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 ^{13}C 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]. Amino-salicylic 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, semidugs 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.

Benzofuran salicylic acid (1-A09)

FIGURE 1

2. Experimental

Melting points were determined in an electrothermal Bibby Stuart scientific melting point SMP (US). The IR spectra recorded for KBr discs on a Perkin Elmer spectrum RXI FT-IR systems no. 53529. $^1\text{H}/^{13}\text{C}$ -NMR was determined for solution in deuterated DMSO with a Bruker DPX-400-FT using TMS as an internal standard solvent (chemical shifts in δ , 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-Thioureido) Benzoic Acid (2a, b & 6) and 2-Hydroxy4-[(4-Pyridoyl) 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 ($\nu\text{ cm}^{-1}$) 3410, 3380 (2 OH), 3320, 3210 (NH), 3030 (Ar-H), 1666 (C=O), 1589 (C=N), 1350 (SO_2NH), 1266 (H-bonding), 1201 (C=S), 810, 800 (Ar-CH), $^1\text{HNMR}$ (δ -ppm): 11.38 (s, 1H, OH), 8.80 (s, 1H, NHSO_2), 8.51 (s, 1H, NHCS), 7.81-8.36 (m, 3H pyrimidine-H), 7.2-7.01 (m, 7H, aromatic H) $^{13}\text{CNMR}$ (δ -ppm): 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. Calcd.

TABLE 1: Antimycobacterial activity versus *M. fortuitum* ATCC 6841 and primary antituberculosis activity screening results.

Compd. no.	MIC/ $\mu\text{g mL}^{-1}$ ^a	MIC/ $\mu\text{g mL}^{-1}$ ^b	Inhibition ^c (%)
2b	64	>12.5	85
3b	64	>12.5	87
4	>128	<12.5	95
6	64	>12.5	85
7	64	>12.5	88
8a	64	<12.5	90
9b	>128	<12.5	92
13	>128	<12.5	96
14	>128	>12.5	90
15	>128	>12.5	90

^a MIC affecting *M. fortuitum* ATCC6841.

^b MIC affecting *M. tuberculosis* H₃₇Rv.

^c Reduction of mycobacterial growth using *M. tuberculosis* H₃₇Rv using 12.5 $\mu\text{g/mL}$ concentration of compounds.

$\text{C}_{18}\text{H}_{15}\text{N}_5\text{S}_2\text{O}_5$ (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 ($\nu\text{ cm}^{-1}$) 3424, 3333 (2 OH), 3074, 3035 (Ar-H), 1670 (C=O), 1588, 1572 (2C=N), 1334 (NHSO_2), 1189 (C=S) 818, 795 (Ar-CH). MS (%): 415 ($\text{M}^+ - \text{CO}_2$, 48.13), 371 (18.8), 215 (11.11), 156 (5.51), 151 (100), 92 (48.51). Anal. Calcd. $\text{C}_{19}\text{H}_{17}\text{N}_5\text{S}_2\text{O}_5$ (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 ($\nu\text{ cm}^{-1}$) 3400-3310 (2 OH), 3210 (NH), 3042, 3035 (Ar-H), 2936, 2888 (aliphatic CH), 1760, 1677 (2C=O), 1609 (C-N), 1492, 1412 (deformation CH_3), 1345, 1310 (NCSN), 1281 (H-bonding), 1186 (C=S), 882, 794 (Ar-CH). $^1\text{HNMR}$ (δ -ppm): 2.55 (s, 3H, CH_3), 4.32 (s, 3H, $\text{CH}_3\text{-N}$), 7.65-7.38 (m, 8H, Ar-H), 8.31 (s, 1H, NH), 11.81 (s, 1H, OH). $^{13}\text{CNMR}$ (δ -ppm): 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. Calcd. $\text{C}_{19}\text{H}_{18}\text{N}_4\text{SO}_4$ (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 ($\nu\text{ cm}^{-1}$) 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). $^1\text{HNMR}$ (δ -ppm): 5.85 (s, 1H, OH), 6.4-7.2 (m, 3H of aryl), 7.7, 7.9 (each dd, 2H, 2H of pyridine), 9.2, 8.8, 8.2 (3s, 3H, 3NH), 11.54 (s, 1H, OH). $^{13}\text{CNMR}$ (δ -ppm): 187.6 (CS), 172.1 (CO), 163.5 (CO), 112.3, 114.2, 117.8, 122.4, 131.4, 142.5, 144.8, 150.3, 159.1 (Ar-C); MS(%): 288 ($\text{M}^+ - \text{CO}_2$, 10.11), 151 (100), 107 (15.10), 106 (38.10), 78 (12.98). Anal. Calcd. $\text{C}_{14}\text{H}_{12}\text{N}_4\text{SO}_4$ (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.

