Research Article

Synthesis of Substituted Thioureas and Their Sulfur Heterocyclic Systems of p-Amino Salicylic Acid as Antimycobacterial Agents


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A series of new N,N’-substituted thioureas (2, 6, and 8) and their sulfur heterocycles as thiobarbituric acids (3, 4, and 7), 2-thioxothiazolidin-4-one (10), thiazolidin-4-one (11), 1,2,4-triazol-5-thione (14), and 1,3,4-thiadiazole (15) of p-Amino salicylic acid (PAS) have been synthesized from treatment with dithiocarbazinate (1, 5 and 12) followed by heterocyclization with dimethyl malonate, chloroacetic acid, and/or trifluoroacetic anhydride. The structures of the newly synthesized compounds were substantiated with IR, 1H, and 13C NMR spectral data and elementary microanalyses. The in vitro antitubercular activity of synthesized compounds against M. tuberculosis strain H37Rv showed moderate-to-good activity.

1. Introduction

Infection of Mycobacterium tuberculosis is more prevalent in the world today than at any other time in human history [1]. Globally, more than one-third of the world’s population is infected with the bacteria that cause TB, and each year approximately 9 million people become ill with the disease, and 2 million of those die [2–5]. TB is the second leading cause of death from an infectious disease worldwide. The frequent coinfection of TB in HIV patients further complicates the selection of an appropriate treatment regimen. In recent years, multidrug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) tuberculosis strains emerged and tuberculosis is considered as one of the most challenging threats to global health [6, 7].

During the last few years, chemical research developed a simple, safe, and efficient method to synthesize new classes of compounds active against M. tuberculosis [8–10]. Aminosalicylic acid (PAS) was introduced as an antitubercular medicine in 1948. It was the second antibiotic found to be effective in the treatment of tuberculosis, after streptomycin. PAS formed part of the standard treatment for tuberculosis prior to the introduction of rifampicin and pyrazinamide. Its potency is less than that of the current five first-line drugs (isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin) for treating tuberculosis, but it is still useful in the treatment of multidrug-resistant tuberculosis. PAS is always used in combination with other anti-TB drugs.

In a recent study, a salicylic acid analog (Figure 1) has been found to show mycobacterium protein tyrosine phosphatase B inhibiting activity. This is relatively a new concept in which the growth of mycobacteria is arrested by a pharmacological activation of the xenophagic pathway [11]. This analog of salicylic acid has provided an innovative therapeutic starting point for the treatment of TB, including MDR and XDR forms, that is not only complementary, but also synergistic with current drugs. It is anticipated that such combination therapy will result in the shorter duration of treatment and recovery time for the TB patient. In an extension of our previous study, in the area of synthesis of drugs, semidrugs and bioactive compounds for the treatment of infectious diseases [12–18], the present work aims at the synthesis of some new thiourea and sulphur-containing heterocyclic systems of p-amino salicylic acid as possible antitubercular agents.
2. Experimental

Melting points were determined in an electrothermal Bibby Stuart scientific melting point SMP (US). The IR spectra recorded for KB r discs on a Perkin Elmer spectrum RXI FT-IR systems no. 53529. $^1$H/$^13$C-NMR was determined for solution in deuterated DMSO with a Bruker DPX-400-FT using TMS as an internal standard solvent (chemical shifts in $\delta$, ppm). Mass spectra were measured on a GCMS-Q 1000-Ex spectrometer. Microanalyses (C, H, N, S, F, and Cl) elements were performed by the Microanalyses Centre of Cairo University, Egypt. The antituberculosis activity was carried out in National Institute of Allergy and Infection Diseases’ Southern Research Institute, Gillis W. Long Hansen's Disease Center, Colorado State University, Birmingham, AL, USA.

General Procedure for the Synthesis of Potassium Dithiocarbamates (1, 5, and 12). Carbon disulfide (0.01 mol) was added slowly with vigorous stirring to a mixture of the appropriate sulfa drug and ethanolic potassium hydroxide (20 mL, 5%) at room temperature. The stirring was continued for 3 h and the precipitated potassium salt was filtered, washed with ether, dried and used for the next step without further purification.

