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Research Article

Synthesis of New Fluorine Substituted Heterocyclic Nitrogen Systems Derived from *p*-Aminosalicylic Acid as Antimycobacterial Agents

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Some new fluorine substituted heterocyclic nitrogen systems **2–17** have been synthesized from ring closure reactions of substituted *p*-amino salicylic acids (PAS). The Schiffs base of PAS was cyclized with chloroacetyl chloride and mercaptoacetic acid to give azetidinone **2**, thiazolidinone **3**, and spiro-fluoroindolothiazoline-dione **10**. However, PAS when reacted directly with 4-fluorobenzoyl chloride and 5-oxazolinone yielded derivatives **4**, **5**, and **7**. Aminomethylation of PAS using formaldehyde and piperidine or piperazine formed N-alkyl and N,N′-dialkyl derivatives (**11** and **12** respectively) upon fluorinated benzoylation gave compounds **13** and **14**. Similarly, treatment of PAS with thiosemicarbazide **15** and subsequent cyclization with diethyl oxalate yielded the fluorinated heterocycle **17**. The structures of the fluorinated heterocyclic systems have been established on the basis of elemental analysis, ^{1}H NMR, ^{13}C NMR, and MS spectral data. Some of the targets exhibited a high inhibition towards *Mycobacterium* strain with favorable log *P* values.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. After AIDS, tuberculosis is the second leading cause of death from an infectious disease worldwide [1–5]. The frequent coinfection of TB in HIV patients further complicates the selection of an appropriate treatment regimen. During, recent years, *Mycobacterium tuberculosis* has developed increased resistance against drugs. The multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of tuberculosis are considered as some of the most challenging threats to global health [6, 7]. Medicinal researchers are continuing all over the world in order to have a safe and effective therapeutic strategy against these resistant strains.

The treatment involves the administration of multiple drugs because it is clear that monotherapy leads to the development of resistance. Aminosalicylic acid (PAS) which was introduced as an antitubercular medicine in 1948 is being used in combination with the second line therapeutic regimen against multidrug-resistant and extensively drug-resistant strains [8]. In a recent study a salicylic acid analog, benzofuran salicylic acid (1-A09; Figure 1), has been found to show *Mycobacterium* protein tyrosine phosphatase B inhibiting activity [9]. 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.

Fluorine is a well-known bioisostere in various organofluorine compounds as antimycobacterial agents [10]. The introduction of fluorine has already shown to modulate the stereoelectronic parameters of organic molecules [11, 12]. Substitution of fluorine into a potential drug molecule not only alters the electronic environment, but also influences the pK_a-value of neighboring Bronsted acid/base centers, polarity, and the influence on lipophilicity as expressed by the distribution coefficient. The introduction of fluorine substituent in bioactive molecules can often improve their pharmacological

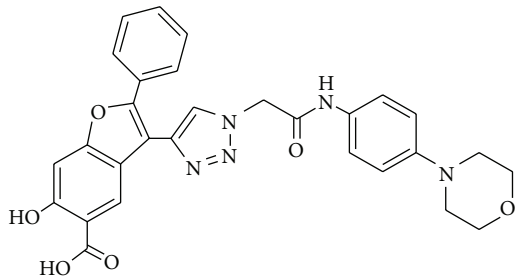


Figure 1: Benzofuran salicylic acid (1-A09).

properties as increased membrane permeability, enhanced hydrophobic binding, and stability against metabolic transformation. Furthermore, it has also been shown that selective organofluorine interactions with protein residues can be used to substantially enhance protein-ligand binding affinity and selectivity [13]. In an extension of our previous study, in the area of synthesis of bioactive compounds for the treatment of infectious diseases [14–20], the present work aims at the synthesis of some new fluorinated heterocyclic systems incorporating PAS as anti-*Mycobacterium* agents.

2. Experimental

Melting points were determined in an electrothermal Bibby Stuart Scientific Melting Point SMP (US). The IR spectra were recorded using KBr discs on a Perkin Elmer Spectrum RXI FT-IR systems number 53529. ^{1}H/^{13}C-NMR was determined in DMSO-d_6 solution using Bruker NMR Advance DPX 600-FT and TMS as an internal standard (Chemical shifts in δ, ppm). Mass spectra were measured on a GCMS-Q 1000-Ex spectrometer. Microanalyses (C, H, N, S, F, and Cl) were performed by the Microanalyses Centre of Cairo University, Egypt.

