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Synthesis and Antimicrobial Evaluation of Some Novel Hydrazone Derivatives of 2,5-Diflurobenzoic acid

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ABSTRACT

Therapeutic eminence of the hydrazide-hydrazone derivatives has been well recognized, in addition, hydrazide-hydrazones were reported to bring forth anticancer, anti-HIV properties and hence they have gained an imperative place in medicinal chemistry. Encouraged by these observations and in continuation of our research on novel hydrazone derivatives derived from 2,5-Difluorobenzoic acid, we intended to synthesize a series of new hydrazide derivatives with the aim of attaining promising antimicrobial compounds to improve actual antimicrobial treatments.

Keywords: Antibacterial Activity, Benzaldehydes, 2,5-Difluorobenzoic acid, Hydrazones.

INTRODUCTION

Hydrazones represent a significant group of biologically active drug molecules [1] which has fascinated consideration of medicinal chemists due to their wide range of pharmacological properties. Therapeutic eminence of the hydrazide-hydrazone derivatives has been well recognized, in addition, hydrazide-hydrazones were reported to bring forth anticancer [2–9], anti-HIV properties [10] and hence they have gained an imperative place in medicinal chemistry. In many reports, hydrazide-hydrazones are considered to be good scaffolds for different pharmaceutical applications, where such compounds were considered as antimicrobial, antifungal, antibacterial, and anticonvulsant agents [11–18]. In recent times hydrazide-hydrazones have gained great importance due to their diverse biological properties, including anti-inflammatory, antimalarial and antituberculotic activities [19–24].

In spite of a large number of antibiotics and chemotherapeutics available for medical use, the antimicrobial resistance has created a substantial need for design of new class of antimicrobials and this field will always remain an area of immense significance. The increased use of antimicrobial agents available in the market has resulted in the development of resistance to the commonly used drugs with important implications for

morbidity, mortality [25, 26] and health care costs. Encouraged by these observations and in continuation of our research [27] on novel hydrazone derivatives derived from 2,5-Difluorobenzoic acid, we intended to synthesize a series of hydrazide derivatives with the aim of attaining promising antimicrobial compounds to improve actual antimicrobial treatments.

MATERIALS AND METHODS

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellet with a perkin-elmer spectrum gx FTIR instrument and only diagnostic and/or intense peaks are reported. ^{1}H NMR spectra were recorded in DMSO- d_6 with a Varian Mercury plus 400 MHz instrument. ^{13}C NMR spectra were recorded in DMSO- d_6 with a Varian Gemini 100 MHz instrument. Signals due to the solvent (^{13}C NMR) or residual protonated solvent (^{1}H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. . The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (*J*) corresponds to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under argon atmosphere. The 2,5 Di-fluorobenzoic acid and 3-chloro-4-hydroxy benzoic acid and all the benzaldehydes used for the preparation of **9a-9o** were purchased from commercial sources.

Synthesis of methyl 2,5-difluorobenzoate 2: To a solution of compound **1** (3 g, 18.98 mmol) in methanol (30 mL) was added sulphuric acid (0.5 mL) and refluxed for 25 h. After completion of reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in ethylacetate (75 mL) and washed with 10% aq; $NaHCO_3$ solution (2 x 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **2.** Colorless liquid, Yield: 2.5 g, 76%; B.p: 211-212 ^{o}C; IR (KBr): υ_{max} 3082, 3005, 2957, 1883, 1739, 1625, 1597, 1496, 1142, 1420, 1312, 1274, 1251, 1188, 1122, 1075, 985, 891, 827, 805, 782, 689, 671 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.46 (m, 1H), 7.62-7.54 (m, 1 H), 7.68-7.64 (m, 1 H), 3.84 (s, 3 H); ESI-MS: m/z (rel.abund.%) 173.10 (M+, 100).