General Procedure for the Synthesis of 2-Hydroxyl-4-(Substituted-Thioarede) Benzoic Acid (2a, b $\phi$ 6) and 2-Hydroxy-4-(4-Pyridyl) Thiosemicarbazido]benzoic Acid (13). A mixture of PAS (0.01 mol) and the appropriate potassium dithiocarbamate (0.01 mol) in ethanol (100 mL) was refluxed for 2 h. The solid which separated on cooling was filtered, washed with water, dried, and recrystallized from ethanol.

2a. Yield 78%; yellow powder, m.p. 230-231°C (decomp); IR (v cm$^{-1}$) 3410, 3380 (2 OH), 3239, 3210 (NH), 3030 (Ar–H), 1666 (C=O), 1589 (C=N), 1350 (SO$_2$NH), 1266 (H-bonding), 1201 (C=S), 810, 800 (Ar–CH). $^1$HNMR (6-pm): 11.38 (s, 1H, OH), 8.80 (s,1H, NH$_2$SO$_2$), 8.51 (s, 1H, NHCS), 7.81–8.36 (m, 3H pyrimidine-H), 7.2–7.01 (m, 7H, aromatic H) $^13$CNMR (6-pm): 206.5 (CS) 172.4 (CO), 110.6, 157.4, 169.3 (pyrimidine-C) 112.5, 113.2, 117.6, 125.6, 125.8, 130.2, 135.4, 142.6, 153.3, 158.7 (Ar–C); Anal. Calc. C$_{18}$H$_{13}$N$_2$O$_2$ (445): C, 48.53; H, 3.37; N, 15.73; S, 14.38. Found: C, 48.38; H, 3.33; N, 15.54; S, 14.09.

2b. Yield 79%, pale yellow powder, m.p. 180–182°C (decomp); IR (v cm$^{-1}$) 3424, 3333 (2 OH), 3074, 3035 (Ar–H), 1670 (C=O), 1588, 1572 (2C=N), 1334 (NH$_2$SO$_2$), 1189 (C=S) 818, 795 (Ar–CH). MS (%): 415 (M$^+$–CO$_2$, 48.13), 371 (18.8), 215 (11.11), 156 (5.51), 151 (100), 92 (48.51). Anal. Calc. C$_{19}$H$_{17}$N$_3$S$_2$O$_2$ (459): C, 49.67; H, 3.70; N, 15.25; S, 13.94. Found: C, 49.29; H, 3.66; N, 15.08; S, 13.75.

6. Yield 88%; yellow crystalline, m.p. 196-197°C (decomp); IR (v cm$^{-1}$) 3400–3310 (2 OH), 3210 (NH), 3042, 3035 (Ar–H), 2936, 2888 (aliphatic CH), 1760, 1677 (2C=N), 1609 (C=N), 1492, 1412 (deformation CH$_2$), 1345, 1310 (NCSN), 1281 (H-bonding), 1186 (C=S), 882, 794 (Ar–CH). $^1$HNMR (6-pm): 2.55 (s, 3H, CH$_3$), 4.32 (s, 3H, CH$_3$–N), 7.65–7.38 (m, 8H, Ar–H), 8.31 (s, 1H, NH), 11.81 (s, 1H, OH). $^{13}$CNMR (6-pm): 15.5 (CH$_3$), 35.3 (N–CH$_3$) 199.9 (CS), 171.7 (CO), 162.84 (CO), 108.7, 110.2, 113.3, 114.1, 118.9, 129.5, 130.3, 131.4, 131.3, 142.3, 146.7, 158.5. Anal. Calc. C$_{19}$H$_{18}$N$_3$SO$_4$ (398): C, 57.28; H, 4.52; N, 14.07; S, 8.04. Found: C, 56.78; H, 4.46; N, 13.91; S, 7.95.