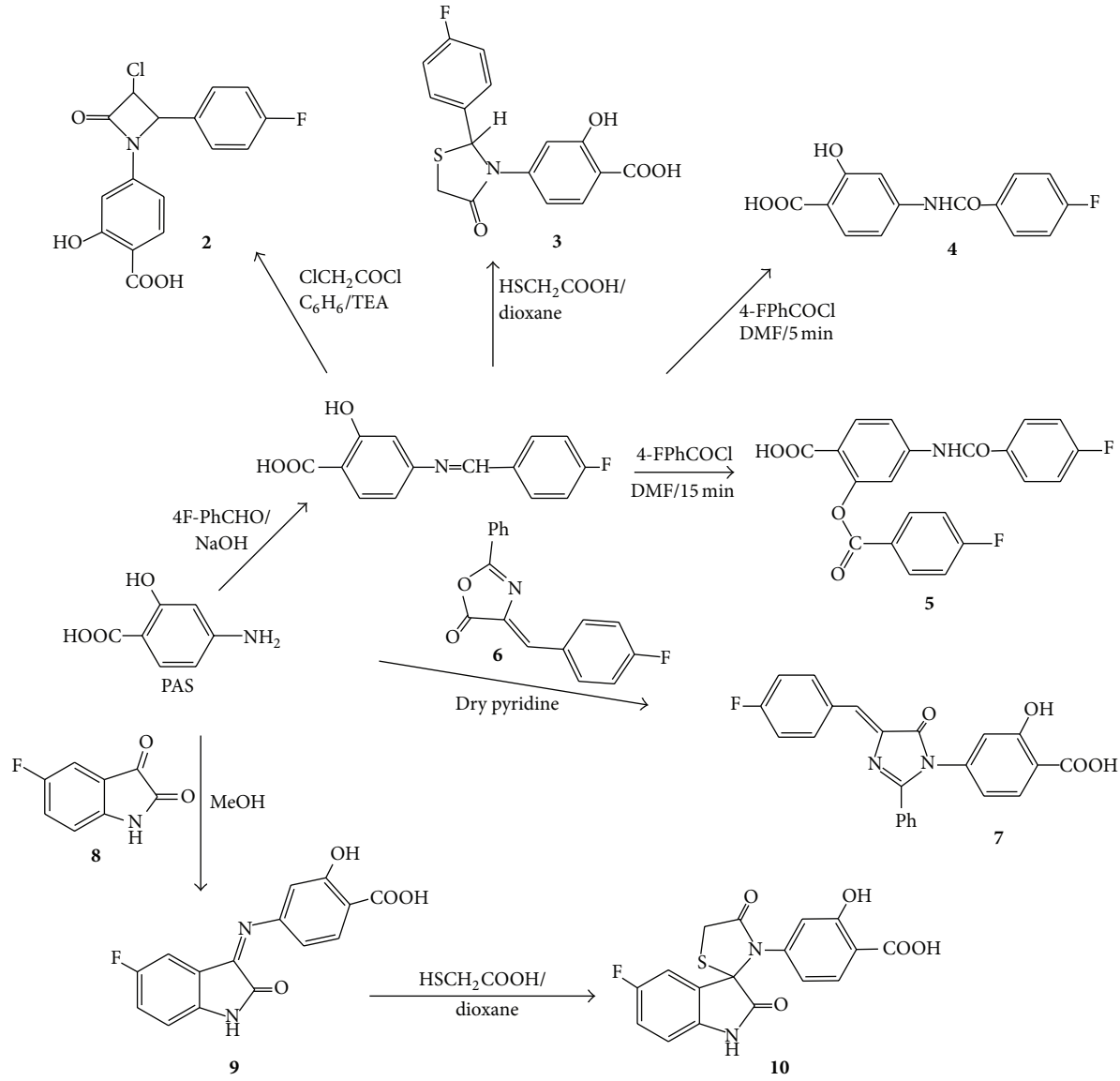
*4-[(4-Fluorobenzylidene)amino]-2-hydroxybenzoic Acid (**1**).* PAS (1.53 g, 0.01 mol) in MeOH (50 mL) and *p*-fluorobenzaldehyde (1.23 g, 0.01 mol) were stirred at room temperature for 24 h (Scheme 1). The precipitate obtained was filtered and crystallized from methanol to give **1** as reddish brown crystals m.p. 273–275°C (decomp.). Yield: 85%. IR (ν cm^{-1}): 3383 (free OH of COOH), 3043 (Ar–CH, str.), 1688 (C=O, COOH), 1602 (C=N), 1427 (aliph. CH), 1238 (C–F); ^{1}H NMR (600 MHz, DMSO) δ ppm: 7.11–8.01 (m, 7H, ArH), 8.30 (s, 1H, CH=N), 5.45 (s, 1H, OH), 11.02 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 109.8, 114.8, 115.4, 116.6, 130.8, 132.1, 133.2, 159.7, 163.5, 165.2 (Ar–C), 160.2 (HC=N), 171.6 (CO). MS: *m/z* (relative intensity) 259.1 (M^+, 12), 260 (M^+ +1, 25). Anal. Calcd. for $C_{14}H_{10}FNO_3$ (259.2): C, 64.86; H, 3.98; N, 5.40; F, 7.33. Found: C, 64.73; H, 3.89; N, 5.38; F, 7.22.

*4-[3-Chloro-2-(4-fluorophenyl)-4-oxo-azetidin-1-yl]-2-hydroxybenzoic Acid (**2**).* To a mixture of PAS (1.52 g, 0.01 mol) in dioxane (10 mL) triethylamine (0.025 mL), was added chloroacetyl chloride (1.35 g, 0.012 mol) dropwise at 10°C. The reaction mixture was stirred for 6 h then poured into crushed ice. The solid separated was dried and recrystallized from dioxane, to give **2** as deep brown powder. m.p. 177–178°C (decomp.). Yield: 55%. IR (ν cm^{-1}): 3268 (free OH of COOH), 3020 (Ar–CH, str.), 2948 (aliph. CH str.), 1777 (C=O of azetidinone), 1669 (C=O of COOH), 1541 (CH), 1243 (C–F), 731 (C–Cl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 5.23 (d, 1H, H-2, J = 9.0 Hz), 5.51 (d, 1H, H-3, J = 9.0 Hz), 7.08–7.96 (m, 7H, ArH), 5.68 (s, 1H, OH), 10.62 (s, 1H, COOH). ^{13}C NMR δ ppm: 62.7 (C-3), 68.2 (C-2), 106.3, 113.5, 114.4, 115.6, 128.5, 131.3, 139.4, 147.9, 161.2, 164.6 (ArC), 162.2 (CO), 170.8 (CO). MS: *m/z* (relative intensity) 235.0 (M^+, 10). Anal. Calcd. for $C_{16}H_{11}ClFNO_4$ (335.7): C, 57.24; H, 3.30; N, 4.16; Cl, 10.56; F, 5.66. Found: C, 57.12; H, 3.11; N, 4.23; Cl, 10.66; F, 5.51.

