyde. It can be concluded that although **5a** is not active enough, it may be considered promising for the development of new antituberculosis agents.

Experimental Section

General Procedures. Melting points were determined on Buchi 530 and are uncorrected. The IR spectra were obtained by potassium bromide pellets using STIR-8-3000 Shimadzu spectrometer. ^{1}H-NMR spectra were recorded on Bruker AC 200L Krotas MS-9/50 using TMS as an internal standard. The mass spectra of **6b** and **8a** were measured on Fisions Instruments VG Platform.II LS-MS. The mass spectra of **5a**, **6c**, **7a, 7b**, and **8b** were measured on Agilent 1100 MSD. LS-MS. Elemental analysis apparatus was Leco CHNS-932. All experiments were followed by TLC using Merck Kieselgel 60 F- 0.2 mm.

Ethyl 4-(benzoylamino) benzoate (1a). Benzocain (0.03 mol) was dissolved in 36 mL ether. Benzoylchloride (3.6 mL diluted in 3.6 mL ether) was added into the mixture drop by drop. After evaporating ether, the final mixture was washed with water until the smell of benzoyl chloride disappeared and then recrystallized from ethanol. Mp 137°C.[27]

4-(Benzoylamino)benzoylhydrazine (2a). 6.0 mL hydrazine hydrate was added to **1a** (0.01 mol). The mixture was refluxed at 110–130 °C for 45 minutes. After adding 10.0 mL ethanol, the mixture was heated in a water steam bath for one hour. The residue was filtered, washed with water and recrystallized from ethanol. Mp 235°C.[27]

1-(4-Benzoylamino)benzoyl-4-alky/arylthiosemicarbazide (3a-e). 80.0 mL ethanol was added to **2a** (0.005 mol) and heated in a water steam bath. Aryl/alkylisothiocyanate was added and refluxed for 2.5 hours. The precipitate was filtered, washed with water, recrystallized from ethanol. Mp 3a 259-260°C [27], 3d 239°C [27], 3e 225-226°C [27], 3b, 208°C [26], 3c, 178°C [28].

2-(Aryl / alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazole (4a-e). 15.0 mL 50 % H_2SO_4 was added to **3a-e** (0.006 mol) and refluxed at 110-150°C for 6 hours. The mixture was neutralized with 2 N NaOH. The precipitate was filtered, washed with water, recrystallized from ethanol. Mp 4a 188°C [29], 4b 225 °C [26], 4c 217-8°C [26], 4d 170°C [30], 4e 218-220°C [31].

Synthesis of Schiff bases (5a,c, 6a-d, 7a-c, 8a-c, 9a-c). 4a-e (0.001 mol) was dissolved in about 10 mL methanol and refluxed with aldehyde (0.001 mol). The precipitate was recrystallized from methanol.

2-Phenylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (5a) was prepared from salicylaldehyde (0.001 mol) and 2-(phenylamino)-5-(4-aminophenyl)-1,3,4-thiadiazol **4a (**0.001 mol). Yield: 41.9 %. Mp 258°C. *Anal. Calcd.* $C_{21}H_{16}N_4OS.1/4\ H_2O$: C, 66.91; H, 4.27; N, 14.86. Found: C, 66.83; H, 3.41; N, 14.57. IR (KBr, cm^{-1}) 3229 (OH), 3166 (NH), 1614 (C=N); ^{1}H-NMR (DMSO-d6) δ 6.95-7.09 (m, 2H, 3`, 5`), 7.38 (t, 2H, 4``, 6`), 7.45 (m, 1H, 4`), 7.56 (d, 2H, 2, 6, *J*=8.5 Hz), 7.61-7.75 (m, 4H, 2``, 3``,5``,6``), 7.96 (d, 2H, 3, 5, *J*=8.5 Hz), 9.04 (s, 1H, N=CH), 10.53 (s, 1H, NH), 12.84 (s, 1H, OH); MS *m/z*: 373.1 (MH^+, 100 %), 269.1 (16 %).