13. Yield 89%, orange powder, m.p. 133-134°C (decomp); IR (v cm$^{-1}$) 3494 (OH), 3386 (NH), 2535 (SH), 1641 (C=O), 1615 (C=N), 1352, (NCS), 1293 (H-bonding), 1227 (N–N), 1194 (C=S), 881, 809, 768 (Ar–CH). $^1$HNMR (6-pm): 5.85 (s, 1H, OH), 6.4–7.2 (m, 3H of aryl), 7.7, 7.9 (each d, 2H, 2H of pyridine), 9.2, 8.8, 8.2 (3s, 3H, 3NH), 11.54 (s, 1H, OH). $^{13}$CNMR (6-pm): 187.6 (CS), 172.1 (CO), 163.5 (CO), 112.3, 114.2, 114.8, 122.4, 131.4, 142.5, 144.8, 150.3, 159.1 (Ar–C); MS(%): 288 (M$^+$–CO$_2$, 10.11), 151 (100), 107 (15.10), 106 (38.10), 78 (12.98). Anal. Calc. C$_{19}$H$_{12}$N$_2$SO$_4$ (332): C, 50.60; H, 3.61; N, 16.86, S, 9.63. Found: C, 49.95; H, 3.54; N, 16.66; S, 9.63.
3-(Aryl/hetaryl)-1-(2-hydroxy benzoic acid)thiobarbituric acid (3a, 3b & 7). A mixture of the appropriate thiourea derivative (0.01 mol) in THF (100 mL) was refluxed with dimethyl malonate (0.01 mol) for 12 h. The obtained solid which separated on cooling was filtered, washed with ethanol, and recrystallized from THF.

3a. Yield 68%; yellow powder, m.p. 240-241°C (decomp); IR (v cm⁻¹) 3420 (OH), 3352 (OH), 3258 (NH₂SO₂), 3074, 3037 (Ar- H), 2936, 2869 (str. CH₃), 1651 (C=O), 1577 (C=N), 1439 (bending CH₂), 1324 (NCSN), 1261 (H-bonding), 1149 (C=S), 841, 820 (Ar-CH). ¹HNMR (δ ppm): 3.17(pyrimidine CH₃), 2.65 (d, 1H, thiazole H-5), 7.5(d, 1H, thiazole H-5), 7.7-8.1(m, 7H, Ar- H), 11.3 (s, 1H, OH), 11.2 (s, 1H, OH). ¹³CNMR (δ ppm): 34.5 (CH₃) 108.4, 138.4, 171.1 (thiazole-C), 107.1, 112.5, 113.8, 120.4, 125.7, 131.9, 134.3, 144.8, 147.6, 159.8, 179.5 (CS), 164.9 (CO). Anal. Calcd: C₇₁H₇₁N₂S₂O₇ (513): C, 49.12; H, 2.92; N, 13.64, S, 12.47. Found: C, 48.85; H, 2.93; N, 13.38; S, 12.21.

3b. Yield 66%; faint yellow; m.p. 214-215°C (decomp); IR (v cm⁻¹) 3410 (OH), 3320 (OH), 3250 (NH₂SO₂), 3060 (Ar- H), 2916, 2899 (str. CH₃), 1661 (C=O), 1587 (C=N), 1488, 1441 (bending CH₂), 1351 (NCSN), 1271 (H-bonding), 1185 (C=S), 821, 808 (Ar-CH). MS(%): 415 (M⁺-CO₂, -C₆H₅O₂) (12.25), 156 (55.13), 151 (100), 108 (13.1). Anal. Calcd: C₂₁H₁₇N₅S₂O₇ (527): C, 50.09; H, 3.22; N, 13.28; S, 12.14. Found: C, 49.69; H, 3.8; N, 13.13; S, 12.06.

7. Yield 65%; yellow powder; m.p. 131-132°C (decomp.); IR (v cm⁻¹) 3305 (OH), 3489 (OH), 3250 (NH₂SO₂), 2977, 2899 (str. CH₃, CH₂), 1650 (C=O), 1616, 1608 (C=C, C=N), 1454 (deform CH₂), 1441 (bending CH₂), 1368 (NCSN), 1279 (H-bonding), 1219 (C=N), 1194 (C=S), 877, 867 (Ar-CH). MS(%): 468 (M⁺ + 21.01), 279 (5.90), 187 (23.11), 151 (100), 84 (1.18). ¹HNMR (δ ppm): 2.2 (s, 3H, Me-C), 3.5 (s, 3H, Me-N), 5.8 (s, 1H, OH), 6.1 (s, 1H, Pyrimidine-H), 6.95-7.9 (m, 8H, aromatic H), 10.6, (s, 1H, OH), 14.2 (s, 1H, pyrimidine OH). ¹³CNMR (δ ppm): 15.3 (Me-C), 35.1 (Me-N), 68.33 (pyrimidine CH=), 105.4 (pyrazolidinone H-4), 129.4 (pyrazolidinone H-3), 107.3, 112.2, 112.9, 113.3, 118.6, 129.1, 131.8, 142.2, 144.5, 159.0 (Ar-C), 160.76 (CO), 164.9 (CO).
164.1 (CO), 174.2 (CO), Anal. Calcd. C_{22}H_{18}N_{3}SO_{6} (466): C, 56.65; H, 3.86; N, 12.01; S, 6.86. Found: C, 56.35; H, 3.79; N, 11.76; S, 6.79.

