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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME NEW HYDRAZIDE-HYDRAZONE DERIVATIVES LINKED WITH BEZAFIBRATE SCAFFOLD

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Abstract

Bezafibrate (marketed as **Bezalip** and various other brand names) is a fibrate drug used for the treatment of hyperlipidaemia. It helps to lower LDL cholesterol and triglyceride in the blood and increase HDL. The present work reports the synthesis, characterization and antibacterial activity of novel hydrazide-hydrazone derivatives **6a-j** derived from the key intermediate related to Bezafibrate.

Keywords: Antibacterial activity, 4-(2-aminoethyl)phenol, Bezafibrate, Benzoylation, Hydrazones

Introduction

Hydrazones, related to ketones and aldehydes belong to a class of organic compounds with the structure, $R1R_2C = NNH_2$ (Uppal et al., 2011). These compounds contain C=N bond, which is conjugated with a lone pair of electrons of the functional nitrogen atom (Corey et al., 1976). The nitrogen atoms of the hydrazones are nucleophilic and the carbon atom has both electrophilic and nucleophilic nature (Corey et al., 1976). The α‑hydrogen of hydrazones is more potent than that of acidic ketones (Belskaya et al., 2010).

The emerging bacterial resistance causes a widespread problem for the treatment of various infections. Therefore, the search for antimicrobials is a never ending task. Now a days a number of hydrazone derivatives have been developed and evaluated for their antibacterial activity (Aslan et al., 2012). Hydrazones possess various biological activities like anthelmintic, anticancer, antimalarial, anticonvulsant, antidepressant, analgesic, anti-HIV, anti-inflammatory, antiplatelet, antimicrobial, antitubercular, vasodialator, antiviral, antischistosomiasis, anthelmintic, antidiabetic and trypanocidal activities (Ali et al., 2012; Ku-mar et al., 2012, 2010).

Inspired by the various pharmacological activities associated with the hydrazone derivative, we report herein the synthesis, characterization and antibacterial activity of novel hydrazide-hydrazone derivatives **6a-j** derived from the key intermediate related to one of the well know drug Bezafibrate. **Bezafibrate** (marketed as **Bezalip** and various other brand names) is a fibrate drug used for the treatment of hyperlipidaemia. It helps to lower LDL cholesterol and triglyceride in the blood and increase HDL (Behar et al., 2000).

Materials and Methods

Solvents and reagents were obtained from commercial source and used without purification. The IR spectra (ϑ_{max}, cm^{-1}) were recorded in solid state KBr dispersion using Perkin Elmer FT-IR spectrometer. The ^{1}H-NMR spectra was recorded on Varian 500 MHz spectrometer. The chemical shifts were reported in δ/ ppm relative to

TMS. The mass spectra were recorded on API 2000 Perkin Elmer PE-Sciex mass spectrometer. The reactions were monitored by Thin–layer chromatography (TLC). Melting points were determined on polman melting point apparatus (Model No MP96) by open capillary method and are uncorrected. All the reactions were carried out under nitrogen atmosphere.

Synthesis of N-(4-hydroxyphenethyl)-4-chlorobenzamide (3):

