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ORIGINAL RESEARCH

Synthesis and antimycobacterial activity of some 2-(4-aminophenyl)-5-substituted amino-1,3,4-thiadiazole derivatives and their coupling products

Sevgi KARAKUŞ*, Sevim ROLLAS

ABSTRACT

In the present study, several 2-(4-aminophenyl)-5-substituted amino-1,3,4-thiadiazoles (**2a-l**) and their coupling products, 2,3,4-pentanetrione-3-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazones (**3a-j**) were synthesized in good yields and characterized by UV, IR, ^{1}H-NMR, mass and elemental analysis. Antitubercular activity of the synthesized compounds was determined in vitro using the BACTEC 460 Radiometric System against *Mycobacterium tuberculosis H37Rv* at 6.25 µg/mL. The antimycobacterial data of screened compounds indicated that 2-(4-aminophenyl)-5-(4-chlorophenyl)amino-1,3,4-thiadiazole **2f** demonstrated the highest inhibition.

Keywords: 1,3,4-thiadiazole, coupling products, antimycobacterial activity

1. Introduction

Tuberculosis (TB) is a serious threat to global public health with 9.6 million new cases of infection and 1.5 million TB-related deaths in 2014 (1). Early detection of the ethiologic agent-*Mycobacterium tuberculosis* is the key to successful treatment and reduction of disease transmission. To date, treatment, prophylaxis and control of TB infection is mainly dependent on the use of first (isoniazid, rifampicin, pyrazinamide, and ethambutol) and second line drugs (ethionamide, prothionamide, thioacetazone, isoxyl, amikacin, kanamycin or capreomycin and some fluoroquinolone derivatives as ofloxacin, levofloxacin, moxifloxacin and gatifloxacin) but increasing resistance to at least isoniazid and rifampicin was revealed the multidrug-resistant (MDR) tuberculosis (TB) (2). In 2006, the first report concerning extensively drug-resistant TB (XDR-TB) was published and this new case was explained as TB caused by MDR strains that are also resistant to any fluoroquinolone (FQ) and any of the second-line injectable drugs, such as capreomycin, kanamycin, or amikacin (3). Here upon WHO has recommended the use of bedaquiline and delamanid (previously OPC-67863) (1). Bedaquiline received approval from the US Food and Drug

Sevgi KARAKUŞ, Sevim ROLLAS
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34668 İstanbul, TURKEY

Corresponding Author: Sevgi KARAKUŞ
E-mail: skarakus@marmara.edu.tr

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Administration in December 2012 and delamanid received approval from the European Medicines Agency and Japan's Pharmaceuticals Medical Devices Agency in 2014 (4).

All these mentioned drugs that have been already approved for TB theraphy are composed of diverse chemical entities and mechanisms of actions. Since last decade, the researchers have developed several compound series originated from target-based screening efforts as nitroimidazopyrans (PA-824), oxazolidinones (linezolid, sutezolid, posizolid), 1,2-ethylenediamine-based compound-SQ109, benzothiazinones (BTZ038, PBTZ169), Imidazopyridine amides (Q203) (5, 6). In addition to the mentioned chemical entities, compounds with 1,3,4-thiadiazole structure are also the subjects of efforts to identify new anti TB drugs and they are being investigated in significant number of works (7-9). Some representatives of 1,3,4-thiadiazole compounds with promising antituberculosis activity were shown in Figure 1.

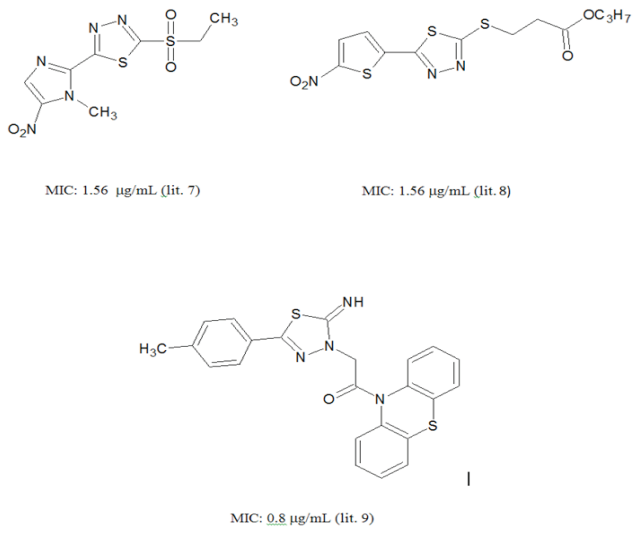


Figure 1. Some representative 1,3,4-thiadiazoles with promising activity against *Mycobacterium tuberculosis H37Rv.*