4-[4,6-Dioxo-3-[4-(pyrimidin-2-ylsulfamoyl)phenyl]-2-thioxocyclohexyl]-2-hydroxybenzoic acid (4). Compound 3 (1 gm) was refluxed with a mixture of trifluoroacetic anhydride (5 mL) and trifluoroacetic acid (5 mL) for 1 h. The precipitated solid, which separated on cooling, was filtered and recrystallized from dioxan as faint yellow needles. Yield 55%; m.p. 211-212°C (decomp); IR(ν cm⁻¹): 3310 w, (OH), 3103 (w, NH), 3035 (Ar–CH), 2877 (aliphatic CH) 1714, 1671 (2C=O), 1604 (C=N), 1525 (C=N), 1499 (deform. CH), 1345 (NCSN), 1319 (NHSO₃), 1280 (C=N), 1181 (C=S), 836, 826, 816 (Ar–CH & hetero–CH). MS(%): 610 (M⁺ + 1, 1.11) 247 (23.27), 169 (35.15), 166 (1.55), 151 (100), 106 (12.25), 79 (76.15), 69 (13.81). \(^1\)HNMR (δ ppm): 4.82 (s, 1H, CH–CO), 6.95 (m, 1H, pyrimidine H-5), 8.43 (dd, 2H, pyrimidine H-4 & H-6), 7.32–7.94 (m, 7H, ArH), 8.23 (s, 1H, of pyrimidine), 11.2 (s, 1H, OH). \(^1\)\(^3\)C NMR (δ ppm): 43.4 (CHCO) 110.3, 157.3, 166.4 (pyrimidine–C), 107.4, 112.3, 113.8, 120.4, 125.6, 131.7, 134.9, 144.0, 148.5, 159.5 (Ar–C),

Scheme 1
Scheme 2

Figure 3: Mass fragmentation pattern of 9a.

Figure 4: Mass fragmentation pattern of compound 13.
2-Hydroxy-4-(Substituted-Ureido- and Thioureido)benzoic Acid (8a-c). A mixture of PAS (0.01 mol) and the appropriate isocyanate or isothiocyanates derivative (0.01 mol) in THF (100 mL) was warmed at 100°C for 2 h, then cooled to room temperature. The precipitate solid obtained was filtered and recrystallized from ethanol.

8a. Yield 90%, faint yellow crystals; m.p. 203-204°C (decomp); IR (v cm⁻¹) 3446 (OH), 3301 (NH), 3010 (Ar–CH), 2900, 2840 (str. CH) 1675 (C=O), 1655 (C=O), 1525 (C=N), 1394 (NCSN), 1286 (H-bonding), 1230 (C–N), 831, 804 (Ar–CH). Anal. Calcd. C₁₅H₁₄N₂O₅ (278): C, 60.43; H, 6.47; N, 10.07. Found: C, 59.79; H, 6.38; N, 9.95.

8b. Yield 88%; orange crystalline, m.p. 164-165°C (decomp); IR (v cm⁻¹) 3399 (OH), 3211 (NH), 3009 (Ar–CH), 1660 (C=O), 1655 (C=O), 1355 (NCSN), 1265 (H-bonding), 1225 (C–N), 1195 (C=S), 830, 810 (Ar–CH), 710 (C=Cl). Anal. Calcd. C₁₅H₁₁N₂ClO₃ (323): C, 52.01; H, 3.40; N, 8.66, S, 9.90, Cl; 11.12. Found: C, 51.88; H, 3.35; N, 8.54; S, 9.83, Cl; 11.00.