To a stirred solution of 4-(2-aminoethyl)phenol **1** (25 g, 0.18 mol) in tetrahydrofuran (100 mL) was added sodiumbicarbonate (16.84 g, 0.20 mol) followed by water (25 mL) and cooled to 0-5 °C. To the above reaction mixture was added benzoylchloride (33.5 g, 0.20 mol) over a period of 20 min and stirred at the same temperature for 3h. After completion of the reaction (monitored by T.L.C) water (225 mL) was added and stirred at 0-5 °C for 1 h, the precipitated solids were filtered and washed with water (50 mL) and dried to isolate compound **3**. Off white solid; Yield: 47g, 93%; M.p: 180-182 ^{o}C; IR (KBr): υ_{max} 3444, 3320, 3245, 3061, 2973, 2909, 2871, 2939, 2764, 2741, 2683, 2634, 1622, 1607, 1595, 1575, 1551, 1528, 1494, 1486, 1470, 1450, 1446, 1406, 1382, 1371, 1362, 1347, 1339, 1331, 1305, 1288, 1267, 1250, 1245, 1200, 1192, 1179, 1118, 1101, 1092, 1019, 969, 962, 930, 919, 877, 872, 868, 839, 834, 824, 813, 807, 769, 755, 719, 713 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 9.17 (s, 1H), 8.60 (t, J = 5.5 Hz, 1H), 7.84-7.82 (m, 2H), 7.54-7.52 (m, 2H), 7.01 (t, J = 8.5 Hz, 2H), 7.0-6.66 (m, 2H), 3.42-3.38 (m, 2H), 2.72 (t, J =7.5 Hz, 2H); ESI-MS: m/z, 273.9 $(M-H)^{+}$.

Ethyl 2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2-methylpropanoate (4)

A mixture of 4-chloro-N-(4-hydroxyphenethyl) benzamide **3** (50 g, 0.18 mol), potassium carbonate (62.6 g, 0.45 mol), methyl isobutyl ketone-MIBK (125 mL) and ethanol(125 mL) was heated to 85°C for 30 min and added tetrabutylamonium bromide-TBAB (11.7 g, 0.0362) followed by ethyl α-bromoisobutyrate (77.8 g, 0.40 mol) at the same temperature. The reaction mixture was heated to 85°C was 24 h, after completion of the reaction (monitored by T.L.C), the reaction mixture was cooled to room temperature and filtered to discard the inorganic salts. The filtrate was concentrated at 40°C to obtain the crude compound which was dissolved in a mixture of acetone (150 mL) and water (500mL), the precipitated solids was filtered and dried at the pump to obtain compound **4**. White solid; Yield: 65g, 91%; M.p: 46-48 ^{o}C; IR (KBr): υ_{max} 3444, 3320, 3245, 3061, 2973, 2909, 2871, 2939, 2764, 2741, 2683, 2634, 1622, 1607, 1595, 1575, 1551, 1528, 1494, 1486, 1470, 1450, 1446, 1406, 1382, 1371, 1362, 1347, 1339, 1331, 1305, 1288, 1267, 1250, 1245, 1200, 1192, 1179, 1118, 1101, 1092, 1019, 969, 962, 930, 919, 877, 872, 868, 839, 834, 824, 813, 807, 769, 755, 719, 713 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 9.17 (s, 1H), 8.60 (t, J = 5.5 Hz, 1H), 7.84-7.82 (m, 2H), 7.54-7.52 (m, 2H), 7.01 (t, J = 8.5 Hz, 2H), 7.0-6.66 (m, 2H), 3.42-3.38 (m, 2H), 2.72 (t, J =7.5 Hz, 2H); ESI-MS: m/z, 273.9 $(M-H)^{+}$.

4-chloro-N-(4-((1-hydrazinyl-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (5)

To a stirred solution of ethylester **4** (20 g, 0.05 mol) in methanol (100 mL) was added 80% aqueous; hydrazine hydride (16.03 g, 0.26 mol) and heated to 45-50°C for 15 h. The completion of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the precipitated solids was filtered and washed with methanol (50 mL) to afford compound **5**. White solid; Yield: 16.35 g, 85%; M.p: 134-136 ^{o}C; IR (KBr): υ_{max} 3247, 3121, 3075, 3009, 2982, 2951, 2933, 2893, 2878, 2148, 1660, 1648, 1631, 1605, 1595, 1576, 1548, 1519, 1508, 1494, 1488, 1404, 1397, 1391, 1373, 1348, 1322, 1255, 1226, 1194, 1188, 1156, 1119, 1107, 1101, 1088, 1033, 1014, 1003, 991, 973, 958, 944, 937, 891, 869, 858, 843, 821, 814, 774, 758, 750, 725, 693, 677 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.63 (t, J = 5.5 Hz, 1H), 7.84 (dd, J = 1.5, 6.5 Hz, 2H), 7.54 (dd, J = 1.5, 6.5 Hz, 2H), 7.12 (d, J = 9.0 Hz, 2H), 6.80 (dd, J = 1.5, 6.5 Hz, 2H), 4.28 (brs, 1H), 3.44 (q, J = 6.5 Hz, 2H), 2.77 (t, J = 7.5 Hz, 2H), 1.40 (s, 6H); ESI-MS: m/z, 375.9 $(M+H)^{+}$.