2-Phenylamino-5-[4-(3-nitrobenzylideneamino)phenyl]-1,3,4-thiadiazole (5c) was prepared from 3-nitrobezaldehyde (0.001 mol) and **4a** (0.001 mol). Yield: 42.6%. Mp 214°C. *Anal. Calcd.* $C_{21}H_{15}N_5\ O_2S$: C, 62.83; H, 3.77; N, 17.45; S, 7.99. Found: C, 62.52; H, 3.49; N, 17.11; S, 7.78. IR (KBr, cm^{-1}) 3246 (NH), 1616 (C=N), 1521 (Ar-NO_2 asymmetric), 1350 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 7.04 (t, 1H, 4``), 7.38 (t, 2H, 3``, 5``), 7.50 (d, 2H, 2, 6, *J*=8.4 Hz), 7.68 (d, 2H, 2``, 6``, *J*=7.8 Hz), 7.86 (t, 1H, 5`), 7.96 (d, 1H, 3, 5, *J*=8.4 Hz), 8.36-8.48 (m, 2H, 4`, 6`), 8.78 (s, 1H, 2`), 8.91 (s, 1H, N=CH), 10.52 (s, 1H, NH).

2-Benzylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (6a) was prepared from salicylaldehyde (0.001 mol) and 2-(benzylamino)-5-(4-aminophenyl)-1,3,4-thiadiazol (**4b**, 0.001 mol). Yield: 46.3 %. Mp 234-235°C. *Anal. Calcd.* $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30. Found: C, 68.38; H, 4.67; N, 14.50; S, 8.18. IR (KBr, cm^{-1}) 3212 (NH, OH), 1617 (C=N); ^{1}H-NMR (DMSO-d6)δ 4.61 (d, 2H, CH_2, *J*=5.7 Hz), 7.03 (t, 2H, 3`, 5`), 7.30 (t, 1H, 4`), 7.36-7.51 (m, 5H, 2``, 3``, 4``, 5``, 6``), 7.57 (d, 2H, 2, 6, *J*=8.5 Hz), 7.72 (d, 1H, 6`), 7.87 (d, 2H, 3, 5, *J*=8.5 Hz), 8.49 (t, 1H, NH), 9.05 (s, 1H, N=CH), 12.90 (s, 1H, OH).

2-Benzylamino-5-[4-(5-nitrofurfurylideneamino)phenyl]-1,3,4-thiadiazole (6b) was prepared from 5-nitrofurfuraldehyde (0.001 mol) and **4b** (0.001 mol). Yield: 87.6 %. Mp 210-211°C. *Anal. Calcd.* $C_{20}H_{15}N_5O_3S$: C, 59.25; H, 3.73; N, 17.27; S, 7.91. Found: C, 60.07; H, 3.25; N, 17.16; S, 7.19. IR (KBr, cm^{-1}) 3245 (NH), 1538 (furfural-NO_2 asymmetric), 1365 (furfural-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 4.61 (d, 2H, CH_2, *J*=5.8 Hz), 7.09-7.69 (m, 8H, 2, 2``, 3``, 4`, 4``, 5``, 6, 6``), 7.80-7.99 (m, 3H, 3, 3`, 5), 8.52 (t, 1H, NH), 8.74 (s, 1H, N=CH). MS *m/z*: 405 (M^+, 6.6%), 91 (100.0 %).

2-Benzylamino-5-[4-(3-nitrobenzylideneamino)phenyl]-1,3,4-thiadiazole (6c) was prepared from 3-nitrobenzaldehyde (0.001 mol) and **4b** (0.001 mol). Yield: 45.2 %. Mp 207-208°C. *Anal. Calcd.* $C_{22}H_{17}N_5O_2S$: C, 63.60; H, 4.12; N, 16.86; S, 7.72. Found: C, 64.49; H, 3.60; N, 16.71; S, 6.83. IR (KBr, cm^{-1}) 3333 (NH), 1633 (C=N), 1524 (Ar-NO_2 asymmetric), 1352 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 4.56 (d, 2H, CH_2), 7.29 (t, 1H, 4``, *J*=5.8 Hz), 7.36-7.54 (m, 6H, 2, 2``, 3``, 5``, 6, 6``), 7.78 (m, 3H, 3, 5, 5`), 8.42 (d, 2H, 4`, 6`, *J*=8.1 Hz), 8.48 (t, 1H, NH), 8.80 (s, 1H, 2`), 8.92 (s, 1H, N=CH). MS *m/z*: 416.2. (MH^+, 100.0 %), 91.1 (23.6 %).