8c. Yield 91%; yellowish crystalline; m.p. 161-162°C (decomp); IR (v cm⁻¹) 3674 (b, OH), 3209 (NH), 3004 (Ar–CH), 2900, 2837 (str. CH), 1655 (C=O), 1620 (C=O), 1355 (CSNH), 1584, 1564 (C=C), 1459, 1416 (deform. Me), 1328 (NCSN), 1286 (H-bonding), 1230 (C–N), 1165 (C=S), 1032 (–OMe), 865, 831, 804 (Ar–CH).¹HNMR (δ ppm): 3.38 (s, 3H–OMe), 6.39–7.81 (m, 7H, ArH), 8.30 (s, 1H, OH phenolic), 9.96, 9.91 (each s, NH, NH), 11.5 (s, 1H, OH).¹³C NMR (δ ppm): 111.2, 113.7, 114.4, 117.9, 126.4, 131.5, 133.3, 145.6, 158.2, 159.4, 179.15 (Ar–C) 171.5(CO), 179.8 (CS). Anal. Calcd. C₁₅H₁₄N₂SO₄ (318): C, 56.60; H, 4.40; N, 8.80; S, 10.06. Found: C, 55.98; H, 4.35; N, .8.60; S, 9.94.

4-Dithiocarboxyamino-2-Hydroxy Benzoic Acid (9a). Equimolar mixture of PAS and CS₂ in ethanolic KOH (50 mL, 5%) and DMF (50 mL) was warmed for 30 minutes and then cooled. The reaction mixture was acidified using dilute AcOH. The resulting solid was filtered and recrystallized from methanol as orange needles. Yield 92%; m.p. 141-142°C (decomp). IR (v cm⁻¹) 3492 (OH), 3385 (NH), 2970, 2522 (SH), 1800 (C=O), 1615 (C=N), 1284 (NCSN), 1225 (C–N), 1197, 1106 (C–S), 878, 817 (Ar–CH). MS (%): 230 (M⁺ +1, 0.85) 196 (5.66), 151 (100), 107 (31.12), 92 (15.28).¹HNMR (δ ppm): 5.98 (s, 1H, SH), 6.25–7.44 (m, 3H,ArH), 8.121 (s, 1H, NH), 11.5 (s, 1H, OH).¹³C NMR (δ ppm): 106.5, 113.6, 117.8, 131.7, 144.5, 158.4 (Ar–C), 174.3 (CO), 198.2 (CS). Anal. Calcd. C₁₅H₁₄N₂O₄ (229): C, 41.92; H, 3.05; N, 6.11; S, 27.94. Found: C, 41.72; H, 3.01; N, 6.06; S, 27.67.

4-Trifluorooctylamino-2-hydroxybenzoic acid (9b). A mixture of PAS (0.01 mol) and trifluoroacetic anhydride (0.01 mol) in THF (100 mL) was warmed for 30 min. and cooled. The produced solid was recrystallized from dioxan as faint yellow needles. Yield 88%; m.p. 119-120°C (decomp); IR (v cm⁻¹) 3410 (OH), 3150 (NH), 2710 (OH), 1701, 1660 (2C=O), 1280 (H-bonding) 1260 (C–F), 810, 805 (Ar–CH), 695 (C–F). MS (%): 250 (M⁺ +1, 5.11) 135 (100), 107 (18.05), 93 (38.27). Anal. Calcd. C₁₅H₁₄NF₃O₄ (249): C, 43.37; H, 2.40; N, 5.62; F, 22.89. Found: C, 42.89; H, 2.37; N, 5.49; F, 22.63.