General Experimental Procedure for the Synthesis of Hydrazide-hydrazones Derivatives 6a-j

To a suspension of 4-chloro-N-(4-((1-hydrazinyl-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (1g, 2.66 mmol) in methanol (10 mL) at 25-27°C was added benzaldehydes **(a-j)** (0.3g 2.92 mmol) and heated to 50°C for 5h. After completion of the reaction (checked by T.L.C), the reaction mixture was concentrated at 45° C, then crude was dissolved in dichloromethane (10 mL) and slowly added n-hexane (20 mL) at 20-25° C. The precipitated solids were filtered at the pump and dried. The yields of the products varied from 80-88%.

(E)-N-(4-((1-(2-benzylidenehydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)-4-chlorobenzamide (6a)

White solid; Yield: 86%; M.p: 193-196 ^{o}C; IR (KBr): υ_{max} 3354, 3304, 3281, 3094, 3065, 3012, 2982, 2962, 2938, 2884, 2857, 2324, 1675, 1655, 1643, 1618, 1610, 1596, 1584, 1570, 1562, 1540, 1518, 1509, 1496, 1489, 1468, 1454, 1411, 1392, 1389, 1383, 1378, 1371, 1341, 1313, 1304, 1297, 1285, 1270, 1259, 1233, 1216, 1207, 1203, 1198, 1182, 1167, 1151, 1124, 1115, 1107, 1096, 1075, 1056, 1033, 1023, 1013, 994, 981, 952, 934 cm^{-1}; ^{1}H

NMR (500 MHz, DMSO-d_6): δ 11.54 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 8.42 (s, 1H), 7.81 (d, J = 6.5 Hz, 2H), 7.65 (dt, J = 2.5, 5.0 Hz, 2H), 7.50 (d, J = 1.5 Hz, 2H), 7.45-7.42 (m, 3H), 7.15 (d, J = 6.5 Hz, 2H), 6.85 (d, J = 6.5 Hz, 2H), 3.46-3.42 (m, 2H), 2.77 (t, J = 7.5 Hz, 2H), 1.48 (s, 6H); ESI-MS: m/z, 464.1 $(M+H)^+$.

(E)-4-chloro-N-(4-((1-(2-(2-methoxybenzylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy) phenethyl)benzamide (6b)

White solid; Yield: 84%; M.p: 180-184 ^oC; IR (KBr): υ_{max} 3338, 3269, 3197, 3117, 3103, 3065, 3269, 3197, 3117, 3103, 3065, 3033, 3018, 3011, 3002, 2991, 2965,2959,2875, 2848, 2839, 2166, 2038, 1665, 1655, 1643, 1618, 1600, 1578, 1555, 1545, 1536, 1519, 1509, 1498, 1488, 1476, 1466, 1441, 1434, 1409, 1398, 1378, 1372, 1362, 1359, 1347, 1339, 1333, 1324, 1318, 1295, 1286, 1280, 1274, 1264, 1252, 1241, 1241, 1229, 1215, 1207, 1197 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.55 (s, 1H), 8.75 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 7.80 (dd, J = 2.5, 6.5 Hz, 3H), 7.70 (dd, J = 3.0, 5.0 Hz, 2H), 7.40-7.38 (m, 1H), 7.16 (d, J = 6.5 Hz, 2H), 7.07 (d, J = 7.5 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H),6.85 (dd, J = 3.5, 5.0 Hz, 2H), 3.82 (s, 3H), 3.45-3.42 (m, 2H), 2.78 (t, J = 8.0 Hz, 2H), 1.48 (s, 6H); ESI-MS: m/z, 495.1 $(M+H)^+$.