*4-[2-(4-Fluorophenyl)-4-oxo-thiazolidin-3-yl]-2-hydroxybenzoic Acid (**3**).* To a solution of PAS (1.53 g, 0.01 mol) in dry dioxane (10 mL), a solution of mercaptoacetic acid (3.5 mL, 0.05 mol) in dry dioxane (10 mL) was added followed by a catalytic amount of anhydrous zinc chloride (0.1 g), and the reaction mixture was refluxed for 8 h. The resulting mixture was evaporated at reduced pressure. The residue was treated with a solution of sodium bicarbonate to remove excess of mercaptoacetic acid. The solid obtained was recrystallized from ethanol to give **3** as reddish orange crystals, m.p. 198–200°C (decomp.). Yield: 60%. IR (ν cm^{-1}): 3440 (free OH, str.), 3063 (Ar–CH, str.), 2880 (aliph. CH str.), 1700 (C=O of thiazole), 1672 (C=O of COOH), 1601 (C–N), 1421 (CH_2), 1253 (C–F), 1158 (C–S), 824 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 3.82 (d, J = 12.0 Hz, 1H, C–H, β-H), 3.92 (d, J = 12.0 Hz, 1H, C–H, α-H), 6.23 (s, 1H, H-2), 6.99–8.54 (m, 7H, ArH), 5.60 (s, 1H, OH), 11.12 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 33.9 (C-5), 72.3 (C-2), 106.2, 113.1, 114.3, 115.6, 130.4, 132.0, 135.1, 148.3, 161.7, 164.5 (ArC), 171.2 (CO), 172.1 (CO). MS: *m/z* (relative intensity) 233.0 (M^+, 18). Anal. Calcd. for $C_{16}H_{12}FNO_4S$ (333.3): C, 57.65; H, 3.60; N, 4.20; F, 5.70; S, 9.60. Found: C, 57.55; H, 3.49; N, 4.26; F, 5.55; S, 9.49.

*4-(4-Fluorobenzoylamino)-2-hydroxybenzoic Acid (**4**).* To a mixture of PAS (1.53 g, 0.01 mol) in DMF (20 mL), 4-fluorobenzoyl chloride (1.53 g, 0.01 mol) was added dropwise. The reaction mixture was warmed for 5 min, cooled, and poured onto ice. The solid thus obtained was filtered and recrystallized from dioxane to give **4** as reddish orange powder. m.p. 210–211°C. Yield: 70%. IR (ν cm^{-1}): 3440 (free OH, COOH), 3180 (NH), 3060 (Ar–CH, str.), 1670 (C=O of COOH), 1599 (C=O of CONH), 1506 (C–N), 1227 (C–F), 848 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 7.42–8.16 (m, 7H, ArH), 8.82 (s, 1H, NH), 5.72 (s, 1H, phenolics OH), 10.43 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 107.2, 113.4, 114.5, 115.2, 130.2, 131.7, 135.4, 147.8, 161.3, 164.2 (ArC), 165.8 (CO), 172.1 (CO). MS: *m/z* (relative intensity) 275.0 (M^+, 12). Anal. Calcd. for $C_{14}H_{10}FNO_4$ (275.2): C, 61.09; H, 3.63; N, 5.09; F, 6.90. Found: C, 60.98; H, 3.51; N, 4.99; F, 6.78.

*4-(4-Fluorobenzoylamino)-2-(4-fluorobenzoyloxy)benzoic Acid (**5**).* To a mixture of PAS (1.53 g, 0.01 mol) in DMF (20 mL), 4-fluorobenzoyl chloride (3.1 g, 0.2 mol) was added dropwise then boiled for 15 min. The reaction mixture was



Scheme 1: Synthesis of *p*-aminosalicylic acid derivatives **1–5**, **7**, **9**, and **10**.

cooled and poured onto ice. The precipitated solid was filtered and recrystallized from THF to give **5** as yellow crystals m.p. 228–230°C. Yield: 60%. IR (ν cm^{-1}): 3405 (free OH, COOH), 3180 (NH), 3040 (Ar–CH, str.), 1762 (ester C=O) 1668 (C=O of COOH), 1639 (C=O of CONH), 1596 (C=C), 1239 (C–F), 1085 (C–O–C) 849, 819 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 7.38–8.26 (m, 11H, ArH), 9.12 (s, 1H, NH), 10.78 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 111.7, 114.9, 115.4, 118.2, 119.4, 125.3, 129.2, 129.7, 130.6, 131.8, 143.4, 154.3, 163.9, 167.4 (ArC), 164.2 (CO), 165.2 (CO), 168.8 (CO) MS: *m/z* (relative intensity) 397.1 (M^+, 9). Anal. Calcd. for $C_{21}H_{13}F_2NO_5$ (397.3): C, 63.48; H, 3.30; N, 3.52; F, 9.57; found: C, 63.56; H, 3.11; N, 3.44; F, 9.37.

4-[4-(4-Fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazol-1-yl]-2-hydroxybenzoic Acid (7). An equimolar mixture of PAS (1.53 g, 0.01 mol) and 4-(4-fluorobenzylidene)-2-phenyloxazol-5(4H)-one (**6**; 2.67 g, 0.01 mol) in dry pyridine (20 mL) was refluxed for 5 h. The reaction mixture was cooled and then neutralized with acetic acid. The produced solid was filtered, washed with cold water, then recrystallized from THF to give **7** as yellow crystals, m.p. 146–148°C (decomp.). Yield: 65%. IR (ν cm^{-1}): 3400–3300 (b, free OH, COOH), 3068 (Ar–CH, str.), 2880 (aliph. –CH str.) 1690 (C=O of imidazole), 1645 (C=O, COOH), 1601 (C=C), 1507–1495 (CH), 1227 (C–F), 833 (*p*-substituted phenyl). ^{1}HNMR (600 MHz, DMSO) δ ppm: 7.12–8.06 (m, 13H, ArH), 7.85 (s, 1H, *H*-C=), 5.48 (s, 1H, OH), 11.20 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 106.3, 113.4, 114.4, 115.6, 117.2, 128.3, 128.7, 130.2, 130.3, 130.6, 131.1, 131.6, 135.3, 139.3, 157.9, 162.2, 164 (ArC), 169.4 (CO), 171.8 (CO). MS: *m/z* (relative intensity) 402.1 (M^+, 16). Anal. Calcd. for $C_{23}H_{15}FN_2O_4$

(402.4): C, 68.65; H, 3.76; N, 6.96; F, 4.72. Found: C, 68.50; H, 3.65; N, 6.76; F, 4.59.