Synthesis of (2,5-difluorophenyl)methanol 3: To a solution of compound **2** (6 g, 34.85 mmol) in methanol (30 mL), cooled to 5-10 ^{o}C was added sodium borohydride (3 g, 37.84 mmol) in six portions. The reaction mixture was allowed to stir at room temperature for 3.5 h. The reaction mixture was quenched with water (2 mL) and evaporated under pressure to obtain yellow residue. The residue was taken in ethyl acetate (100 mL) and washed with water followed by brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to compound **3**. Colorless liquid,Yield: 4.5 g, 90%; B.p: 196 - 197 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60-7.21 (m, 3 H), 5.40 (t, 1 H, *J* = 5.8 Hz), 4.58 (d, 2 H, *J* = 4.8 Hz);

Synthesis of 2-(bromomethyl)-1,4-difluorobenzene 4: A mixture of compound **3** (5 g, 34.70 mmol) in 33% HBr in acetic acid (25 mL) was heated to 55 ^{o}C for 2.5 h. The reaction mixture was diluted with water (25 mL) and extracted with dichloro methane (2 x 20 mL). The organic layer washed with 10% aq; $NaHCO_3$ solution (3 x 15 mL) followed by water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **4**. Reddish brown liquid,, Yield: 5 g, 69%; B.p: 27 - 28 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.22 (m, 2H), 7.48-7.42 (m, 1 H), 4.62 (s, 2 H);

Synthesis of methyl 3-chloro-4-hydroxybenzoate 6: To a solution of compound **5** (5 g, 29.06 mmol) in methanol (50 mL) was added sulphuric acid (0.1 mL) and refluxed for 6 h. After completion of the reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in

ethylacetate (60 mL,), washed with 10% aq; $NaHCO_3$ solution (3 x 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **6.** White solid, Yield: 4.0 g, 73%; M.p: 108 - 110 ^oC; IR (KBr): υ_{max} 3346, 2957, 1690, 1604, 1577, 1512, 1443, 1418, 1355, 1292, 1268, 1189, 1126, 1054, 972, 909, 877, 830, 806, 765, 711, 656, 633, 544; ^1H NMR (400 MHz, $CDCl_3$): δ 11.30 (br.s, 1 H), 7.87 (d, 1 H, *J* = 2.4 Hz), 7.67 (dd, 1 H, *J* = 2.0, 2.4 Hz), 7.05 (d, 1 H, *J* = 12.0 Hz), 3.82 (s, 3 H);

Synthesis of methyl 4-(2,5-difluorobenzyloxy)-3-chlorobenzoate 7: To a stirred mixture of compound **6** (2.4 g, 12.85 mol) and potassium carbonate (1.77 g, 12.85 mol) in dimethyl formamide (15 mL), at room temperature was added compound **4** (2 g, 9.66 mmol) over a period of 5 min. The reaction mixture was heated to 70 ^oC for 2 h. The reaction mixture was diluted with water (25 L) and extracted with ethyl acetate (30 mL). Organic layer was separated and washed with water (3 x 25 mL) followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo* to give crude compound, which was purified by flash chromatography eluting with hexane-ethyl acetate (90:10) to afford compound **8** as a white solid. White solid, Yield: 2 g, 60%; M.p: 124 - 125 ^oC; IR (KBr): υ_{max} 3087, 3030, 2956, 1715, 1504, 1489, 1458, 1435, 1408, 1383, 1286, 1294, 1235, 1191, 1183, 1122, 1091, 1063, 1033, 978, 957, 905, 877, 817, 788, 763, 729, 647, 568; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98-7.92 (m, 2H), 7.48-7.42 (m, 2 H), 7.40-7.28 (m, 2 H), 5.40 (s, 2 H), 3.86 (s, 3 H); ESI-MS: m/z, 313.10 (M+1).

Synthesis of 4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide 8: To a solution of compound **7** (1.5 g, 4.80 mmol) in ethanol (15.0 mL) was added hydrazine hydrate (14.40 m mol) and heated to reflux for 10 h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound **8**. The crude compound was slurred in n-Hexane, filtered at the high vaccum pump and dried to obtain compound **8.** White solid, Yield: 1.3 g, 86%; M.p: 87 - 88 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 9.80 (s, 1 H), 7.94 (s, 2 H), 7.84 (d, 1 H, *J* = 4.2 Hz), 7.50-7.18 (m, 4 H), 5.25 (s, 2 H), 4.50 (s, 2 H); ESI-MS: m/z, 313.00 (M+1).