(E)- 4-chloro-N-(4-((1-(2-(4-methoxybenzylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6c)

White solid; Yield: 85%; M.p: 163-165 ^oC; IR (KBr): υ_{max} 3338, 3305, 3282, 3117, 3060, 3046, 2982, 2960, 2932, 2848, 2837, 2651, 2557, 2200. 2024, 1647, 1620, 1606, 1583, 1568, 1563, 1544, 1523, 1508, 1494, 1488, 1470, 1463, 1428, 1421, 1403, 1378, 1370, 1360, 1346, 1312, 1288, 1278, 1271, 1251, 1240, 1230, 1207, 1180, 1176, 1170, 1164, 1156, 1122, 1112, 1102, 1092, 1079, 1062, 1047, 1032, 1021, 1015, 987, 975, 958, 950, 897, 841, 836, 784 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.40 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 8.34 (s, 1H), 7.81 (d, J = 6.5 Hz, 2H), 7.60 (dd, J = 3.0, 4.5 Hz, 2H), 7.50 (dt, J = 2.5, 5.0 Hz, 2H), 7.14 (d, J = 6.5 Hz, 2H), 7.00 (d, J = 6.5 Hz, 2H), 6.85 (d, J = 6.5 Hz, 2H), 3.80 (s, 3H), 3.46-3.42 (m, 2H), 2.77 (t, J = 7.0 Hz, 2H), 1.48 (s, 6H); ESI-MS: m/z, 495.1 $(M+H)^+$.

(E)- 4-chloro-N-(4-((1-(2-(2-hydroxy-3-methoxy benzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6d)

White solid; Yield: 80%; M.p: 120-124 ^oC; IR (KBr): υ_{max} 3273, 3060, 2984, 2935, 2869, 2848, 2840, 1676, 1644, 1609, 1597, 1578, 1534, 1506, 1486, 1463, 1380, 1361, 1252, 1227, 1180, 1152, 1110, 1091, 1014, 983, 957, 924, 866, 846, 779, 757, 731 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.83 (s, 1H), 10.97 (s, 1H), 8.62 (s, 2H), 7.80 (dd, J = 2.5, 7.5 Hz, 2H), 7.50 (dd, J = 2.0, 7.0 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.05 – 7.01 (m, 2H), 6.87 – 6.83 (m, 3H), 3.80 (s, 3H), 3.45 (t, J = 7.0 Hz, 2H), 2.77 (t, J = 7.0 Hz, 2H), 1.49 (s, 6H); ESI-MS: m/z, 510.1 $(M+H)^+$.

(E)-4-chloro-N-(4-((1-(2-(3,4-diethoxybenzylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6e)

White solid; Yield: 80%; M.p: 163-164 ^oC; IR (KBr): υ_{max} 3346, 3277, 3212, 3083, 3033, 2987, 2930, 2876, 2597, 2446, 1666, 1644, 1596, 1535, 1512, 1435, 1394, 1378, 1342, 1329, 1316, 1269, 1230, 1185, 1151, 1132, 1111, 1091, 1064, 1041, 1013, 964, 941, 898, 860, 846, 808, 777, 759, 725, 693, 683 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.40 (s, 1H), 8.63 (t, J = 5.5 Hz, 1H), 8.31 (s, 1H), 7.80 (d, J = 9.0 Hz, 2H), 7.52 (d, J = 6.5 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.01 (dd, J = 1.5, 8.0 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 4.04 (q, J = 6.5 Hz, 4H), 3.43 (t, J = 7.5 Hz, 2H), 2.77 (t, J = 6.5 Hz, 2H), 1.48 (s, 6H), 1.32 (t, J = 6.5 Hz, 6H); ESI-MS: m/z, 552.2 $(M+H)^+$.