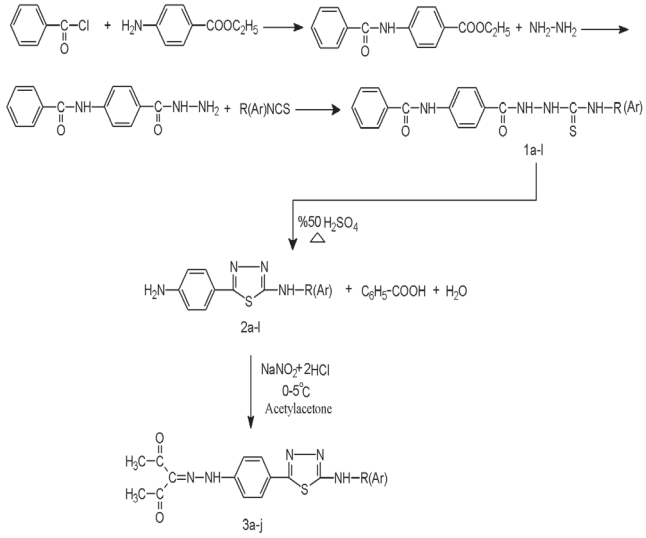
In the light of encouraging literature data; we synthesized novel 1,3,4-thiadiazole compounds and evaluated them for their anti-TB activity against *Mycobacterium tuberculosis H37Rv.*

2. Chemistry

In the first part of our research, benzoyl chloride and ethyl 4-aminobenzoate were reacted according to the literatüre (10). The obtained product was refluxed with hydrazine hydrate to prepare 4-(benzoylamino) benzoylhydrazine. 1-[4-(Benzoylamino)benzoyl]-4 alkyl-/arylthio-semicarbazides **(1a-l)** were then gained by condensing methyl, ethyl, propyl, cyclohexyl, phenyl, benzyl, 4-fluorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methylphenyl, 4-methoxyphenyl and 4-nitrophenyl isothiocyanates to 4-(benzoylamino)benzoylhydrazine (11). From **1a-l,** 2-(aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles **(2a-l)** were synthesized by acid catalyzed cyclization. In the second part, 2,3,4-pentanetrione-3-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazones **(3a-j)** were obtained through the coupling reaction of acetylacetone and the diazonium salts of aromatic primary amines **(2a-l)** (12). The synthetic route to **2a-l** and **3a-j** is presented in Scheme 1.

Compounds **2a-f,** had been described previously by Rollas (13) and Özger (14). The researchers have obtained these compounds at the end of a six steps reaction procedure by reducing 2-substituted amino-5-[p-(1'-phenyl-3',5'-dimethyl-4'-(1*H*)-pyrazolylazo)phenyl]-1,3,4-thiadiazoles with hydrazine hydrate without catalyst in ethanolic medium. On the other hand; we have achieved compounds **2a-l** by employing a short and economical reaction procedure comprising of four steps. According to our new reaction procedure; 1-[4-(benzoylamino)benzoyl]-4-alkyl-/arylthiosemicarbazides **(1a-l)** were heated in 50% H_2SO_4 solution at 110-150 °C.

The purity of the synthesized compounds was determined by HPLC. The structures of the synthesized **2g-l** and **3a-j** were confirmed using UV, IR, ^{1}H-NMR and MS spectral data besides elemental analysis.



Scheme 1. Synthetic route to the compounds **2a-l** and **3a-j**

3. In vitro evaluation of antimycobacterial activity against *M. tuberculosis H37Rv*

Primary screen was conducted at 6.25 μg/mL against *M. tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system (15, 16). Compounds denoting < 90% inhibition in the primary screen (MIC > 6.25 μg/mL) are not considered for further evaluation. Compounds demonstrating at least 90% inhibition in the primary screen are re-tested at lower concentrations (MIC) in a broth microdilution assay alamar Blue. The MIC is defined as the lowest concentration inhibiting 99% of the inoculum.

BACTEC radiometric method of susceptibility testing. Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium. The culture was mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampin (0.25 μg/mL). A control vial was inoculated with a 1:100 dilution of the culture. A suspension equivalent to a Mc Farland no. 1 standart was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used.

Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control reads at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret results:

ΔGI control > ΔGI drug = susceptible

ΔGI control < ΔGI drug = resistant

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control GI is 30 the vials read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

4. Results and discussion

A series of 2-(4-aminophenyl)-(5-substituted amino)-1,3,4-thiadiazoles **(2a-l)** and their coupling products **(3a-j)** have been synthesized and their antitubercular activity was determined *in vitro* using the BACTEC 460 Radiometric System against *M. tuberculosis* H37Rv at 6.25 μg/mL.

The structures of the synthesized compounds were determined on the basis of spectral data analysis; such as UV, IR, ^{1}H-NMR and MS.