2-Benzylamino-5-[4-(3-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (6d) was prepared from 3-hydroxybenzaldehyde (0.001 mol) and **4b** (0.001 mol). Yield: 53.2 %. Mp 230°C. *Anal. Calcd.* $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30. Found: C, 68.45; H, 4.34; N, 14.15; S, 7.73. IR (KBr, cm^{-1}) 3395 (NH, OH), 1624 (C=N); ^{1}H-NMR (DMSO-d6) δ 4.59 (d, 2H, CH_2, *J*=5.8 Hz), 6.99 (d, 1H, 4`), 7.25-7.52 (m, 10H, 2, 2`, 2``,3``, 4``, 5`, 5``, 6, 6`, 6``), 7.81 (d, 2H, 3, 5, *J*=8.4 Hz), 8.46 (t, 1H, NH), 8.62 (s, 1H, N=CH), 9.71 (s, 1H, OH);

2-(4-Methylphenylamino)-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (7a) was prepared from salicylaldehyde (0.001 mol) and 2-(4-metylphenylamino)-5-(4-aminophenyl)-1,3,4-thiadiazol (**4c**, 0.001 mol). Yield: 72.1 %. Mp 254-255°C. *Anal. Calcd.* $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30. Found: C, 68.36; H, 4.28; N, 14.45; S, 8.32. IR (KBr, cm^{-1}) 3433 (NH, OH), 1617 (C=N); ^{1}H-NMR (DMSO-d6) δ 2.29 and 2.38 (two s, 3H, CH_3), 6.89-7.98 (m, 12H, 2, 2``, 3, 3`, 3``,4`, 5, 5`, 5``, 6, 6`, 6``), 8.92 and 9.03 (two s, 1H, N=CH), 10.42, 14.08 (two s, 1H, OH); 12.52 and 12.98 (two s, 1H, NH); MS *m/z*: 387.2 (MH^+, 100.0 %), 186 (10.9 %).

2-(4-Methylphenylamino)-5-[4-(5-nitrofurfurylideneamino)phenyl]-1,3,4-thiadiazole (7b) was prepared from 5-nitrofurfuraldehyde (0.001 mol) and **4c** (0.001 mol). Yield: 54.3 %. Mp 224-225°C. *Anal. Calcd.*$C_{20}H_{15}N_5O_3S$: C, 59.25; H, 3.72; S, 7.90. Found: C, 59.12; H, 3.44; S, 7.38. IR (KBr, cm^{-1}) 3387 (NH), 1616 (C=N), 1538 (furfural-NO_2 asymmetric), 1352 (furfural-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 2.32 (s, 3H, CH_3); 7.21 (d, 2H, 2``, 6``, *J*=8.4 Hz); 7.46-7.64 (m, 5H, 2, 3``,4`, 5``, 6), 7.86 (d, 1H, 3`, *J*=3.9 Hz), 7.97 (d, 2H, 3, 5, *J*=8.5 Hz) 8.75 (s, 1H, N=CH), 10.46 (s, 1H, NH); MS *m/z*: 406.1 (MH^+, 100.0 %), 282 (10.1 %).

2-(4-Methylphenylamino)-5-[4-(3-nitrobenzylideneamino)phenyl]-1,3,4-thiadiazole (7c) was prepared from 3-nitrobezaldehyde (0.001 mol) and **4c** (0.001 mol). Yield: 41.2 %. Mp 255°C. *Anal. Calcd.* $C_{22}H_{17}N_5O_2S$: C, 63.60; H, 4.12; N, 16.86; S, 7.72. Found: C, 63.87; H, 3.95; N, 16.86; S, 7.21. IR (KBr, cm^{-1}) 3382 (NH), 1612 (C=N), 1517 (Ar-NO_2 asymmetric), 1350 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 2.27 (s, 3H, CH_3), 7.21 (d, 2H, 2``, 6``, *J*=8.4 Hz), 7.51 (d, 2H, 2, 6, *J*=8.5 Hz), 7.58 (d, 2H, 3``,5``, *J*=8.4 Hz), 7.88 (t, 1H, 5`), 7.98 (d, 2H, 3, 5, *J*=8.5 Hz), 8.38-8.49 (m, 2H, 4`,6`), 8.81 (s, 1H, 2`), 8.93 (s,1H, N=CH), 10.44 (s, 1H, NH).