*4-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylideneamino)-2-hydroxybenzoic Acid (**9**).* A mixture of PAS (1.53 g, 0.01 mol) and 5-fluoroisatin **8** (1.50 g, 0.01 mol) in methanol (20 mL) was heated on a water bath for 30 min. The reaction mixture was removed from water bath and allowed to acquire room temperature. The solid thus obtained was filtered and recrystallized from MeOH to give **9** as orange crystals, m.p. 230–231°C (decomp.). Yield: 80%. IR (ν cm^{-1}): 3550–3198 (b, OH, NH), 1714 (C=O of COOH), 1624 (CONH), 1256 (C–F), 899 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 6.98–8.12 (m, 6H, ArH), 8.12 (s, 1H, NH), 5.62 (s, 1H, OH), 10.74 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 109.7, 110.3, 112.5, 114.6, 116.4, 116.8, 117.9, 133.4, 154.2, 158.4, 159.6, 163.3 (ArC), 164.2 (C=N), 161.9 (CO), 172.3 (CO). MS: *m/z* (relative intensity) 300.1 (M$^+$, 22). Anal. Calcd. for $C_{15}H_9FN_2O_4$ (300.2): C, 60.00; H, 3.02; N, 9.33; F, 6.33. Found: C, 59.91; H, 2.95; N, 9.08; F, 6.18.

*3′-(3-Hydroxy-4-carboxyphenyl-1-yl)spiro[5-fluoro-3H-indole-2,3′-thiazolidine]-2-(1H)-4′-(5′H)-dione (**10**).* A mixture of **9** (3 g, 0.01 mol) and thioglycolic acid (1.4 mL, 0.02 mol) in dry dioxane (100 mL) was refluxed for 8 h. The reaction mixture was cooled and poured onto ice. The solid thus produced was filtered and recrystallized from ethanol to give **10** as yellow crystals, m.p. 198–200°C (decomp.). Yield: 65%. IR (ν cm^{-1}): 3450–3269 (b, OH, NH), 2890 (aliph. –CH str.) 1681, 1667, 1612 (2C=O, CONH), 1612 (N–C), 1485 (CH_2), 1286 (C–F), 1189 (C–S), 814 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 3.84 (d, J = 12.4 Hz, 1H, C–H, β-H), 3.95 (d, J = 12.4 Hz, 1H, C–H, α-H), 6.98–8.12 (m, 6H, ArH), 8.12 (s, 1H, NH), 5.38 (s, 1H, OH), 10.74 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 30.1 (C-5), 86.7 (S–C–N), 106.2, 111.2, 113.8, 114.1, 114.6, 116.7, 129.4, 132.2, 136.6, 148.2, 159.2, 163.9 (ArC), 168.4 (CO), 171.2 (CO), 171.9 (CO). MS: *m/z* (relative intensity) 376 (M$^+$ + 2, 1.75). Anal. Calcd. for $C_{17}H_{11}FN_2O_5S$ (374.3): C, 54.54; H, 2.94; N, 7.48; S, 8.55; F, 5.08. Found: C, 54.33; H, 2.88; N, 7.39; S, 8.39; F, 5.00.

*2-Hydroxy-4-[(piperidin-1-ylmethyl)amino]benzoic Acid (**11**).* To a solution of PAS (1.53 g, 0.01 mol) in MeOH (20 mL), piperidine (0.85 g, 0.01 mol) and formaldehyde (37%, 2 mL) were added. The reaction mixture was stirred at room temperature for 5 h. To this mixture an excess amount of distilled water was added and the mixture was left overnight. The resulting solid was filtered and recrystallized from methanol to give **11**, as faint yellow crystals, m.p. 278–280°C (decomp.). Yield: 85%. IR (ν cm^{-1}): 3345 (free, OH, COOH), 3210 (NH) 2880 (aliph. C–H str.) 1671, (C=O, COOH), 1576 (C=C), 1487, 1433 (CH_2). ^{1}H NMR (600 MHz, DMSO) δ ppm: 1.34–1.73 (m, 6H, piperidine H-3,4,5), 2.65–2.81 (m, 4H, piperidine H-2,6), 4.12 (s, 2H, CH_2), 6.23 (s, 1H, NH), 6.23–7.78 (m, 3H, ArH), 5.49 (s, 1H, OH), 10.91 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 24.6, 25.8, 54.3 (piperidine C), 72.5 (CH_2), 98.4, 104.8, 106.4, 132.3, 153.9, 165.7 (Ar–C), 172.4 (CO). MS: *m/z* (relative intensity) 250.1 (M$^+$, 14). Anal. Calcd. for $C_{13}H_{18}N_2O_3$ (250.3): C, 62.38; H, 7.25; N, 11.19. Found: C, 62.19; H, 7.14; N, 10.99.