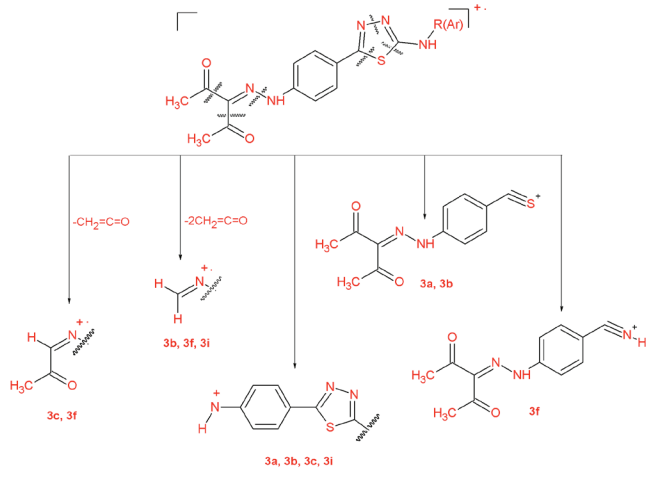
Investigations on IR spectra of compounds **2a-l** revealed that there were no bands characterising amide moiety but bands at 3483 - 3401 cm^{-1} and 3401 - 3282 cm^{-1} could be dedicated to asymmetric and symmetric stretching vibrations of primary aromatic amine respectively.

According to the ^{1}H-NMR spectra of the compounds **2a-l**; the singlet signals between 3.10- 5.83 ppm possessing the integration of 2H were attributed to primary aromatic amine. The N-H protons of secondary amine were determined between 8.16-13.84 ppm as singlet with the integration of 1H (16- 18).

The IR spectra of compounds **3a-j** exhibited hydrazone (-NH–N=C<) group at 3201-3166 cm^{-1} and carbonyl groups of acetyl moiety >C=O bands at the 1689-1666 cm^{-1} ve 1655-1625 cm^{-1} (20). The absorption bands of other functional groups also appeared in the expected regions.

^{1}H-NMR spectra of the cyclization products **3a-j** displayed the resonances of hydrazone N–H at 13.02-14.75 ppm and methyl protons at 2.41-2.54 ve 2.52-2.64 ppm except for compounds **3e** and **3g**. Methyl protons of compounds **3e** and **3g** were determined as singlets at 2.57 ppm and 2.52 ppm possesing integration equivalent to 6H respectively (21).

EI-MS spectra of **2a-l** showed molecular ion (M^+) peaks which confirmed their molecular weights. In the EI-MS spectra of compounds **3a-j** (except for compound **3f**) detected molecular ions (M^+) peaks confirmed their molecular weights. The common fragmentation pathway of these compounds was existed by the cleavage between nitrogen atoms of hydrazone moiety (12, 22). CI-MS spectrum of compound **3f** revealed nearly the same cleavage pathways as compounds **3a-j.** Fragmentation patterns of thiadiazole ring were found in accordance with the literatüre (11, 18).



Scheme 2. Proposed mass fragmentation pathways of compounds **3a-j.**

The synthesized compounds **2a-l** and **3a-j** were tested for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv. As provided in antituberculosis data which was reported in Table 1, among the 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazole series **2a-l**, the 4-chloro substituted compound **2f** showed the highest inhibition. The 4-nitro substituted compound **2l** showed 37% inhibition. The benzyl substituted compound **2g** was as active as compound **2l**. The methyl and ethyl substituted compounds **2a** and **2b** also exhibited inhibition however the propyl substituted compound **2c** did not show any inhibition. The 2-methyl- substituted compound **2i** displayed no inhibition, either. The 4-methyl-substituted compound **2j** showed 22% inhibition.

The highest inhibition in the hydrazone series **3a-j** was observed for derivatives bearing an alkyl group at the 5th- position of the thiadiazole ring. Longer alkyl chains caused a decrease in inhibition. Compounds **3a-c** bearing methyl, ethyl, propyl groups respectively showed higher inhibition than their corresponding amine derivatives **2a-c**. Compounds **3d** and **3f** bearing cyclohexyl, 4-chlorophenyl groups respectively showed lower inhibition than their corresponding amine derivatives **2d** and **2f**.