2-Ethylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (8a) was prepared from salicylaldehyde (0.001 mol) and 2-ethylamino-5-(4-aminophenyl)-1,3,4-thiadiazol (**4d**, 0.001 mol). Yield: 82.3 %. Mp 216°C. *Anal. Calcd.* $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; S, 9.88. Found: C, 63.41; H, 5.70; N, 17.40; S, 9.40. IR (KBr, cm^{-1}) 3353 (NH, OH), 1617 (C=N); ^{1}H-NMR (DMSO-d6) δ 1.27 (t, 3H, CH_3), 3.39-3.43 (m, 2H, CH_2), 6.98-7.12 (m, 2H, 3`, 5`),

7.42-7.63 (m, 3H, 2, 4`, 6), 7.74 (m, 1H, 6`), 7.87 (d, 2H, 3, 5, *J*=8.6 Hz), 7.98 (t, 1H, NH), 9.07 (s, 1H, N=CH), 12.93 (s, 1H, OH); MS *m/z*: 324 (M^+, 65.6%), 296, 221, 120 (78.3%),102 (67.4%), 60 (100%), 41 (86.4%).

2-Ethylamino-5-[4-(5-nitrofurfurylideneamino)phenyl]-1,3,4-thiadiazole (8b) was prepared from 5-nitrofurfuraldehyde (0.001 mol) and **4d** (0.001 mol). Yield: 87.4 %. Mp 220°C. *Anal. Calcd.* $C_{15}H_{13}N_5O_3S.H_2O$: C, 49.86; H, 4.15; S, 8.86. Found: C, 50.63; H, 3.86; S, 8.46. IR (KBr, cm^{-1}) 3251 (NH), 1655 (C=N), 1549 (Ar-NO_2 asymmetric), 1354 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 1.25 (t, 3H, CH_3), 3.38-3.55 (m, 2H, CH_2), 7.46-7.55 (m, 3H, 2, 4`, 6), 7.81-7.92 (m, 3H, 3, 3`, 5), 7.97 (t, 1H, NH), 8.73 (s, 1H, N=CH); MS *m/z*: 344.1 (MH^+, 100.0 %), 220.1 (9.2 %).

2-Ethylamino-5-[4-(3-nitrobenzylideneamino)phenyl]-1,3,4-thiadiazole (8c) was prepared from 3-nitrobenzaldehyde (0.001 mol) and **4d** (0.001 mol). Yield: 58.2 %. Mp 205-206°C. *Anal. Calcd.* $C_{17}H_{15}N_5O_2S$: C, 57.78; H, 4.28; N, 19.82; S, 9.07. Found: C, 57.37; H, 3.77; N, 19.55; S, 9.07. IR (KBr, cm^{-1}) 3200 (NH), 1635 (C=N), 1533 (Ar-NO_2 asymmetric), 1353 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 1.27 (t, 3H, CH_3), 3.32-3.48 (m, 2H, CH_2), 7.32 (d, 2H, 2, 6, *J*=8.3 Hz), 7.72 (t, 1H, NH), 7.79 (d, 3H, 3, 5, 5`), 8.29 (t, 2H, 4`, 6`), 8.69 (s, 1H, 2`), 8.76 (s, 1H, N=CH).

2-Methylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole (9a) was prepared from salicylaldehyde (0.001 mol) and 2-(methylamino)-5-(4-aminophenyl)-1,3,4-thiadiazol (**4e**, 0.001 mol). Yield: 71.9 %. Mp 204-205°C. *Anal. Calcd.* $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05; S, 10.33. Found: C, 61.99; H, 4.57; N, 18.02; S, 10.40. IR (KBr, $cm^{-1)}$ 3180 (NH, OH), 1615 (C=N); ^{1}H-NMR (DMSO-d6) 2.93 and 2.99 (two d, 3H, CH_3, *J*=4.7 Hz, 4.7 Hz); 5.54 and 6.65 (s and d, 1H, NH); 6.91-7.18 (m, 2H, 3`, 5`); 7.40-7.52 (m, 1H, 4`); 7.55 (d, 2H, 2, 6, *J*=8.5 Hz); 7.72 (d, 1H, 6`, *J*=7.6 Hz); 7.91 (d, 2H, 3, 5, *J*=8.5 Hz); 9.05 and 10.29 (two s, 1H, N=CH); 10.71 and 12.91 (two s, 1H, OH).