*1,4-Di[(4-methylamino-2-hydroxybenzoic acid)]piperazine (**12**).* To a solution of PAS (3.06 g, 0.02 mol) in MeOH (50 mL), piperazine (0.86 g, 0.01 mol) and formaldehyde (37%, 4 mL) were added. The reaction mixture was stirred at room temperature for 12 h. To the resulting reaction mixture crushed ice was added. The precipitated solid was filtered and recrystallized from ethanol to give **12** as yellow crystals, m.p. 300–302°C (decomp.). Yield: 89%. IR (ν cm^{-1}): 3450–3180 (b, OH, NH), 3210 (NH), 3040 (Ar–CH str.), 2936 (C–H str.), 2795 (C–H str.), 1662 (C=O, COOH), 1411 (CH_2), 1282 (C–N), 831 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 2.48 (s, 8H, piperazine H), 5.2 (s, 2H, CH_2), 5.98 (s, 1H, NH), 6.36–7.88 (m, 6H, ArH), 5.35 (s, 1H, OH), 10.83 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 52.4 (piperazine C), 75.1 (CH_2), 99.2, 104.3, 106.3, 132.6, 154.4, 165.5 (ArC), 171.6 (CO). MS: *m/z* (relative intensity) 417 (M$^+$ +1, 11). Anal. Calcd. for $C_{20}H_{24}N_4O_6$ (416.4): C, 57.69; H, 5.81; N, 13.46. Found: C, 57.72; H, 5.78; N, 13.37.

*4-[(4-Fluorobenzoyl)piperidin-1-ylmethylamino]-2-hydroxy Benzoic Acid (**13**).* To a solution of **11** (2.5 g 0.01 mol) in dry pyridine (20 mL), *p*-fluorobenzoyl chloride (153, 0.01 mol) was added drop-wise. The reaction mixture was refluxed for 1 h cooled, and then poured onto ice. The solid produced was filtered and recrystallized from THF to give **13** as faint yellow crystals, m.p. 209–210°C (decomp.). Yield: 78%. IR (ν cm^{-1}): 3352 (free OH, COOH), 3180 (NH), 3040 (Ar–CH str.) 2936 (asymmetric C–H str.), 1700, 1670, (2C=O), 1603 C=C, 1488, 1444 (CH_2), 1232 (C–F), 852 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 1.52–1.63 (m, 6H, piperidine H-3,4,5), 2.55–2.61 (m, 4H, piperidine H-2,6), 4.72 (s, 1H, N–CH–N), 5.98 (s, 1H, NH), 7.48–9.02 (m, 7H, ArH), 5.56, (s, 1H, OH), 11.13 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 24.5, 25.8, 51.6, (piperidine C), 77.3 (CH_2), 106.3, 113.7, 114.1, 115.8, 129.4, 131.2, 132.1, 145.4, 164.7, 166.0 (ArC), 171.5 (CO), 195.5 (CO). MS: *m/z* (relative intensity) 372.1 (M$^+$, 15). Anal. Calcd. for $C_{20}H_{21}FN_2O_4$ (372.4): C, 64.51; H, 5.68; N, 7.52, F, 5.10. Found: C, 64.41; H, 5.53; N, 7.52, F, 5.01.

*1,4-Di{4-[(4-fluorobenzoyl)methylamino]-2-hydroxybenzoic acid}]piperazine (**14**).* To a solution of **12** (4.16 g, 0.01 mol) in dry pyridine (20 mL), *p*-fluorobenzoyl chloride (3.1 g 0.02 mol) was added drop-wise. The reaction mixture was refluxed for 2 h, cooled and then poured onto ice. The solid produced was filtered and recrystallized from dioxane to give **14** as yellow crystals, m.p. 228–230°C. IR (ν cm^{-1}): 3480 (free, OH, COOH), 3150, 3130 (2NH), 2851 (C–H str.), 1710–1673 (4C=O), 1599 (C=C), 1508, 1424 (CH_2), 1220 (C–F), 847 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 2.67 (s, 8H, piperazine H), 5.26 (s, 1H, N–CH–N), 6.76 (s, 1H, NH), 7.43–7.87 (m, 14H, ArH), 5.62 (s, 1H, OH), 11.02 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 49.8 (piperazine C), 99.8 (HC=N), 106.2, 113.6, 114.2, 115.8, 129.3, 131.3, 131.9, 145.9, 164.7, 166.2 (ArC), 171.7 (CO), 196.2 (CO). MS: *m/z* (relative intensity) 660.2 (M$^+$, 12). Anal. Calcd. for

$C_{34}H_{30}F_2N_4O_8$ (660.6): C, 61.81; H, 4.58; N, 8.48, F, 5.75. Found: C, 61.71; H, 4.44; N, 8.39, F, 5.55.

*1-(2-Hydroxybenzoic acid-4-yl)-4-(4′-fluorophenyl)thiosemicarbazide (**16**).* A mixture of PAS (1.53 g, 0.01 mol) and 4-(4′-fluorophenyl)thiosemicarbazide **15** (1.8 g, 0.01 mol) in ethanol (50 mL) was refluxed for 1 h. The resulting reaction mixture was cooled to give a brownish yellow solid. The solid was filtered and recrystallized from ethanol to give **16** as yellow crystals, m.p. 162–164°C, (decomp.). Yield: 65%. IR (ν cm^{-1}): 3450 (NH), 3040 (Ar–CH str.), 1674, (C=O, COOH), 1601 (C=C), 1509 (N–N), 1226 (C–F), 1156 (C–S) 849 (*p*-substituted phenyl). ^{1}H NMR (600 MHz, DMSO) δ ppm: 4.22 (s, 1H, NNHCS), 5.13 (s, 1H, CSNH), 5.18 (s, 1H, NHCS), 6.76 (s, 1H, NH), 6.62–7.89 (m, 7H, ArH), 5.58 (s, 1H, OH), 11.16 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 97.7, 106.2, 108.8, 115.2, 131.0, 132.3, 134.1, 157.6, 164.7, 166.2 (ArC), 172.4 (CO), 181.9 (CS). MS: *m/z* (relative intensity) 321.1 (M^+, 13.6). Anal. Calcd. for $C_{14}H_{12}FN_3O_3S$ (321.3): C, 52.33; H, 3.73; N, 13.08, S, 9.96; F, 5.91. Found: C, 52.26; H, 3.59; N, 12.88; S, 9.69; F, 5.69.