Table 1. Primary antitubercular activity screening results of **2a-l** and **3a-j**

Compounds	R(Ar)	MIC (μg/mL)	Inhibition (%)
2a	CH_3	>6.25	29
2b	C_2H_5	>6.25	34
2c	C_3H_7	>6.25	0
2d	C_6H_{11}	>6.25	41
2e	C_6H_5	>6.25	16
2f	C_6H_5-Cl(4)	>6.25	57
2g	CH_2-C_6H_5	>6.25	37
2h	C_6H_5-F(4)	>6.25	3
2i	C_6H_5-CH_3(2)	>6.25	0
2j	C_6H_5-CH_3(4)	>6.25	22
2k	C_6H_5-OCH_3(4)	>6.25	7
2l	C_6H_5-NO_2(4)	>6.25	37
3a	CH_3	>6.25	46
3b	C_2H_5	>6.25	39
3c	C_3H_7	>6.25	36
3d	C_6H_{11}	>6.25	11
3e	C_6H_5	>6.25	0
3f	C_6H_5-Cl(4)	>6.25	15
3g	CH_2-C_6H_5	>6.25	0
3h	C_6H_5-F(4)	>6.25	0
3i	C_6H_5-CH_3(2)	>6.25	5
3j	C_6H_5-CH_3(4)	>6.25	0
Rifampicin		0.25	98

5. Experimental

Acetylacetone, benzocaine and hydrazine hydrate were purchased from Merck. All other chemicals were purchased from Fluka. Melting points were determined by using a Büchi-530 melting point apparatus (open capilleries) and were uncorrected. UV spectra were determined on a Shimadzu UV 2100 S spectrophotometer. IR spectra were run on a Perkin Elmer 1600 spectrophotometer as KBr pellets. ^{1}H-NMR spectra were obtained on a Bruker DP X-400 spectrometer at MHz using TMS as the internal reference. Mass spectra were determined at 70 eV on a VG Zabspec Double Focussing Magnetic Sector spectrometer.

HPLC apparatus and conditions: All measurements were performed by HPLC apparatus consisting of a Waters Model 600 pump, a Waters Model 481 UV dedector and a Rheodyne Model 7725 injector. An integrator (Unicam 4880 Chromatography Data Handling System) was used for data collection. A reversed-phase μ-Bondapak C_{18} column (150 mm x 3.9 mm ID; Waters Assoc. Milford, MA, USA) was used for the analysis. The mobile phase consisted of acetonitrile-water (60:40, v/v). The solvent flow-rate was 0.6 mL/min. The mobile phase was degassed in an ultrasonic bath (Bransonic 221) prior to use. The UV detector was set at 254 nm.

5.1. General procedure for the preparation of 1-[4-(benzoylamino)benzoyl]-4-alkyl-/arylthio-semicarbazides (1a-l)

Compound 4-(benzoylamino)benzoylhydrazine was heated with substituted isothiocyanates under reflux for 2h in ethanol. The crude product was filtered and crystallized from ethanol (11).

5.2. General procedure for the preparation of 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (2g-l)

To 0.006 mol of 1a-l, 50% H_2SO_4 (15 mL) was added and the mixture and was refluxed for 5h at 110-150 °C. It was cooled and neutralized with 2N NaOH. The precipitate was filtered, washed with water and recrystallized from ethanol (11).

5.2.1. 2-(4-Aminophenyl)-5-benzylamino-1,3,4-thiadiazole (2g) M.p. 225 °C, yield 58%, HPLC t_R (min): 2.98; UV (EtOH, $\lambda_{max,}$): 326 (ε 25470), 204 (ε 29875) ; IR (KBr): 3425, 3307, 3248, 3095, 1631, 1602, 1555, 1467, 1331, 826, 738 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ): 4.48 (2H, s, -CH_2-), 5.58 (2H, s, -NH_2), 6.58 (2H, d, *J: 8.5 Hz* protons in ortho position of aromatic primary amine), 7.18-7.40 (7H, m, Ar-H), 8.16 (1H, s, -NH); MS (EI) m/z 282 (M^+), 267, 257, 136, 135, 118, 91, 77, 65, 63. Anal.calc. for $C_{15}H_{14}N_4S$ (282.26): C, 63.80; H, 5.00; N, 19.84; S, 11.36 %. Found C, 63.95; H, 5.51; N, 18.80; S, 11.63%.

5.2.2. 2-(4-Aminophenyl)-5-(4-fluorophenyl)amino-1,3,4-thiadiazole (2h)

M.p. 193-195 °C; yield 63%; HPLC t_R (min): 3.30; UV (EtOH, $\lambda_{max,}$): 339 (ε 3579), 231 (ε 1717), 216, (ε 2090); IR (KBr): 3412, 3319, 3248, 3037, 1624, 1602, 1572, 1502, 1467, 1308, 1214 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ): 5.75 (2H, s, -NH_2), 6.71 (2H, d, *J: 8.6 Hz* protons in ortho position of aromatic primary amine), 7.28 (2H, t, *J: 8.9 Hz* protons in ortho position of fluorine atom), 7.53 (2H, d, *J: 8.6 Hz* protons in meta position of aromatic primary amine), 7.67-7.76 (m, 2H, protons in meta position of fluorine atom), 10.43 (1H, s, -NH-). Anal.calc. for $C_{14}H_{11}FN_4S$. H_2O (304.34): C, 55.25; H, 4.31; N, 18.41; S, 10.54 %. Found C, 55.42; H, 3.80; N, 18.45; S, 11.05%.