General Experimental Procedure for the Synthesis of Hydrazone derivatives (9a-9o): To a stirred solution of compound **8** (100 mg, 0.32 mmol) in ethanol was added corresponding benzaldehydes (1.0 mmol) and refluxed for 3 h. The reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 85 and 97%.

(E)-N'-(2,4-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9a): White solid; Yield: 85%; M.p: 117-118 ^oC; IR (KBr): υ_{max} 3179, 3043, 2978, 2838, 1658, 1636, 1602, 1558, 1434, 1456, 1383, 1275, 1209, 1189, 1156, 1107, 1064, 1034, 937, 926, 833, 756, 730. cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1 H), 8.78 (s, 1 H), 8.06 (s, 1 H), 7.83 (dd, 1 H, *J* = 1.6, 8.8 Hz), 7.80 (d, 1 H, *J* = 9.2 Hz), 7.48 - 7.26 (m, 4 H), 6.65 (s, 2 H), 3.80 (s, 3 H), 3.84 (s, 3 H), 5.30 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 162.44, 160.79, 159.25, 159.13, 157.52, 156.86, 155.64, 155.09, 143.26, 129.17, 128.21, 127.0, 126.63, 121.39, 115.07, 113.73, 64.26, 55.73, 55.14; ESI-MS: m/z, 461.10 (M+1).

(E)-N'-(2,5-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9b): White solid; Yield: 90%; M.p: 107-108 ^oC; IR (KBr): υ_{max} 3181, 3078, 2991, 2835, 1644, 1609, 1561, 1493, 1463, 1431, 1358, 1276, 1243, 1222, 1189, 1171, 1110, 1045, 961, 925, 878, 812, 763, 756, 708, 690, 651, 610, 595, 437 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1 H), 8.80 (s, 1 H), 8.08 (dd, 1 H, *J* = 2.0 Hz), 7.96 (dd, 1 H, *J* = 1.6, 8.0 Hz), 7.48-7.26 (m, 5 H), 7.08 – 7.02 (m, 2 H), 5.30 (s, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H); ^{13}C NMR ($CDCl_3$): δ 161.02, 159.23, 157.49, 156.86, 155.77, 155.08, 153.23 (2C), 152.28 (2C), 143.05 (2C), 129.24, 128.31, 126.79, 122.84, 121.43, 113.76, 113.40, 109.21, 64.28, 56.20, 55.43; ESI-MS: m/z, 461.10 (M+1).