2-Hydroxy-4-(4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (10). A mixture of 9a (0.01 mol) and chloroacetic acid (0.01 mol) in DMF (100 mL) was heated at 100°C for 4 h. After being cooled to room temperature, the reaction mixture was poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from THF as yellow needles. Yield 80%; m.p. >280°C; IR (v cm⁻¹): 3392 (OH), 2975 (str. CH₂), 1724
2-Hydroxy-4-(4-oxo-3-p-methoxyphenylthiazolidin-2-ylideneamino)benzoic acid (11). A mixture of 8c (0.01 mol) and chloroacetic acid (0.01 mol) with anhydrous sodium acetate (10 g) and absolute ethanol (50 mL) was refluxed for 2 h, cooled and then poured on ice. The solid obtained was filtered off and crystallized from ethanol to give 11. Yield 65%, yellowish powder, m.p. 151-152 °C (decomp.); IR (v cm⁻¹): 3300 (OH), 2977-2837 (str. CH₂), 1724 (C=O), 1597 (C=N), 1454 (defrom. CH₂), 1370 (NCS), 1297 (H-bonding), 1145 (C=N), 1140 (C=S), 1075 (C=O–Me), 883, 826 (Ar–CH).¹ HNMR (δ ppm): 3.72 (s, 3H, OCH₃), 4.16 (s, 2H, thiazolidine H-4), 6.55-7.89 (m, 3H, ArH) 10.89 (s, 1H, OH).¹ CNCN (δ ppm): 56.4 (OCH₃), 68.5 (thiazolidinone C-4), 163.6 (thiazolidinone C-2) 109.2, 113.2, 114.5, 116.1, 132.2, 134.5, 150.2, 152.3, 160.4, 172.07 (CO), 194.7 (CO), 171.35, 162.61, 159.37, 156.15, 154.56, 131.38, 129.152, 127.07, 121.80, 114.28, 113.63, 112.20, 111.56, 109.11, 108.35, 104.49. Anal. Calcd. C₁₇H₁₄N₂O₇ (358); C, 56.98; H, 3.91; N, 7.82; S, 8.93. Found: C, 56.48; H, 3.86; N, 7.73; S, 8.48.

3. Results and Discussion

3.1. Chemistry. A series of new 2-hydroxy-4-(substituted thiourido) benzoic acid (2, 6 & 8) were synthesized via the reaction of PAS with the appropriate potassium dithiocarbamates (1, 5) and addition of isocyanate/isothiocyanates to PAS in THF under reflux (Schemes 1 and 2). The IR spectra showed two absorption bands at 3209–3258 cm⁻¹, and 3310–3674 cm⁻¹ for the HN and OH groups, respectively, along with a characteristic thiourea carbolnyl absorption at 1150–1195 cm⁻¹. Their ¹H-NMR spectra exhibited beside the aromatic protons a singlet of one proton intensity at δ 11.35–511.81. The structures of the above compounds were further confirmed from their ¹³C NMR and MS data (Section 2).

Ring closure reactions of compounds 2 and 6 with dimethyl malonate in refluxing THF produced the corresponding 1,3-disubstituted thiobarbituric acids 3 and 7, respectively. Their IR spectra showed two OH absorption bands at 3305–3352 cm⁻¹ and 3410–3489 cm⁻¹, and a thio-carbonyl band at 1149–1194 cm⁻¹ in addition to a carbolnyl absorption at 1650–1661 cm⁻¹. The structures of the above compounds were further confirmed by their ¹H-NMR, ¹³C NMR, and MS data. The fluorination of 3a by boiling with trifluoroacetic anhydride afforded 1,3-disubstituted-5-trifluoroacetyl-thiobarbituric acid 4 (Scheme 1). The IR spectrum showed beside the HN and OH bands at 3103 and 3310, two carbonyl absorption bands at 1671 and 1714 in addition to thio-carbonyl band at 1181 cm⁻¹. The mass fragmentation pattern of compound 4 is shown in Figure 2. The structure of 4 was further confirmed from its ¹H-NMR and ¹³C NMR data.

Careful treatment of PAS with CS₂ (DMF) and trifluoroacetic anhydride in THF afforded 4-dithiocarboxyaminom-2-hydroxybenzoic acid 9a and 2-hydroxy-4-trifluoroacetyl amino benzoic acid 9b, respectively. Moreover, ring closure reaction of 9a by refluxing it with chloroacetic acid in DMF produced 2-hydroxy-4-(4-oxo-2-thioxothiazolidin-3-yl)benzoic acid 10 (Scheme 2). The IR spectrum of 9a showed two absorption bands at 1800 cm⁻¹ and 1284 cm⁻¹ due to the CO and CS groups, respectively, while the IR spectrum of 9b exhibited two carbonyl bands at 1701 cm⁻¹ and 1660 cm⁻¹. The structures of 9a and 9b were further confirmed from their MS analyses which exhibited molecular ion peaks at m/z 230 and m/z 250, respectively (Figure 3).