2-Methylamino-5-[4-(5-nitrofurfurylideneamino)phenyl]-1,3,4-thiadiazole (9b) was prepared from 5-nitrofurfuraldehyde (0.001 mol) and **4e** (0.001 mol). Yield: 62.7 %. Mp 210-211°C. *Anal. Calcd.* $C_{14}H_{11}N_5O_3S$: C, 51.06; H, 3.37; N, 21.27; S, 9.74. Found: C, 51.66; H, 3.54; N, 21.34; S, 9.90. IR (KBr,cm^{-1}) 3350 (NH), 1624 (C=N), 1534 (Ar-NO_2 asymmetric and N-H), 1347 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 2.73 (d, 3H, CH_3, *J*=4.8 Hz); 7.17-7.34 (m, 3H, 2, 4`, 6); 7.52-7.75 (m, 4H, NH, 3, 3`, 5), 8.47 (s, 1H, N=CH).

2-Methylamino-5-[4-(3-nitrobenzylideneamino)phenyl]-1,3,4-thiadiazole (9c) was prepared from 3-nitrobenzaldehyde (0.001 mol) and **4e** (0.001 mol). Yield: 75.1 %. Mp 226°C. *Anal. Calcd.* $C_{16}H_{13}N_5O_2S$: C, 56.63; H, 3.86; N, 20.64; S, 9.45. Found: C, 56.32; H, 3.55; N, 20.18; S, 9.19. IR (KBr, cm^{-1}) 3324 (NH), 1624 (C=N), 1525 (Ar-NO_2 asymmetric), 1351 (Ar-NO_2 symmetric); ^{1}H-NMR (DMSO-d6) δ 3.08 (d, 3H, CH_3, *J*=4.8 Hz), 7.58 (d, 2H, 2, 6, *J*=8.5 Hz), 7.93-8.03 (m, 4H, NH, 3, 5, 5`), 8.53 (d, 2H, 4`, 6`, *J*=8.4 Hz), 8.89 (s, 1H, 2`), 9.02 (s, 1H, N=CH).

Antituberculosis activity assays. Tuberculosis Activity Antimicrobial Acquisition and Coordinating Facility (TAACF) of Southern Research evaluated all of the compounds for in vitro

antituberculosis activity against *mycobacterium tuberculosis H37Rv*. Primary screening was conducted at 6.25 μg/mL against *mycobacterium tuberculosis H37Rv* in BACTEC 12B medium using broth microdilution assay .[34]

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References

1. Abd El Rahman, A. H.; Ismail E. M. *Arzneim.-Forsch/Drug Res.* **1976**, *26*, 756.
2. Dogan, H. N.; Buyuktimkin, S.; Rollas, S.; Yemni, E.; Cevikbas, A. *Farmaco* **1997**, *52,* 565.
3. Atta, S. M. Sh.; Ammen, A. *Arzneim.-Forsch./Drug Res.* **1993**, *43*,1354.
4. Dhapalapur, M. G.; Sabnis, S. S.; Deliwala, C. V. *J. Med. Chem.* **1968**, *11*, 1014.
5. Patole J.; Shingnapurkar D.; Padhye S.; Ratledge C. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1514.
6. Foroumadi, F.; Daneshtalab, M.; Shafiee, A. *Arzneim.-Forsch./Drug Res.***1999**, *49*, 1035.
7. Foroumadi, F.; Mirzaei, M.; Shafiee, A. *Farmaco* **2001**, *56*, 621.
8. Karakus, S.; Rollas, S. *Farmaco* **2002**, *57*, 577.
9. Oruc, E. E.; Rollas, S.; Kandemirli, F.; Shvets, N.; Dimoglo, A. S. *J. Med. Chem.* **2004**, *47*, 6760.
10. Miyamoto, K.; Koshiura, R.; Moro, M.; Yokoi, H.; Mori, C.; Hasegawa, T.; Takatori, K. *Chem. Pharm. Bull.* **1985**, *33*, 5126.
11. Chou, J. Y.; Lai, S. Y.; Pan, S. L.; Jow, G. M.; Chern, J. W.; Guh, J. H. *Biochem Pharmacol*. **2003**, *66*, 115.
12. Desai, K.; Baxi, A. J. *Indian J. Pharm. Sci*. **1992**, *54*, 183.
13. Gawande, N. G.; Shingare, M. S. *Indian J. Chem*. **1987**, *26B*, 387.
14. Mamolo, M. G.; Vio, L.; Banfi, E. *Farmaco* **1996**, *51*, 71.
15. Demirbas N.; Demirbas A. ; Karaoglu S.A. ; Celik E. *Arkivoc* **2005**, *(i),* 75.
16. Mullican, M. D.; Wilson, M. W.; Connor, D. T.; Kostlan, C. R.; Schrier, D. J.; Dyer, R.D. *J. Med. Chem*. **1993**, *36*, 1090.
17. Song, Y.; Connor, D. T.; Sercel, A. D.; Sorenson, R. J.; Doubleday, R.; Unangst, P. C.; Roth, B. D.; Beylin, V. G.; Gilbertsen, R. B.; Chan, K.; Schrier, D.J.; Guglietta, A.; Bornemeier, D. A.; Dyer, R. D. *J. Med. Chem*. **1999**, *42*, 1161.