(E)-N'-(2,6-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9c): White solid; Yield: 93%; M.p: 127-128 ^{o}C; IR (KBr): υ_{max} 317,2 3012, 2938, 2837, 1645, 1597, 1556, 1497, 1469, 1431, 1379, 1312, 1257, 1189, 1142, 1115, 1064, 1034, 956, 942, 910, 877, 810, 778, 755, 731, 649, 477 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1 H), 8.60 (s, 1 H), 8.08 (s, 1 H), 7.92 (d, 1 H, *J* = 5.4 Hz), 7.50-7.22 (m, 5 H), 6.72 (d, 2 H, *J* = 5.8 Hz), 5.20 (s, 2 H), 3.80 (s, 6 H); ESI-MS: m/z, 461.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(3,4,5-trimethoxybenzylidene)-3-chlorobenzohydrazide(9d): White solid; Yield: 96%; M.p: 122-124 ^{o}C; IR (KBr): υ_{max} 3439, 3207, 3063, 2948, 2839, 1649, 1600, 1501, 1468, 1364, 1297, 1272, 1188, 1128, 1068, 1056, 997, 947, 900, 881, 841, 828, 818, 788, 743, 722, 751, 703, 669, 644, 605, 518, 492 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.90 (d, 1 H, *J* = 7.2 Hz), 7.50-7.24 (m, 4 H), 7.0 (s, 1 H), 5.20 (s, 2 H), 3.84 (s, 6 H), 3.82 (s, 3 H); ^{13}C NMR ($CDCl_3$): δ 161.21, 159.24, 157.05, 156.84, 155.75, 155.01, 153.18 (3C), 147.79 (2C), 139.24, 129.77, 129.25, 128.30, 126.93, 125.17, 121.46, 113.80, 104.28, 64.28, 60.10, 55.93 (2C); ESI-MS: m/z, 491.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-ethoxy-3-methoxybenzylidene)-3-chlorobenzohydrazide(9e): **W**hite solid; Yield: 97%; M.p: 97-98 ^{o}C; IR (KBr): υ_{max} 3217, 3078, 2974, 2936, 1642, 1599, 1573, 1544, 1500, 1476, 1456, 1420, 1385, 1369, 1334, 1297, 1272, 1214, 1191, 1174, 1140, 1107, 1064, 1035, 969, 961, 932, 906, 858, 873, 805, 754, 766, 731, 696, 651, 621, 514, 488, 448 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.94 (d, 1 H, *J* = 8.4 Hz), 7.58-7.22 (m, 5 H), 7.20 (d, 1 H, *J* = 8.8 Hz), 5.38 (s, 2 H), 4.08 (q, 2 H, *J* = 6.8 Hz), 3.80 (s, 3 H), 1.40 (t, 3 H, *J* = 6.6 Hz); ESI-MS: m/z, 475.20 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(3-methoxy-4-propoxybenzylidene)-3-chlorobenzohydrazide (9f): White solid; Yield: 94%; M.p: 132-133 ^{o}C; IR (KBr): υ_{max} 3226, 3074, 2937, 1644, 1599, 1574, 1545, 1502, 1469, 1456, 1421, 1384, 1372, 1332, 1297, 1273, 1239, 1191, 1174, 1140, 1064, 1034, 969, 904, 873, 862, 846, 797, 731, 685, 873, 862, 846, 797, 731, 685, 651, 596, 566, 518, 491, 448 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.92 (d, 1 H, *J* = 9.2 Hz), 7.54-7.24 (m, 5 H), 7.20 (d, 1 H, *J* = 8.0 Hz), 7.0 (d, 1 H, *J* = 8.2 Hz), 5.38 (s, 2 H), 4.02 (q, 2 H, *J* = 7.2 Hz), 3.80 (s, 3 H), 1.70 (q, 2 H, *J* = 6.8 Hz), 1.0 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$): δ 161.05, 159.24, 157.82, 156.86, 155.69, 150.18, 149.19, 148.01, 129.20, 128.24, 127.04, 126.86, 125.01, 121.91, 121.44, 113.79 (2C), 112.42 (2C), 108.44 (2C), 69.63, 55.48, 22.0, 10.39; ESI-MS: m/z, 489.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-tert-butylbenzylidene)-3-chlorobenzohydrazide (9g): White solid; Yield: 92%; M.p: 92-94 ^{o}C; IR (KBr): υ_{max} 3358, 3079, 2963, 1667, 1609, 1598, 1565, 1532, 1495, 1459, 1432, 1388, 1371, 1306, 1262, 1189, 1178, 1147, 1134, 1064, 1029, 976, 961, 939,881, 836, 825, 805, 751, 734, 723, 708, 647, 593, 510, 448 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1 H), 8.42 (s, 1 H), 8.04 (s, 1 H), 7.92 (d, 1 H, *J* = 9.6 Hz), 7.66 (d, 2 H, *J* = 8.0 Hz), 7.52-7.26 (m, 6 H), 5.38 (s, 2 H), 1.0 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 161.16, 159.24, 157.50, 156.87, 155.76, 155.09, 152.90, 147.76, 131.55, 129.25, 128.29, 126.92 (2C), 125.63 (2C), 125.18, 121.46, 113.78 (2C), 64.30, 34.58, 30.93 (4C); ESI-MS: m/z, 457.20 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9h): White solid; Yield: 95%; M.p: 138-140 ^{o}C; IR (KBr): υ_{max} 3219, 3062,1649, 1597, 1544, 1502, 1434, 1415, 1403, 1378, 1360, 1333, 1293, 1275, 1233, 1192, 1152, 1114, 1070, 1017, 996, 961, 943, 905, 881, 842, 833, 785, 751, 720, 678, 640, 599, 504, 474, 447 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.0 (s, 1 H), 8.60 (s, 1 H), 8.07 (s, 1 H), 7.98-7.61 (m, 3 H), 7.81 (d, 2 H, *J* = 8.4 Hz), 7.49-7.26 (m, 4 H), 5.40 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 161.13, 159.24, 157.43, 156.85, 155.93, 155.08, 138.25, 129.83, 129.51, 129.35, 128.44, 127.63, 126.60, 125.72, 125.68, 125.43, 125.41, 125.07, 122.72, 121.50, 113.80, 64.30; ESI-MS: m/z, 469.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-(trifluoromethoxy)benzylidene)-3-chlorobenzohydrazide (9i): White solid; Yield: 89%; M.p: 143-145 ^{o}C; IR (KBr): υ_{max} 3222, 3064, 1647, 1608, 1598, 1544, 1502, 1434, 1403, 1363, 1309, 1290, 1272, 1233, 1193, 1151, 1103, 1057, 1018, 998, 957, 937, 906, 881, 846, 813, 785, 752, 720, 704, 649, 517,446 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1 H), 8.48 (s, 1 H), 8.06 (s, 1 H), 7.96 (d, 1 H, *J* = 6.4 Hz), 7.84 (d, 2 H, *J* = 7.2 Hz), 7.50-7.24 (m, 6 H), 5.40 (s, 2 H); ESI-MS: m/z, 485.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(3-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9j): White solid; Yield: 92%; M.p: 136-138 ^{o}C; IR (KBr): υ_{max} 3183, 2998, 1652, 1603, 1566, 1501, 1493, 1456, 1431, 1369, 1328, 1313, 1273, 1243, 1215, 1191, 1166, 1147, 1120, 1097, 1068, 1030, 954, 910, 879, 862, 823, 806, 757, 721, 713, 697, 670, 626, 495 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.0 (s, 1 H), 8.50 (s, 1 H), 8.14-7.92 (m, 4 H), 7.84-7.68 (m, 2 H), 7.56-7.24 (m, 4 H), 5.30 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 167.43, 161.44, 159.25, 157.52, 156.85, 155.91, 155.11, 153.33, 145.93, 135.47, 131.06, 130.04, 129.34, 128.45, 126.62, 125.36, 129.14, 122.95, 121.49, 113.80, 64.32; ESI-MS: m/z, 469.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(2-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9k): White solid; Yield: 88%; M.p: 103-105 ^{o}C; IR (KBr): υ_{max} 3184, 3014, 1651, 1603, 1563, 1500, 1493, 1462, 1430, 1377, 1312, 1271, 1214, 1192, 1176, 1163, 1120, 1145, 1066, 1032, 942, 911, 886, 863, 822, 810, 766, 770, 756, 712, 682, 642, 625, 595, 571, 448, 409 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.10 (s, 1 H), 8.80 (s, 1 H), 8.26 (d, 1 H, *J* = 7.2 Hz), 8.08 (s, 1 H), 7.96 (d, 1 H, *J* = 6.8 Hz), 7.66 (t, 1 H, *J* = 6.4 Hz), 7.56-7.32 (m, 4 H), 5.30 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 161.42, 159.24, 157.52, 156.52, 156.85, 155.98 (2C), 155.09, 142.64, 132.82, 132.15, 130.08, 129.31, 128.50, 126.86, 126.45, 125.88, 125.53, 124.88, 121.50, 116.99, 113.83, 64.30; ESI-MS: m/z, 469.00 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-fluorobenzylidene)-3-chlorobenzohydrazide (9l): White solid; Yield: 91%; M.p: 112-114 ^{o}C; IR (KBr): υ_{max} 3220, 3071, 3039, 1649, 1606, 1598, 1565, 1548, 1511, 1500, 1415, 1433, 1403, 1365, 1296, 1275, 1233, 1217, 1191, 1157, 1143, 1099, 1057, 1015, 998, 958, 933, 904, 879, 832, 815, 790, 752, 721, 664, 644, 522, 462 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (s, 1 H), 8.42 (s, 1 H), 8.04 (s, 1 H), 7.92 (d, 1 H, *J* = 6.2 Hz), 7.80 (t, 2 H, *J* = 4.8 Hz), 7.42 (d, 2 H, *J* = 6.8 Hz), 7.40-7.24 (m, 4 H), 5.30 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 164.35, 161.88, 161.24 (2C), 159.25, 157.52, 156.85, 155.80 (2C), 155.11, 146.52 (2C), 130.89, 129.27, (2C), 128.34 (2C), 126.81, 121.47, 113.80, 64.29; ESI-MS: m/z, 419.10 (M+1).