5.2.3. 2-(4-Aminophenyl)-5-(2-methylphenyl)amino-1,3,4-thiadiazole (2i)

M.p. 183 °C; yield 57 %; HPLC t_R (min): 4.28; UV (EtOH, $\lambda_{max,}$): 335 (ε 24695), 205 (ε 30137); IR (KBr): 3436, 3354, 3225, 1636, 1608, 1590, 1531, 1496, 1461, 1331, 826, 750 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.33 (3H, s, -CH_3), 3.10-4.20 (2H, s, -NH_2), 6.73 (2H, d, *J: 8.4 Hz* protons in ortho position of aromatic primary amine), 7.10 (1H, t, *J: 7.5 Hz* proton in meta position of –CH_3), 7.28 (1H, t, *J: 8.3 Hz* proton in para position of –CH_3), 7.30 (1H, d, *J: 8.0 Hz* proton in meta position of –CH_3), 7.60 (2H, d, *J: 8.0 Hz* protons in meta position of aromatic primary amine), 7.92 (1H, d, *J: 7.8 Hz* proton in ortho position of –CH_3), 9.20-9.70 (s, -NH-); MS (EI) m/z 282 (M^+), 263, 240, 210, 196, 165, 149, 105, 91, 77, 69, 57. Anal.calc. for $C_{15}H_{14}N_4S$. H_2O (300.38): C, 59.98; H, 5.37; N, 18.65; S, 10.67 %. Found C, 60.86; H, 5.36; N, 18.09; S, 10.44%.

5.2.4. 2-(4-Aminophenyl)-5-(4-methylphenyl)amino-1,3,4-thiadiazole (2j)

M.p. 217-218 °C; yield 56 %; HPLC t_R (min): 3.40; UV (EtOH, $\lambda_{max,}$): 341 (ε 35325), 259 (ε 8866), 204 (ε 37555) nm; IR (KBr): 3436, 3331, 3213, 2919, 1619, 1602, 1508, 1478, 1437, 1325, 820, 738 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.42 (3H, s,–CH_3), 5.61(2H, s, -NH_2), 6.47 (2H, d, *J: 8.5 Hz* protons in ortho position of aromatic primary amine), 6.99 (2H, d, *J: 8.5 Hz* protons in meta position of –CH_3), 7.23 (2H, d, *J: 8.1 Hz* protons in ortho position of –CH_3), 7.39 (2H, d, *J: 8.2 Hz* protons in meta position of aromatic primary amine), 13.84 (1H, s, -NH-); MS (EI) m/z 282 (M^+), 280, 266, 249, 223, 209, 195, 164, 119, 118, 106, 91, 77, 65, 63. Anal.calc. for $C_{15}H_{14}N_4S$. ½ H_2O (291.37): C, 61.83; H, 5.19; N, 19.23; S, 11.00%. Found C, 62.01; H, 5.13; N, 18.13; S, 11.23%.

5.2.5. 2-(4-Aminophenyl)-5-(4-methoxyphenyl)amino-1,3,4-thiadiazole (2k)

M.p. 255-257 °C; yield 41 %; HPLC t_R (min): 2.21; UV (EtOH, $\lambda_{max,}$): 337 (ε 33749), 205 (ε 39948) nm; IR (KBr): 3436, 3342, 3236, 3142, 2966, 2931, 1631, 1608, 1514, 1461, 1437, 1331, 1249, 1026, 832, 726 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 3.87 (3H, s, -OCH_3), 5.61 (2H, s, -NH_2), 6.51 (2H, d, *J: 8.5 Hz* protons in ortho position of aromatic primary amine), 7.00 (2H, d, *J: 8.6 Hz* protons in ortho position of -OCH_3), 7.10 (2H, d, *J: 8.9 Hz* protons in meta position of -OCH_3), 7.27 (2H, d, *J: 8.9 Hz* protons in meta position of aromatic primary amine); MS (EI) m/z 298 (M^+), 297, 282, 266, 225, 165, 133, 118, 106, 91, 78, 68. Anal.calc. for $C_{15}H_{14}N_4O_2S$. ½ H_2O (307.37): C, 58.61; H, 4.92; N, 18.23; S, 10.43%. Found C, 58.31; H, 4.45; N, 17.39; S, 10.62%.