*4-[4-(4-Fluorophenyl)-5,6-dioxo-3-thioxo[1,2,4]triazian-1-yl]-2-hydroxybenzoic Acid (**17**).* Equimolar amounts of **16** and diethyl oxalate in THF (100 mL) were heated under reflux for 4 h. The reaction mixture was cooled to give a white solid which was filtered and recrystallized from ethanol-water to give **17** as white crystals, m.p. 280–282°C (decomp.). Yield: 66%. IR (ν cm^{-1}): 3450 (OH), 3210, 3180 (NH) 3060 (Ar–CH str.), 1640, 1663 (C=O), 1317 (NCSN), 779 (C–F). ^{1}H NMR (600 MHz, DMSO) δ ppm: 5.76 (s, 1H, NH), 6.56–8.53 (m, 7H, ArH), 5.53 (s, 1H, OH), 10.92 (s, 1H, COOH). ^{13}C NMR (600 MHz, DMSO) δ ppm: 98.3, 106.1, 108.8, 115.8, 130.3, 132.4, 133.9, 143.0, 162.2, 165.4 (ArC), 155.8 (CO), 157.5 (CO), 171.8 (CO), 182.3 (CS). MS: *m/z* (relative intensity) 377 (M^+ + 2, 3.9). Anal. Calcd. for $C_{16}H_{10}FN_3O_5S$ (375.3): C, 51.20; H, 2.69; N, 11.20, S, 8.53; F, 5.06. Found: C, 51.13; H, 2.65; N, 11.33; S, 8.41; F, 4.89.

Antimycobacterial Activity. The antimycobacterial activity was carried out in National Institute of Allergy and Infection Disease Southern Research Institute, GWL Hansen's Disease Center, Colorado State University, Birmingham, AL, USA. All the new compounds obtained were tested for *in vitro* anti-tuberculosis activity against *M. tuberculosis* H37Rv using the BACTEC 12β medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [21, 22]. Rifampicin was used as the standard (Table 1). Of these compounds, the ones which exhibited >90% inhibition in the primary screen (MIC < 6.25 μg/mL) were considered at lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual MIC, using MABA in the level 2 of the screening (Table 2). Rifampin (RMP) was used as the reference compound (RMP MIC = 0.015–0.125 mg/mL).

Table 1: Results of the primary antituberculosis screening of compounds **1–17**.

Compound	MIC (μg/mL)[a]	GI (%)[b]
1	<6.25	95
2	<6.25	96
3	<6.25	98
4	<6.25	98
5	<6.25	98
7	<6.25	94
9	<6.25	94
10	<6.25	98
11	<6.25	92
12	<6.25	92
13	<6.25	98
14	<6.25	95
16	<6.25	96
17	<6.25	100

[a]MIC (minimum inhibitory concentration) of Rifampicin: 0.125–0.25 μg/mL versus *M. tuberculosis* H37Rv.
[b]Growth inhibition of virulent H37Rv strains of *M. tuberculosis*.

Table 2: Results of second level antituberculosis assay.

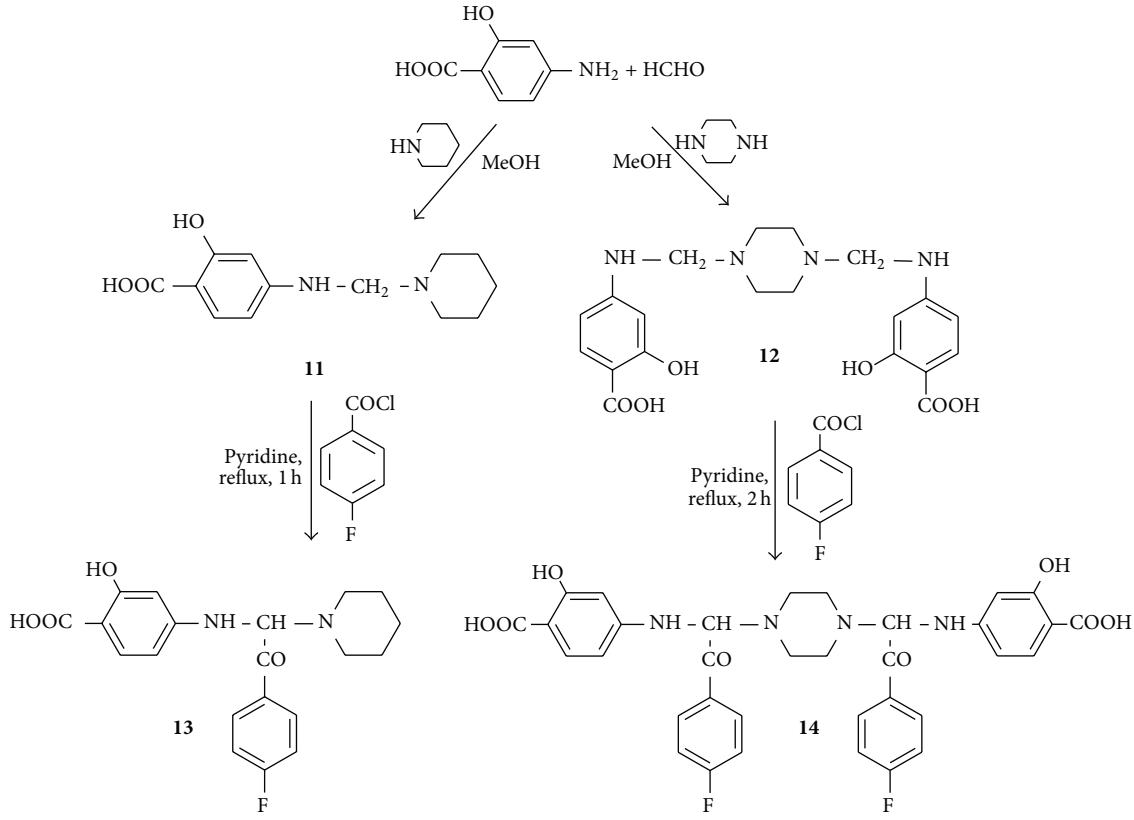
Compound	MIC (μg/mL)[a]	IC_{50} (μg/mL)[a]	SI (IC_{50}/MIC)	log P[b]
3	6.25	29	4.64	2.82
4	6.25	27	4.32	2.17
5	4.25	14	3.29	4.2
10	6.25	27	4.32	1.93
13	3.78	12	3.17	3.37
17	6.25	30	4.80	2.12

[a]Actual minimum inhibitory concentration (MABA assay).
[b]Calculated log P.