(E)-N'-(2,4-difluorobenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9m): White solid; Yield: 93%; M.p: 101-102 ^{o}C; IR (KBr): υ_{max} 3228, 3086, 1639, 1618, 1599, 1569, 1565, 1459, 1426, 1408, 1388, 1366, 1301, 1280, 1270, 1243, 1188, 1175, 1143, 1092, 1062, 1032, 967, 871, 915, 850, 814, 756, 732, 682, 651, 613, 509, 487, 445, 487, 421 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.95 (s, 1 H), 8.64 (s, 1 H), 8.08 (s, 1 H), 8.04-7.90 (m, 2 H), 7.50-7.18 (m, 6 H), 5.30 (s, 2 H); ESI-MS: m/z, 437.0 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-bromobenzylidene)-3-chlorobenzohydrazide (9n): White solid; Yield: 90%; M.p: 87-88 ^{o}C; IR (KBr): υ_{max} 3183, 3064, 1644, 1598, 1566, 1546, 1498, 1431, 1405, 1362, 1295, 1270, 1213, 1190, 1143, 1099, 1055, 1010, 1001, 974, 940, 894, 884, 862, 818, 782, 748, 721, 702, 669, 515, 466, 443, 414 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 8.12 (d, 1 H, *J* = 7.2 Hz), 7.74-7.62 (m, 4 H), 7.38-7.22 (m, 4 H), 5.30 (s, 2 H); ESI-MS: m/z, 479.0 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-((benzofuran-2-yl)methylene)-3-chlorobenzohydrazide (9o): White solid; Yield: 96%; M.p: 111-112 ^{o}C; IR (KBr): υ_{max} 3228, 3050, 2553, 1650, 1619, 1598, 1556, 1535, 1496, 1457, 1446, 1382, 1353, 1307, 1266, 1239, 1190, 1148, 1108, 1034, 926, 962, 888, 855, 820, 802,

