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

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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-pentanetione-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, 1H-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, thiacetazone, 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
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 therapy 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.

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. In the first part of our research, benzoyl chloride and ethyl 4-aminobenzoate were reacted according to the literature (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/arylaminio-1,3,4-thiadiazoles (2a-l) were synthesized by acid catalyzed cyclization. In the second part, 2,3,4-pentanetrione-3-[4-(5-alkyl/arylaminio-1,3,4-thiadiazole-2-yl)phenyl]hydrazones (3a-j) were obtained through the coupling reaction of acetylacetonate 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’-(1H)-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% H2SO4 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, 1H-NMR and MS spectral data besides elemental analysis.

![Scheme 1. Synthetic route to the compounds 2a-l and 3a-j](image)
3. In vitro evaluation of antitubercular 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 McFarland no. 1 standard 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 CO₂ 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 antituberculc 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, ¹H-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 ¹H-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.

¹H-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 possessing 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 literature (11, 18).
The synthesized compounds 2a-l and 3a-j were tested for antimycobacterial activity against Mycobacterium tuberculosis H37Rv. As provided in antibacterial data which was reported in Table 1, among the 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazole series 2a-l, the 4-chlororo substituted compound 2f showed the highest inhibition. The 4-nitro substituted compound 2l showed 37% inhibition. The methyl substituted compound 3a-c bearing cyclohexyl, 4-chlorophenyl groups respectively showed lower 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.

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.

### 5. Experimental
Acetylaceton, 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 capillaries) 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. 1H-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 detector and a Rheodyne Model 7725 injector. An integrator (Unicam 4880 Chromatography Data Handling System) was used for data collection. A reversed-phase µ-Bondapak C18 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 (Brasonic 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-/aryltiosemicarbazides (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% H2SO4 (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).

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<th>Compounds</th>
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<th>MIC (µg/mL)</th>
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<tr>
<td>2f</td>
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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, λ_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; ¹H-NMR (DMSO-d_6, 400 MHz, δ): 5.75 (2H, s, -NH₂), 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), 6.70-7.76 (m, 2H, protons in meta position of fluorine atom), 10.43 (1H, s, -NH-). Anal.calc. for C₁₅H₁₄N₄S. ½ H₂O (291.37): C, 61.83; H, 5.19; N, 19.23; S, 11.05%.

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

M.p. 183 °C; yield 57%; HPLC t_R (min): 4.28; UV (EtOH, λ_max): 335 (ε 24695), 205 (ε 30137); IR (KBr): 3436, 3354, 3225, 1636, 1608, 1590, 1546, 1461, 1331, 826, 750 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d_6, δ): 3.10-4.20 (2H, s, -NH₂), 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 -CH₃), 7.28 (1H, t, J: 8.3 Hz proton in para position of -CH₃), 7.30 (1H, d, J: 8.0 Hz proton in meta position of -CH₃), 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 -CH₃), 9.20-9.70 (s, -NO₂); MS (EI) m/z 298 (M⁺), 297, 282, 266, 225, 165, 133, 118, 106, 91, 78, 68. Anal.calc. for C₁₄H₁₁FN₄S. ½ H₂O (300.38): C, 53.66; H, 3.54; N, 22.35; S, 10.23%. Found C, 53.52; H, 3.70; N, 10.28%.

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, λ_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; ¹H-NMR (400 MHz, DMSO-d_6, δ): 2.42 (3H, s, -CH₃), 5.61(2H, s, -NH₂), 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 -CH₃), 7.23 (2H, d, J: 8.1 Hz protons in ortho position of -CH₃), 7.39 (2H, d, J: 8.2 Hz protons in meta position of aromatic primary amine), 8.04 (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 C₁₄H₁₁N₅O₂S (313.33): C, 53.66; H, 3.54; N, 22.35; S, 10.23%. Found C, 53.52; H, 3.70; N, 10.28%.