5.2.6. 2-(4-Aminophenyl)-5-(4-nitrophenyl)amino-1,3,4-thiadiazole (2ı)

M.p. > 300 °C; yield 47 %; HPLC t_R (min): 3.28; UV (EtOH, $\lambda_{max,}$): 375 (ε 2890), 287 (ε 23222), 205 (ε 33319); IR (KBr): 3483, 3401, 3330, 3049, 1614, 1514, 1461, 1331, 838, 750 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 5.83 (2H, s, -NH_2), 6.69 (2H, d, *J: 8.6 Hz* protons in ortho position of aromatic primary amine), 7.58 (2H, d, *J: 8.6 Hz,* protons in meta position of –NO_2), 7.96 (2H, d, *J: 7.2 Hz* protons in meta position of aromatic primary amine), 8.33 (2H, d, *J: 7.3 Hz* protons in ortho position of –NO_2), 11.02 (1H, s, -NH-). Anal.calc. for $C_{14}H_{11}N_5O_2S$ (313.33): C, 53.66; H, 3.54; N, 22.35; S, 10.23 %. Found C, 53.25; H, 3.70; N, 22.28; S, 10.36%.

5.3. 2,3,4-Pentanetrione-3-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3a-j)

To a cooled solution of compounds 2a-l (0.01 mol) in 2 ml of hydrochloric acid (37%), an ice-cold solution of 10 mL of sodium nitrite (10%) were added. The reaction mixture was then poured into the mixture of 1 mL of acetylacetone and 50 g of sodium acetate in ethanol (50%) by vigorous stirring. This mixture was allowed to stand in a refrigerator for 24 h. Precipitated solid was collected, washed with water, dried and washed with ethanol to give 3a-j (12).

5.3.1. 2,3,4-Pentanetrione-3-[4-(5-methylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3a)

M.p. 185-188 °C; yield 65 %; HPLC t_R (min): 5.05; UV (EtOH, $\lambda_{max,}$): 393 (ε 6116), 247 (ε 1586), 208 (ε 2135) ; IR (KBr): 3178, 1672, 1602, 1573, 1508, 1461, 1450, 1343, 1261, 844, 768 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.54 (3H, s, -CO$\underline{CH_3}$), 2.64 (3H, s, -CO$\underline{CH_3}$), 3.78 (3H, s, -NH-$\underline{CH_3}$), 4.15 (1H, s, -$\underline{NH}$-CH_3), 7.54 (2H, d, *J: 8.5 Hz* protons in meta position of thiadiazole ring), 8.05 (2H, d, J: 8.5 Hz protons in ortho position of thiadiazole ring), 14.68 (s, =N-NH-), MS (EI) m/z 317 (M^+), 247, 205, 132, 118, 104, 88, 63.

5.3.2. 2,3,4-Pentanetrione-3-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3b)

M.p. 178-180 °C; yield 63 %; HPLC t_R (min): 10.76; UV (EtOH, $\lambda_{max,}$): 398 (ε 2485), 252 (ε 397), 208 (ε 563); IR (KBr): 2978, 2931, 1678, 1637, 1602, 1578, 1508, 1472, 1355, 1267, 850, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCI_3$, δ): 1.22-1.75 (3H, 2t, $-CH_2-\underline{CH_3}$), 2.54 (3H, s, $-CO\underline{CH_3}$), 2.64 (3H, s, $-CO\underline{CH_3}$), 4.33-5.20 (2H, 2q, $-NH-\underline{CH_2}-$), 7.53 (2H, d, *J: 8.7 Hz* protons in meta position of thiadiazole ring), 8.04 (2H, t, *J: 8.6 Hz* protons in ortho position of thiadiazole ring), 14.69 (1H, s, =N-NH-); MS (EI) m/z 331 (M^+), 249, 248, 247, 220, 219, 150, 149, 118, 92, 69, 63, 43.

5.3.3. 2,3,4-Pentanetrione-3-[4-(5-propylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3c)

M.p. 166-168 °C; yield 65 %; HPLC t_R (min): 10.70; UV (EtOH, $\lambda_{max,}$): 395 (ε 23873), 286 (ε 4491), 247 (ε 7101); IR (KBr) 3619-3353, 3049, 2962-2923, 2865, 1676, 1600, 1581, 1524, 1505, 1429, 822, 733 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCI_3$, δ): 0.91-1.09 (3H, 2t, $-CH_2-CH_2-\underline{CH_3}$), 1.64-2.19 (2H, 2m, $-CH_2-\underline{CH_2}-CH_3$), 2.54 (3H, s, $-CO\underline{CH_3}$), 2.64 (3H, s, $-CO\underline{CH_3}$), 4.23-5.08 (2H, 2t, $-\underline{CH_2}-CH_2-CH_3$), 7.54 (d, 2H, *J: 8.5 Hz* protons in meta position of thiadiazole ring), 8.05 (2H, t, *J: 8.5 Hz* protons in ortho position of thiadiazole ring), 14.69 (s, =N-NH-), MS (EI) m/z 345 (M^+), 303, 247, 234, 233, 191, 141, 136, 135, 118, 116, 115, 103, 77, 73.