3. Results and Discussion

3.1. Chemistry. The condensation of *p*-aminosalicylic acid (PAS) with *p*-fluorobenzaldehyde in methanol produced the Schiff base **1**. The IR spectra showed two absorption bands at 3383 cm^{-1} and 1668 cm^{-1} for the OH and CO groups, respectively along with a characteristic C=N absorption at 1602 cm^{-1}. Their ^{1}H NMR spectra exhibited beside the aromatic protons a singlet of one proton intensity at δ 8.30 for the CH=N as well as two exchangeable singlets at δ 11.02 and δ 5.45 for the COOH and the phenolic OH, respectively. The structure of the above compound was further confirmed from its ^{13}C NMR and MS data (experimental section).

Similarly, cycloaddition [23] of compound **1** with chloroacetyl chloride in dry benzene afforded the azetidinone **2**, while with thioglycolic acid in dry dioxane it afforded the 4-[2-(4-fluorophenyl)-4-oxo-thiazolidin-3-yl]-2-hydroxybenzoic acid **3**. The IR spectra of **2** and **3** showed two carbonyl absorptions at 1700–1777 cm^{-1} and 1669–1672 cm^{-1} for the azetidinone and COOH groups, respectively, as well as OH bands in the regions 3268–3383 cm^{-1}. The ^{1}H NMR spectra of **2** exhibited beside the aromatic protons at δ 7.08–7.96 two doublets at δ 5.23 and 5.51 (J = 9.0 Hz) for H-2 and H-3 protons, respectively. On the other hand the thiazolidine derivative **3** showed beside the seven aromatic protons at



Scheme 2

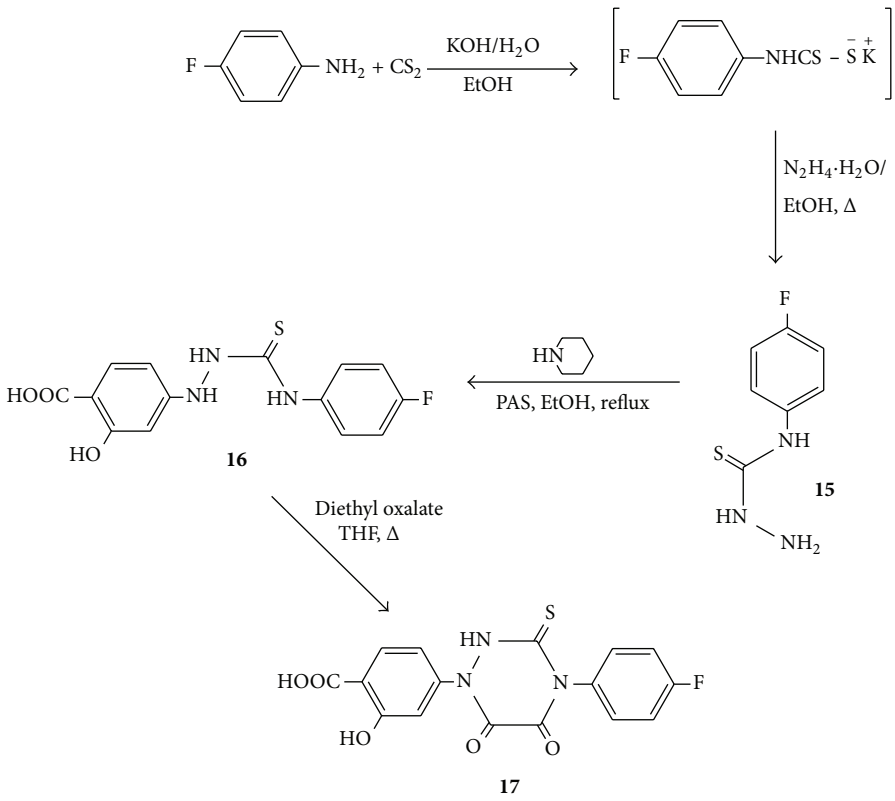
δ 6.99–8.54 two doublets at δ 3.82 and 3.92 for the β and α proton, respectively, of C-5 methylene of the thiazolidine ring. The C2 proton of the same ring appears at δ 6.23 as a singlet. The ^{13}C NMR of **2** exhibited beside the aromatic carbons two signals at δ 62.7 and 68.2 for C-3 and C-2, respectively, of the azetidinone moiety, while compound **3** showed two signals at δ 33.9 and 72.3 for C-5 and C-2, respectively. The structures were further confirmed from MS data.

However, fluorination of PAS *via* warming with 4-fluorobenzoyl chloride in DMF yielded the 4-(4-fluorobenzoylamino)-2-hydroxybenzoic acid **4** or 4-(4-fluorobenzoylamino)-2-(4-fluorobenzoyl-oxy)benzoic acid **5** depending on the time the reaction has been allowed to go (Scheme 1). The IR spectra of **4** and **5** showed two carbonyl absorption at 1700–1777 cm^{-1} and 1668–1673 cm^{-1} for the azetidinone and COOH carbonyl groups, respectively, as well as OH bands in the regions 3268–3383 cm^{-1}. Compound **4** also exhibited an absorption band at 3440 cm^{-1} which is attributed to a free OH group. However, compound **5** exhibited a third carbonyl at 1762 cm^{-1} for the ester group. The structures of the above compounds were further confirmed by their ^{1}H NMR, ^{13}C NMR and MS data.