FIGURE 2: Mass fragmentation pattern of compound **4**.TABLE 2: Level 2 antituberculosis acidity assay results of **4**, **9b**, **13**, and **14** (Level 2 assay).

Compd. no.	MIC/ $\mu\text{g mL}$	IC ₅₀ ($\mu\text{g mL}$)	SI
4	6.25	>10	1.5
9b	>12.5	—	—
13	>12.5	—	—
14	6.25	10	1.6

The assay was performed using BACTEC in which MIC of RMP was found as 0.125 $\mu\text{g/mL}$.

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 ($\nu \text{ cm}^{-1}$) 3420 (OH), 3352 (OH), 3258 (NHSO₂), 3074, 3037 (Ar–H), 2936, 2869 (str. CH₂) 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 CH₂), 6.5 (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 (CH₂) 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. C₂₁H₁₅N₅S₂O₇ (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 ($\nu \text{ cm}^{-1}$) 3410 (OH), 3320 (OH), 3250 (NHSO₂), 3060 (Ar–H), 2916, 2899 (str. CH₂) 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, –C₃H₂O₂) (12.25), 156 (55.13), 151 (100), 108 (13.1). Anal. Calcd. C₂₂H₁₇N₅S₂O₇ (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 ($\nu \text{ cm}^{-1}$) 3305 (OH), 3489 (OH), 3250 (NHSO₂), 2977, 2899 (str. CH₂, CH₃), 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⁺ + 2 1.01), 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),

SCHEME 1

164.1 (CO), 174.2 (CO). Anal. Calcd. $C_{22}H_{18}N_4SO_6$ (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 (NHSO₂), 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). ¹HNMR (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). ¹³CNMR (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 2

FIGURE 3: Mass fragmentation pattern of **9a**. FIGURE 4: Mass fragmentation pattern of compound **13**.

SCHEME 3

127.9 (CF₃), 168.9, 172.5 (2CO), 204.1 (CO–CF₃), 184 (CS). Anal. Calcd. C₂₃H₁₄N₅S₂F₃O₈ (609): C, 45.32; H, 2.29; N, 11.49; S, 10.50; F, 9.35. Found: C, 44.89; H, 2.26; N, 11.22; S, 10.38; F, 9.25.

2-Hydroxyl-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 (ν 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. C₁₄H₁₈N₂O₄ (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 (ν 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. C₁₄H₁₁N₂SClO₃ (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 (ν 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). ¹³CNMR (δ-ppm): 112.2, 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 (ν cm⁻¹) 3492 (OH), 3385 (NH), 2970, 2522 (SH), 1800 (C=O), 1615 (C=N), 1284 (NCS), 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). ¹³CNMR (δ-ppm): 106.5, 113.6, 117.8, 131.7, 144.5, 158.4 (Ar–C), 174.3 (CO), 198.2 (CS). Anal. Calcd. C₈H₇NS₂O₃ (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-Trifluoroacetyl-amino-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 (ν 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. C₉H₆NF₃O₄ (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(ν cm⁻¹): 3392 (OH), 2975 (str. CH₂), 1724

(C=O), 1607 (C=C), 1492 (deform. CH₂), 1371 (NCS), 1227 (H-bonding), 1160 (C=S), 1078 (C–S–C). MS (%): 270 (M⁺ +1, 1.55), 151 (100), 72 (18.28). Anal. Calcd. C₁₀H₇NS₂O₄ (269): C, 44.60; H, 2.60; N, 5.20; S, 23.79. Found: C, 44.41; H, 2.57; N, 5.19; S, 23.55.

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 (ν cm⁻¹): 3300 (OH), 2977–2837 (str. CH₂), 1724 (C=O), 1597 (C=N), 1454 (deform. CH₂), 1370 (NCS), 1297 (H-bonding), 1145 (C–N), 1140 (C=S), 1075 (C–O–Me), 883, 826 (Ar–CH). ¹H-NMR (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). ¹³C-NMR (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, 135.4, 150.2, 153.2, 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, 111.36, 109.11, 108.35, 104.49. Anal. Calcd. C₁₇H₁₄N₂SO₅ (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.