5.3.4. 2,3,4-Pentanetrione-3-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3d)

M.p. 223 °C; yield 55 %; HPLC t_R (min): 6.11; UV (EtOH, $\lambda_{max,}$): 404 (ε 43475), 298 (ε 14563), 202 (ε 23001); IR (KBr): 3166, 3060, 2919, 2837, 1666, 1590, 1525, 1461, 1273, 844, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCI_3$, δ): 1.00-2.23 (10H, m, cyclohexyl $-CH_2-$), 2.51 (3H, s, $-CO\underline{CH_3}$), 2.62 (3H, s, $CO\underline{CH_3}$), 3.74 (1H, s, cyclohexyl –CH-), 5.39 (1H, s, -NH-), 7.45 (2H, d, *J: 8.3 Hz* protons in meta position of thiadiazole ring), 7.84 (2H, d, *J: 8.0 Hz* proton in ortho position of thiadiazole ring), 14.72 (s, =N-NH-). Anal.calc. for $C_{19}H_{23}N_5O_2S$. ½ H_2O (394.49): C, 57.85; H, 6.13; N, 17.75; S, 8.13 %. Found C, 58.12; H, 5.95; N, 17.30; S, 8.24%.

5.3.5. 2,3,4-Pentanetrione-3-[4-(5-phenylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3e)

M.p. 273 °C; yield 69 %; HPLC t_R (min): 6.56; UV (EtOH, $\lambda_{max,}$): 405 (ε 41946), 309 (ε 16109), 247 (ε 16592), 203 (ε 33564). IR (KBr): 3243, 3194, 3077, 1673, 1567, 1503, 1442, 1260, 836, 750 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.57 (6H, s, $-COCH_3$), 7.09-7.93 (10H, m, Ar-H and –NH-), 13.81 (s, =N-NH-); MS (EI) m/z 379 (M^+), 303, 273, 246, 230, 191, 157, 136, 135, 108, 90, 83, 67. Anal.calc. for $C_{19}H_{17}N_5O_2S$. 3/2 H_2O (406.46): C, 56.14; H, 4.96; N, 17.23; S, 7.89 %. Found C, 56.51; H, 4.53; N, 16.13; S, 8.03%.

5.3.6. 2,3,4-Pentanetrione-3-[4-[5(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3f)

M.p. 206-209 °C; yield 84 %; HPLC t_R (min): 7.35; UV (EtOH, $\lambda_{max,}$): 376 (ε 29838), 204 (ε 35632); IR (KBr): 3084, 2990, 1689, 1525, 1490, 1437, 1261, 1085, 838, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCI_3$, δ): 2.47 (3H, s, $-COCH_3$), 2.60 (3H, s, $-COCH_3$), 7.18-7.51 (9H, m, Ar-H and –NH-), 14.62 (1H, s, =N-NH-); MS (CI) m/z 442 ($M+C_2H_5$)$^+$, 414, 373, 331, 230, 153, 114. Anal.calc. for $C_{19}H_{16}ClN_5O_2S$ (413.88): C, 55.14; H, 3.90; N, 16.92; S, 7.75 %. Found C, 54.94; H, 3.55; N, 17.00; S, 8.05%.

5.3.7. 2,3,4-Pentanetrione-3-[4-(5-benzylamino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3g)

M.p. 205 °C; yield 75 %; HPLC t_R (min): 5.90; UV (EtOH, $\lambda_{max,}$): 400 (ε 5784), 298 (ε 1967), 252 (ε 1298), 210 (ε 1770); IR (KBr): 3331, 3201, 3002, 1672, 1637, 1537, 1508, 1420, 1296, 826, 767 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.52 (6H, s, $-COCH_3$), 4.61 (2H, d, *J: 5.8 Hz*, $-CH_2-$), 7.29-7.47 (5H, m, $-CH_2-\underline{C_6H_5}$), 7.65 (2H, d, J*: 8.7 Hz* protons in meta position of thiadiazole ring), 7.88 (2H, d, *J: 8.8Hz* protons in ortho position of thiadiazole ring), 8.52 (1H, t, -NH-), 13.90 (s, =N-NH-); MS (EI) m/z 393(M^+), 384, 356, 346, 281, 258, 250, 223, 161, 139, 133, 125, 122, 105, 91, 79, 77, 65, 57, 43. Anal.calc. for $C_{20}H_{19}N_5O_2S$ (393.46): C, 61.05; H, 4.87; N, 17.80; S, 8.15%. Found C, 61.41; H, 4.04; N, 16.83; S, 7.88%.