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, λ_max): 337 (ε 35749), 205 (ε 39948) nm; IR (KBr): 3436, 3342, 3236, 3142, 2966, 2931, 1631, 1608, 1514, 1461, 1437, 1331, 1249, 1026, 832, 726 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d_6, δ): 3.87 (3H, s, -OCH₃), 5.61 (2H, s, -NH₂), 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 -OCH₃), 7.10 (2H, d, J: 8.9 Hz protons in meta position of -OCH₃), 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 C₁₄H₁₁O₃N₄S. ½ H₂O (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%.
5.3.2. 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, λ_max): 398 (ε 2485), 252 (ε 397), 208 (ε 563); IR (KBr): 2978, 2931, 1678, 1637, 1502, 1472, 1355, 1267, 850, 750 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ): 1.22-1.75 (3H, 2t, -CH₂CH₂CH₂-), 2.54 (3H, s, -COCH₃), 4.61 (2H, d, J: 8.7 Hz, protons in ortho position of thiadiazole ring), 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+), 257, 256, 230, 126, 118, 117, 81, 80, 63, 57.

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

M.p. 166-168 °C; yield 65 %; HPLC t_R (min): 10.70; UV (EtOH, λ_max): 395 (ε 23873), 286 (ε 4491), 247 (ε 7101); IR (KBr): 3619-3353, 3049, 2962-2923, 2865, 1676, 1600, 1581, 1525, 1405, 822, 733 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ): 0.91-1.09 (3H, 2t, -CH₂CH₂CH₂-), 1.64-1.75 (2H, 2m, -CH₂-CH₂-), 4.23-5.08 (2H, 2t, -CH₂-CH₂-CH₃), 7.54 (d, 2H, J: 8.5 Hz protons in ortho 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+), 272, 261, 250, 223, 161, 139, 133, 125, 122, 105, 91, 79, 77, 65, 57, 43.

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

M.p. 206-209 °C; yield 84 %; HPLC t_R (min): 7.35; UV (EtOH, λ_max): 376 (ε 29838), 204 (ε 35632); IR (KBr): 3084, 2990, 1689, 1525, 1490, 1437, 1261, 1085, 838, 750 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ): 2.47 (3H, s, -COCH₃), 2.60 (3H, s, -COCH₃), 7.18-7.51 (9H, m, Ar-H and –NH-), 14.62 (1H, s, =N-NH-); MS (EI) m/z 442 (M+C₂H₅)⁺, 414, 373, 331, 230, 153, 114. Anal.calc. for C₁₉H₁₇N₅O₂S (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%.

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

M.p. 223 °C; yield 55 %; HPLC t_R (min): 6.11; UV (EtOH, λ_max): 404 (ε 43475), 298 (ε 14563), 202 (ε 23001); IR (KBr): 366, 3060, 2919, 2837, 1660, 1590, 1525, 1461, 1273, 844, 750 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ): 0.00-2.27 (10H, m, cyclohexyl -CH₂-), 2.51 (3H, s, -COCH₃), 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, t, J: 8.0 Hz proton in ortho position of thiadiazole ring), 14.72 (s, =N-NH-). Anal.calc. for C₁₉H₂₁N₅O₂S.H₂O (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%.

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

M.p. 217-219 °C; yield 59 %; HPLC t_R (min): 7.55; UV (EtOH, λ_max): 397 (ε 18314), 247 (ε 6832), 213 (ε 8084) nm; IR (KBr): 3530-3331, 3178, 2919, 1666, 1631, 1584, 1508, 1461, 1431, 1372, 1296, 785, 750 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ): 2.27 (s, Ar-CH₃), 2.41 (s, -COCH₃), 2.52 (s, -COCH₃), 7.11-7.82 (9H, m, Ar-H and –NH-), 14.30-14.75 (1H, s, =N-
Antimycobacterial activity of some 1,3,4-thiadiazole derivatives and their coupling products


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 C₃₀H₂₉N₅O₅S. 1/2 H₂O (402.47): C, 59.68; H, 5.01; N, 17.40; S, 8.17%. 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, λmax): 373 (e 53512), 253 (e 2676), 211 (e 2400); IR (KBr): 3084, 2919, 1649, 1578, 1508, 1437, 1355, 1302, 838, 750 cm⁻¹; ¹H-NMR (400 MHz, DMSO- d₆, δ): 2.42-2.46 (CH₃ 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 C₂₀H₁₉N₅O₂S (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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References

14. Özger Y, Rollas S. Reductive cleavage of azo compounds with hydrazine and some 1,3,4-thiadiazoles derivatives III. J Pharm Univ Mar 1988; 5: 133-41.