Refluxing 8c with chloroacetic acid and sodium acetate in ethanol under neutral conditions furnished 2-hydroxy-4-(4-oxo-3-p-methoxyphenylthiazolidin-2-ylideneamino) benzoic acid 11 (Scheme 2). The IR spectrum showed cyclic carbolnyl absorption at 1724 cm⁻¹ as well as OH absorption band at 3300 cm⁻¹. The structure was further supported by its ¹³C NMR data.
Wadher et al. reported the synthesis of 4-thiazolidinone derivatives of \( p \)-Aminosalicylic acid which showed \textit{in vitro} a significant antimicrobial activity [19]. We have further extended this work by refluxing PAS with potassium dithiocarbazinate 12 in EtOH to yield \( N^1 \)-(4'-pyridinol)-\( N^1 \)-(2-hydroxybenzoic acid)thiosemicarbazide 13. The compounds 9 and 13 has analogous mass fragmentation patterns (Figure 4).

The cyclization of 13 in alkaline medium by refluxing it with aqueous \( NaOH \) produced 2-hydroxy-4-(3-(pyridin-4-yl)-5-thioxo-1H,1,2,4-triazol-4(5H)-yl) benzoic acid 14. However, the cyclization in the acidic medium using cold \( H_2SO_4 \) at room temperature, resulted in the formation of 2-hydroxy-4-(5-pyridin-4-yl-1,3,4 thiazadiazol-2-ylamino)benzoic acid 15 (Scheme 3). The structures of compounds 13–15 were characterized by IR, \( ^1H \), and \( ^13C \) NMR as well as by their mass fragmentation patterns.

3.2. Antimycobacterial Activity. All the new compounds obtained were tested for \textit{in vitro} antimycobacteria activity against \textit{M. tuberculosis} H37Rv using the BACTEC 12B medium using a broth microdilution assay, the microplate alamar blue assay (MABA) [20, 21]. Rifampicin was used as the standard (Table 1). The antimycobacterial activities of the synthesized compounds were also investigated against \textit{M. fortuitum} ATCC6841, a rapidly growing strain, by the use of microdilution broth susceptibility methods [22–25]. Lowenstein-Jensen egg Medium and DFM for control purposes were used at 37 C. Of these compounds, the ones which exhibited > 90% inhibition in the primary screen (MIC > 12 \( \mu \)g/mL) were considered for further evaluation in the Level 2 of the screening (Table 2).

Compounds 2–4, 6, 8, 9, and 13 effecting 80–90% inhibition in the primary screen at a concentration of 12.5 \( \mu \)g/mL were resteted at lower concentrations against \textit{M. tuberculosis} H37Rv to determine the actual MIC (Table 2). The compound, 4 and 14 have shown a marked improvement in the antibacterial activity. In this case it has been demonstrated that substituted PAS may be of interest as antibacterial where the incorporation of isoniazid (INH) and \( p \)-Aminosalicylic acid in a simple molecule was aimed to enhance possible antibacterial activity which might arise from each side. Moreover, bulky groups introduced at nitrogen atom of PAS led to a decrease in activity which may be attributed to a steric hindrance and that causes an increased lipophilicity of this system [26–33].

From the preliminary data obtained from the level 1 screening, it was also found that compounds 2, 3, and 6–8 showed moderate activity against \textit{M. fortuitum}. However, compounds 4, 9b, and 13–15 showed weak activity against the same strain.

4. Conclusion

\( p \)-Aminosalicylic acid is an important second-line treatment against mycobacterium as it may prevent the emergence of resistance to other antimycobacterial agents and enhances the efficacy of first-line drug isoniazid. Incorporation of structural features from both structures has seemingly improved the activity profiles in compound, 4 and 14. From the previously data, we can infer that both PAS bearing thiourea, thiobarbituric acids, and mercapto-1,2,4-triazole contributed significantly to exhibit the antibacterial activity. Compounds 4 and 14 were selected for further structure optimization.

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