747, 733, 679, 613, 443 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.0 (s, 1 H), 8.50 (s, 1 H), 8.10 (s, 1 H), 7.96 (d, 1 H, *J* = 6.6 Hz), 7.80-7.62 (m, 2 H), 7.52-7.24 (m, 7 H), 5.40 (s, 2 H); ESI-MS: m/z, 441.10 (M+1).

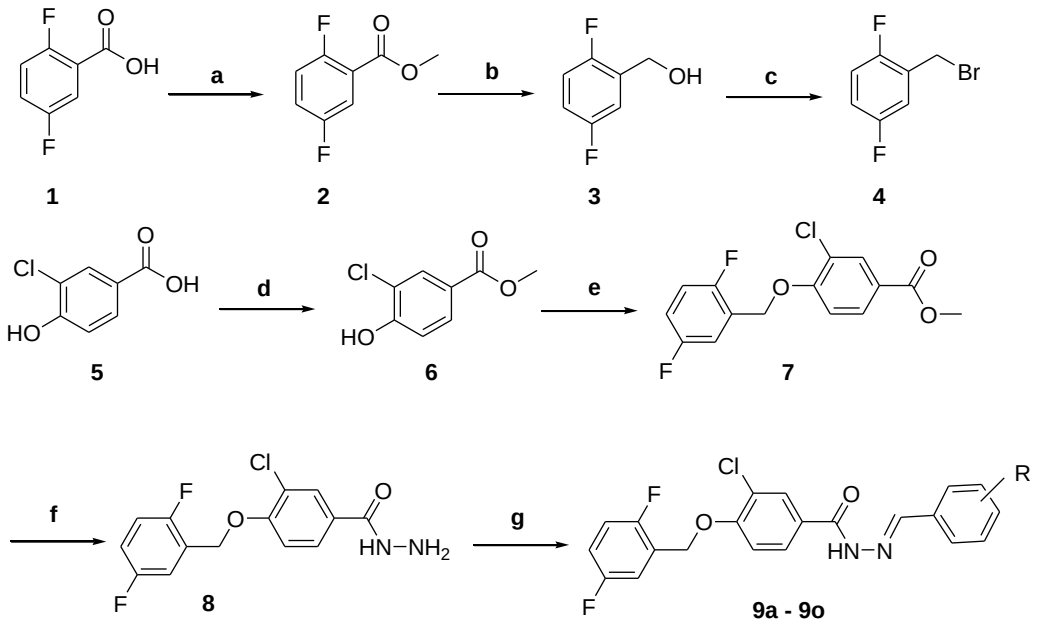
Antimicrobial Bioassay: The antimicrobial activities of the synthesized compounds were determined by agar well diffusion method [28-30]. The compounds were evaluated for antibacterial activity against *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Staphylococcus pyogenes* (MTCC 442). The antibiotic Ampicillin (250 µg/mL) was used as reference drug for antibacterial activity. Dimethyl sulphoxide (1%, DMSO) was used as a control without compound. The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 oC for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at a fixed concentration of 250 µg mL^{-1} separately for each bacterial strain. All the plates were incubated at 37±0.5 oC for 24 h. Zone of inhibition of compounds in mm were noted.