5.3.8. 2,3,4-Pentanetrione-3-[4-[5(4-fluorophenyl)amino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3h)

M.p. 165-168 °C; yield 52 %; HPLC t_R (min): 5.31; UV (EtOH, $\lambda_{max,}$): 372 (ε 32193), 250 (ε 15580), 214 (ε 13195); IR (KBr): 3084, 2998, 2974, 1674, 1606, 1594, 1576, 1478, 1263, 1166, 763 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.49 (3H, s, $-COCH_3$), 2.53 (3H, s, $-COCH_3$), 7.30-7.77 (8H, m, Ar-H), 8.89 (s, -NH-), 13.81 (1H, s, =N-NH-); MS (EI) m/z 397(M^+), 366, 365, 322, 266, 254, 253, 226, 197, 184, 170, 136, 122, 118, 109, 95, 82, 75, 70, 69. Anal.calc. for $C_{19}H_{16}FN_5O_2S$ (397.43): C, 57.42; H, 4.06; N, 17.62; S, 8.07%. Found C, 58.36; H, 3.51; N, 16.94; S, 8.68%.

5.3.9. 2,3,4-Pentanetrione-3-[4-[5(2-methylphenyl)amino-1,3,4-thiadiazole-2-yl)phenylhydrazone (3i)

M.p. 217-219 °C; yield 59 %; HPLC t_R (min): 7.55; UV (EtOH, $\lambda_{max,}$): 397 (ε 18314), 247 (ε 6832), 213 (ε 8084) nm; IR (KBr): 3530-3331, 3178, 2919, 1666, 1631, 1584, 1555, 1508, 1461, 1431, 1372, 1296, 785, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCI_3$, δ): 2.27 (s, Ar-CH_3), 2.41 (s, $-CO\underline{CH_3}$), 2.52 (s, $-CO\underline{CH_3}$), 7.11-7.82 (9H, m, Ar-H and –NH-), 14.30-14.75 (1H, =N-

NH-); MS (EI) m/z 393(M^+), 367, 356, 283, 282, 281, 250, 123, 118, 105, 91, 78, 77, 65, 59, 51, 45. Anal.calc. for $C_{20}H_{19}N_5O_2S$. 1/2 H_2O (402.47): C, 59.68; H, 5.01; N, 17.40; S, 7.97%. Found C, 59.59; H, 4.91; N, 16.74; S, 8.01%.

5.3.10. 2,3,4-Pentanetrione-3-[4-[5(4-methylphenyl)amino-1,3,4-thiadiazole-2-yl)phenyl]hydrazone (3j)

M.p. 242-244 °C; yield 63 %; HPLC t_R (min): 6.06; UV (EtOH, $\lambda_{max,}$): 373 (ε 53512), 253 (ε 2676), 211 (ε 2400); IR (KBr): 3084, 2919, 1684, 1649, 1578, 1508, 1437, 1355, 1302, 838, 750 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO- d6 , δ): 2.42-2.46 (CH_3 protons were over shadow by DMSO peak), 6.46-8.07 (9H, m, Ar-H and N-H), 13.02-14.46 (d and broad singlet ,=N-NH-); MS (EI) m/z 393(M^+), 295, 281, 282, 267, 265, 164, 163, 149, 132, 118, 107, 91, 64. Anal.calc. for $C_{20}H_{19}N_5O_2S$ (393.46): C, 61.05; H, 4.87; N, 17.80; S, 8.15 %. Found C, 60.28; H, 4.59; N, 16.92; S, 8.57%.

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Bazı 2-(4-aminofenil)-5-sübstitüe amino-1,3,4-tiyadiazol türevlerinin ve kenetlenme ürünlerinin sentezi ve antimikobakterial etkileri

ÖZ

Bu çalışmada, çeşitli 2-(4-aminofenil)-5-sübstitüe amino-1,3,4-tiyadiazoller (**2a-l**) ile onun kenetlenme ürünleri 2,3,4-pentantrion-3-[4-(5-alkil/arilamino-1,3,4-tiyadiazol-2-il)fenil]hidrazonlar (**3a-j**) yüksek verimlerle sentezlendi ve bileşiklerin yapıları UV, IR, ^{1}H-NMR, kütle spektroskopisi ve elementel analiz yöntemleri kullanılarak aydınlatıldı. Bileşiklerin, *in vitro Mycobacterium tuberculosis* H37Rv suşuna karşı antimikobakteriyel etkileri BACTEC 460 Radyometrik Sistemi kullanılarak 6.25 µg/mL derişimde değerlendirildi. 2-(4-Aminofenil)-5-(4-klorofenil)amino-1,3,4-tiyadiazol (**2f**) bileşiğinin en yüksek inhibisyon gösterdiği tespit edildi.

Anahtar kelimeler: 1,3,4-tiyadiazol, kenetlenme ürünleri, antimikobakteriyel etki

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