2-Hydroxy-4-(3-(Pyridin-4-yl)-5-Thioxo-1H-1,2,4-Triazol-4(5H)-yl)benzoic Acid (14). Compound **13** (1 gm) in aqueous NaOH (50 mL 5%) was warmed at 80°C for 30 min, cooled then acidified by dilute hydrochloric acid. The isolated solid was filtered, washed with water, and recrystallized as faint yellow needles from THF. Yield 55%; m.p. 199–200°C (decomp); IR (ν cm⁻¹): 3300–2942 (b, OH, NH), 2624 (SH), 1680 (C=O), 1609, 1572 (C=N), 1346 (NCS), 1282 (H-bonding), 1234 (C–N), 1188 (C–S), 883, (Ar–CH). ¹H-NMR (δ-ppm): 7.34–7.86 (m, 3H, Ar–H), 8.02, 8.76 (2d, each of 2H, pyridine–H), 8.3 (s, 1H, NH), 11.6 (s, 1H, OH). ¹³C-NMR (δ-ppm): 108.5, 111.9, 113.9, 121.5, 126.6, 130.7, 140.3, 143.2, 148.6, 155.9, 157.9, 166.7 (CS), 171.8 (CO). Anal. Calcd. C₁₄H₁₀N₄SO₃ (314): C, 53.50; H, 3.18; N, 17.83; S, 10.19. Found: C, 52.28; H, 3.08; N, 17.63; S, 10.07.

2-Hydroxy-4-(5-pyridin-4-yl-[1,3,4] thiadiazol-2-ylamino)benzoic acid (15). To an ice-cooled stirred solution of conc., H₂SO₄ (1.0 mL) was added in small portions of the thiosemicarbazide derivative **13** (1 g) over a period of 15 min. Stirring was maintained for further 1 h at room temperature, then the reaction mixture was then poured onto ice. The resulting solid product was filtered, washed with water, dried, and recrystallized from dioxan as orange needles. Yield 62%; m.p. 204–205°C (decomp); IR (ν cm⁻¹): 3350–3150 (b, OH, NH), 1652 (C=O), 1508 (C=N), 1350 (NCS), 1258 (H-bonding), 1214 (C–N), 1085 (C–S–C), 868, 850, 779 (hetero/aryl CH). ¹H-NMR (δ-ppm): 7.51, 8.25 (m, 4H, of pyridine), 8.14 (s, 1H, NH), 11.1 (s, 1H, OH). ¹³C-NMR (δ-ppm): 102.4, 107.5, 122.4, 132.1, 136.3, 141.3,

143.2, 150.3, 153.4, 154.1, 159.3, 163.6, 172.3 (CO). Anal. Calcd. C₁₄H₁₀N₄SO₃ (314): C, 53.50; H, 3.18; N, 17.83; S, 10.19. Found: C, 52.95; H, 3.13; N, 17.83; S, 10.08.

3. Results and Discussion

3.1. Chemistry. A series of new 2-hydroxyl-4-(substituted thioureido) 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 carbonyl 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 thiocarbonyl band at 1149–1194 cm⁻¹ in addition to a carbonyl 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 thiocarbonyl 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-dithiocarboxyamino-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 carbonyl 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 *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'-pyridinoyl)- N^4 -(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] thiadiazol-2-ylamino)benzoic acid **15** (Scheme 3). The structures of compounds **13**–**15** were characterized by IR, 1H , and ^{13}C NMR as well as by their mass fragmentation patterns.

3.2. Antimycobacterial Activity. All the new compounds obtained were tested for *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv using the BACTEC 12 β medium using a broth microdilution assay, the microplate alamar blue assay (MABA) [20, 21]. Rifampicin was used as the standard (Table 1). The antitubercular activities of the synthesized compounds were also investigated against *M. fortuitum* ATCC6841, a rapidly growing strain, by the use of microdilution broth susceptibility methods [22–25]. Lowenstein-Jensen egg Medium and DMF 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 retested at lower concentrations against *M. tuberculosis* H37Rv to determine the actual MIC (Table 2). The compound, **4** and **14** have shown a marked improvement in the antitubercular 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 antitubercular 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 *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 antitubercular activity. Compounds **4** and **14** were selected for further structure optimization.

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