RESULTS AND DISCUSSION

Synthesis: The reaction progression for the synthesis of fifteen new hydrazone derivatives **9a-9o** is presented in **Scheme 1**. 2,5-Difluorobenzoic acid **1** was converted to corresponding methyl benzoate derivative **2** in presence of H_2SO_4 (catalytic quantity) in methanol. The methyl benzoate derivative **2** was treated with sodium borohydride in methanol to afford the benzyl alcohol derivative **3**. Benzyl alcohol derivative **3** was converted to benzyl bromide derivative **4** using 33% HBr in acetic acid. Esterification of 3-chloo-4-hydoxy-benzoic acid **5** was carried out using conc. H_2SO_4 in methanol to afford methyl benzoate derivative **6**. Coupling of benzylbromide derivative **4** with methyl benzoate derivative **6** using potassium carbonate in DMF gave compound **7**. Compound **7** was treated with hydrazine hydrate in ethanol to afford the key intermediate hydrazide derivative **8**. Condensation reaction between hydrazide **8** and various benzaldehydes **a-o**, lead to hydrazide-hydrazone derivatives **9a-9o**. The synthesized hydrazide-hydrazone derivatives **9a-9o** was characterized by Mass, IR, ^{1}H NMR and ^{13}C NMR spectral data. The mass spectra of compounds showed (M+1) peaks, in agreement with their molecular formula. The -N=C*H*- proton was observed as singlet in the region at 8.40-8.80 ppm. The -N*H*-N= proton was observed as a broad singlet in the region at 11.60- 12.10 ppm. All the other aliphatic and aromatic protons were observed at expected regions. The ^{1}H-NMR data were also consistent with the assigned structures. In the ^{13}C NMR chemical shift values of the carbon atoms at around 161.21-162.44 ppm (hydrazide *C*=O), and about 143.26-147.79 ppm (imine N=*C*H) corroborate the hydrazide character deduced from the ^{1}H NMR data.

Scheme 1:

Experimental Conditions: a) Conc. H_2SO_4, Methanol, reflux, 25 h; b) Sodium borohydride, Methanol, room temperature, 3.5 h; c) 33% HBr in acetic acid, 55 ^oC, 2.5 h ; d) Conc. H_2SO_4, Methanol, reflux, 6 h; e) K_2CO_3, **4**, DMF, 70 ^oC, 2 h; f) $NH_2NH_2.H_2O$, ethanol, reflux, 10 h; g) Benzaldehydes **a-o**, ethanol, reflux, 3 h.