18. Labanauskas, L.; Kalcas, V.; Udrenaite, E.; Gaidelis, P.; Brukstus, A.; Dauksas, A. *Pharmazie* **2001**, *56*, 617.
19. Turner, S.; Myers, M., Gadie, B.; Nelson, A. J.; Pape, R.; Saville, J. F.; Doxey, J. C.; Berridge, T. L. *J. Med. Chem*. **1988**, *31*, 902.
20. Turner, S.; Myers, M., Gadie, B.; Hale, S. A.; Horsley, A.; Nelson, A. J.; Pape, R.; Saville, J. F.; Doxey, J.C.; Berridge, T.L. *J. Med. Chem*. **1988**, *31*, 907.
21. Mazzone, G.; Pignatello, R.; Mazzone, S.; Panico, A.; Penisi, G.; Castana, R.; Mazzone, P. *Farmaco* **1993**, *48*, 1207.
22. Chapleo, C. B.; Myers, M.; Myers, P. L.; Saville, J. F.; Smith, A. C. B.; Stillings, M. R.; Tulloch, I. F.; Walter, D. S.; Welbourn, A. *J. Med. Chem*. **1986**, *29*, 2273.
23. Chapleo, C. B.; Myers, P. L.; Smith, A. C. B.; Stillings, M. R.; Tulloch, I. F.; Walter, D. S. *J. Med. Chem*. **1988**, *31*, 7.
24. Dogan, H. N.; Duran, A.; Rollas, S.; Sener, G.; Uysal, M. K.; Gulen, D. Bioorg. Med. Chem. **2002**, *10*, 2893.
25. Carvalho, S.; Silva E.F.; Santa-Rita R.M.; Castro S.L.; Fraga C.A.M. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 5967.
26. Karakus, S. Ph.D. thesis, Marmara University, Institution of Healt Sciences 2001.
27. Kalyoncuoglu, N.; Rollas, S.; Sur-Altıner, D.; Yegenoglu, Y.; Ang, O. *Pharmazie* **1992**, *47*, 796.
28. Kucukguzel, I..; Kucukguzel, S.G.; Rollas, S.; Otuk-Sanis, G.; Ozdemir, O.; Bayrak, I., Altug , T.; Stables, J. P. *Il Farmaco* **2004**, *59*,893.
29. Rollas, S. *J. Pharm. Univ. Mar.* **1985**,*1,* 59.
30. Rollas, S.; Topaloglu , Y, *J. Pharm.Univ. Mar.* **1986**, *2*, 1.
31. Ozger, Y.; Rollas, S. *Mar. Univ. J. Sci. and Tech*. **1998**, *5*, 133.
32. Szady-Chelmieniecka, A.; Grech, E.; Rozwadowski, Z.; Dziembowska, T.; Schilf, W.; Kamienski, B. *Journal of Molecular Structure* **2001**, 565-566, 125.
33. Terzioglu, N.; Karali, N.; Gursoy, A.; Otuk, G.; Kiraz, M.; Erturan, Z. *Acta Pharm. Turcia*, **1998**, *40*, 77.
34. (a) Collins, L. ; Franzblau, S. G. *Antimicrob. Agents Chemother.* 1997, 1004. (b) TAACF web site www.taacf.org