The treatment of PAS with oxazolone **6** in refluxing dry pyridine afforded the imidazolone **7**. Its IR spectra showed two carbonyl absorptions at 1690 cm^{-1} (C=O of imidazolone) and 1665 cm^{-1} (C=O, COOH). The ^{1}H NMR spectra of **2** exhibited beside the aromatic protons at δ 7.12–8.06 two exchangeable singlets at δ 11.20 and δ 5.48 for the COOH and the phenolic OH groups, respectively. The structure of **7** was further supported by the ^{13}C NMR spectral data which showed the expected number of aliphatic and aromatic carbons.

Similarly, condensation of 5-fluoroisatin **8** with PAS in methanol yielded 5-fluoroisatin Schiff base **9** which upon cycloaddition with thioglycolic acid in dry dioxane afforded the spirothiazolidine derivative **10**. Compound **10** can be also obtained directly from refluxing of compound **8** and PAS with thioglycolic acid in dry dioxan in one step (Scheme 1). The ^{13}C NMR spectra of Schiff base **9** showed beside the aromatic carbons two carbonyl carbons at δ 161.9 and δ 172.3 as well as a C=N signal at δ 164.2. The spiroderivative **10** exhibited three carbonyl signals at δ 168.4, δ 171.2, and 171.9 in addition to a methylene carbon signal at δ 30.1. However, the ^{1}H NMR spectrum of compound **10** showed very characteristic two doublets of C-5 proton at δ 3.84 and 3.95 showing geminal coupling (J = 12.4 Hz). The structures of compounds **9** and **10** were further confirmed by their MS spectra which showed the molecular ion peak $M^+ + 2$ at m/z 376.

The aminomethylation of PAS using formaldehyde and piperidine or piperazine in methanol produced the N-alkyl **11** and N,N′-dialkyl **12** derivatives, respectively. Benzoylation of compounds **11** and **12** on warming it with 4-fluorobenzoyl chloride in DMF led to the formation of the benzoyl- or dibenzoyl derivatives **13** or **14**, respectively (Scheme 2). The structures of the above compounds **11–14** were confirmed by



Scheme 3: Synthesis of *p*-amino salicylic acid derivatives **16**-**17**.

their IR, ^{1}H NMR, ^{13}C NMR and MS data (see Experimental section).

The treatment of PAS with 4-(4′-fluorophenyl)thiosemicarbazide **15** in refluxing ethanol yielded 1,4-diarylthiosemicarbazide **16** which upon heterocyclization with diethyl oxalate in THF afforded 4-[4-(4-fluorophenyl)-5,6-dioxo-3-thioxo[1,2,4]triazian-1-yl]-2-hydroxybenzoic acid **17** (Scheme 3). The IR spectra of the triazines derivative **17** showed beside the two carbonyl absorptions at 1640 cm^{-1} and 1663 cm^{-1} a C=S band at 1317 cm^{-1}. The structure of the above compound was further confirmed from its ^{13}C NMR which showed the expected number of aliphatic and aromatic carbons as well as a thiocarbonyl signal at δ 182.3 in addition to three carbonyl signals at δ 155.8, 157.5, and 171.8 (carboxyl) (Scheme 3). Further confirmation of the structure of **17** was done by its MS spectral data.

3.2. Antimycobacterial Activity. The results of the *in vitro* evaluation of antituberculosis activity are reported in Tables 1 and 2. During the preliminary screening compounds **1-5**, **7**, **9–14**, and **17** were tested (Table 1) for their antimycobacterial activity; one of the compounds **17** has exhibited 100% inhibition at this concentration while other compounds exhibited between 92 and 98% inhibition at the same concentration. Compounds **3–5**, **10**, **13**, and **17** have shown inhibition between 98 and 100%. Therefore, these are selected for the second level screening to determine the actual minimum inhibitory concentration (MIC). Compounds **5** and **13** have shown a slight improvement in the antitubercular activity in the second level and were found to be the most promising candidates of PAS analogs with MIC values 4.25 μM and 3.78 μM, respectively (Table 2).

The IC_{50} and MIC data are used to calculate the selectivity index (SI) of each compound as an estimate of a therapeutic window and a mechanism to identify candidates for efficacy studies *in vivo* (Table 2). Compounds **3–5**, **10**, **13**, and **17** have shown selectivity index values 4.64, 4.32, 3.29, 4.32, 3.17, and 4.80 respectively. Furthermore, all compounds have shown log *P* values in the accepted range (1.93–4.2) of druglikeness. However compounds **4**, **10**, and **17** show medium log *P* value (~2.0) and make them suitable candidates for a possible oral drug.

In our previous research work we prepared *p*-amino-salicylic acid analogs keeping in mind the mutual prodrug concept [24]. However, this paper includes the introduction of fluorine in almost all the PAS analogs. The reason for the induction of fluorine into these analogs is due to the fact that fluorine is much more lipophilic than hydrogen, so incorporating fluorine atoms in PAS analogs make them more fat soluble. This means it partitions into membranes much more readily, and hence these analogs have a higher bioavailability and metabolic stability.

4. Conclusion

Fluorine substituted heterocyclic systems containing *p*-amino salicylic acid were synthesized as antimycobacterial agents. Some derivatives selected for the second level

screening have shown favorable partition coefficient values to support druglikeness of these compounds. However their selectivity index is not very high. Further optimization of these PAS analogs is recommended in order to have a compound with the optimum structure features and the required biological activity.

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