9a: R= 2,4-di-Methoxy; **9b:** R= 2,5-di-Methoxy; **9c**: R = 2,6-di-methoxy; **9d**: R= 3,4,5 tri-Methoxy; **9e:** 3-methoxy-4-ethoxy; **9f:** 3-methoxy-4-propoxy; **9g:** 4-t-butyl; **9h:** R= 4-CF_3; **9i:** R= 4-OCF_3; **9j:** R= 3-CF_3; **9k:** R= 2-CF_3; **9l**: R= 4-F; **9m:** R= 2,4-di-Fluoro;**9n:** R= 4-Br; **9o:** R= Benzofuran

Scheme 1. Synthesis of Hydrazone derivatives **9a – 90**

Antibacterial evaluation: The outcome of antibacterial screening of fifteen newly synthesized entities **9a-90** are accessible in Table 1. Results disclose that four chosen bacterial strains like, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* has revealed different patterns of activities against the control drug Ampicillin. For, *E. coli*, compound **9h** (R = 4-CF_3), **9k** (R = 2- CF3) and **9m** (R = 2,4-di-Fluoro) displayed excellent activity (zone of inhibition: 21-24 mm), while compounds **9d** (R = 3,4,5-OMe), **9i** (R = 4-OCF_3), and **9o** (R = Benzo[b]furan) showed equipotent activity (zone of inhibition: 19-22 mm) and the compounds **9e, 9f, 9j** and **9l** (R = 3-OMe,4-OEt, 3-OMe,4-OPr, 3-CF_3, 4-F respectively) exhibited good activity (zone of inhibition: 17 – 20 mm) and the remaining entities in the series such as **9a** (R = 2,4-OMe), **9b** (R = 2,5-OMe) and **9c** (R = 2,6-OMe) showed moderate activity (zone of inhibition: 13-16 mm) when compared to the standard drug ampicillin. Similar trends of antibacterial activity were observed when tested against the following bacterial strains such as *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Staphylococcus pyogenes* (MTCC 442). Compounds **9g** (R = t-butyl) and **9n** (R = Br) was found to be inactive against all the tested bacterial strains.

It is noteworthy to observed from **Table 1** that the compounds possessing fluorine moiety **9h, 9k, and 9m** exhibited excellent antibacterial activity with zone of inhibition 21-24 mm, while the compounds **9d**, **9i** and **9o** having –OCF_3, 3,4,5-tri-OMe and Benzo[b] furan moiety displayed good activity towards all the tested bacterial strains. As most of the tested entities emerged as active against all the tested microorganisms, it indicates that this essential scaffold can be a promising anti bacterial drug agent. Hence hydrazones derivative with an appropriate R group may emerge as a good antibacterial agent for all the *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* bacterial strains.

Table 1. Results of Antibacterial Bioassay of Compounds **9a-9o**
(Concentration Used 250 μg mL^{-1} of DMSO).

Compound No.	**R**	*Gram negative*		*Gram positive*	
		E.Coli ***MTCC 443***	*P.aeruginosa* ***MTCC 424***	*S.Aureus* ***MTCC 96***	*S.Pyogenes* ***MTCC 442***
		Zones of Inhibition of compounds 9a – 9o in mm			
9a	2,4-OMe	16	15	16	13
9b	2,5-OMe	15	14	12	13
9c	2,6-OMe	16	15	15	14
9d	3,4,5-OMe	22	20	21	19
9e	3-methoxy-4-ethoxy	20	18	19	17
9f	3-methoxy-4-propoxy	19	19	18	17
9g	4-t-butyl	--	--	--	--
9h	4-CF_3	24	21	22	21
9i	4-OCF_3	22	20	21	19
9j	3-CF_3	19	18	20	18
9k	2-CF_3	23	22	24	21
9l	4-F	20	18	20	18
9m	2,4-di-Fluoro	24	21	23	21
9n	4-Bromo	--	--	--	--
9o	Benzo[b]furan	22	19	21	19
Standard Drug	Ampicillin (250 μg/mL of DMSO)	22	20	21	19

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antibacterial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

In summary, we have synthesized some fifteen new hydrazone derivatives **9a-9o** and evaluated for their antibacterial activities against four selected bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*, at the concentrations 250 μg/mL with reference to the antibiotic drug Ampicillin. The results revealed that hydrazone derivatives possessing fluorine moiety **9h, 9k, and 9m** exhibited excellent antibacterial activity with zone of inhibition 21-24 mm, while the compounds **9d**, **9i** and **9o** having $–OCF_3$, 3,4,5-tri-OMe and Benzo[b] furan moiety displayed good activity towards all the tested bacterial strains. Thus, hydrazone derivative with a appropriate R group may emerge as a good antibacterial agent it may be considered as a promising lead for further design and development of new antimicrobial agents.

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