(E)-4-chloro-N-(4-((1-(2-(2-chlorobenzylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6f)

White solid; Yield: 84%; M.p: 195-196 ^oC; IR (KBr): υ_{max} 3344, 3250, 3209, 3108, 3067, 3011, 2993, 2973, 2959, 2944, 2938, 2923, 2919, 2884, 2853, 2541, 1677, 1674, 1663, 1652, 1640, 1617, 1610, 1605, 1597, 1584, 1546, 1516, 1506, 1495, 1488, 1475, 1471, 1452, 1443, 1438, 1434, 1410, 1382, 1371, 1364, 1344, 1319, 1308, 1296, 1264, 1227, 1211, 1190, 1171, 1158, 1132, 1128, 1120, 1112, 1105, 1093, 1085, 1080, 1059, 1048, 1038 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.86 (s, 1H), 8.58 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 7.98 (dd, J = 2.5, 7.5 Hz, 1H), 7.80 (dd, J = 1.5, 6.5 Hz, 1H), 7.80 (dd, J = 2.5 Hz, 2H), 7.51 (dd, J = 3.0, 6.5 Hz, 2H), 7.45-7.42 (m, 2H), 7.16 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 6.5 Hz, 2H), 3.46-3.42 (m, 2H), 2.78 (t, J = 8.0 Hz, 2H), 1.49 (s, 6H); ESI-MS: m/z, 499.1 $(M+H)^+$.

Synthesis of 6g: R = 3-Cl-Benzaldehyde

(E)-4-chloro-N-(4-((1-(2-(3-chlorobenzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6g)

White solid; Yield: 85%; M.p: 160-162 ^oC; IR (KBr): υ_{max} 3328, 3259, 3200, 3113, 3082, 3072, 3032, 3003, 2991, 2956, 2934, 2894, 2873, 2488, 2450, 2353, 1673, 1657, 1637, 1617, 1609, 1602, 1595, 1577, 1538, 1517, 1508, 1498, 1492, 1474, 1470, 1438, 1429, 1393, 1378, 1371, 1357, 1336, 1313, 1293, 1279, 1255, 1227, 1201, 1183, 1168, 1154, 1126, 1112, 1108, 1092, 1084, 1069, 1043, 1037, 1029, 1013, 1003, 998, 991, 973, 963, 948, 916,

895, 878, 859, 850 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.70 (s, 1H), 8.62 (t, J = 5.5 Hz, 1H), 8.40 (s, 1H), 7.80 (dd, J = 1.5, 6.5 Hz, 2H), 7.70 (d, J = 2.0 Hz, 1H), 7.62-7.60 (m, 1H), 7.52-7.47 (m, 4H), 7.16 (d, J = 6.5 Hz, 2H), 6.85 (dd, J = 2.5, 5.0 Hz, 2H), 3.46-3.41 (m, 2H), 2.77 (t, J = 7.0 Hz, 2H), 1.48 (s, 6H); ESI-MS: m/z, 499.1 $(M+H)^+$.

(E)-4-chloro-N-(4-((1-(2-(4-chlorobenzylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)benzamide (6h)

White solid; Yield: 84%; M.p: 154-158 ^oC; IR (KBr): υ_{max} 3345, 3203, 3035, 2991, 2979, 2942, 2931, 2873, 2453, 1676, 1650, 1609, 1596, 1546, 1507, 1488, 1435, 14040, 1378, 1360, 1343, 1315, 1300, 1281, 1229, 1179, 1157, 1110, 1087, 1058, 1014, 975, 952, 847, 831, 821, 778, 722, 712, 673 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.62 (s, 1H), 8.61 (t, J = 5.5 Hz, 1H), 8.60 (s, 1H), 7.80 (t, J = 8.5 Hz, 2H), 7.66 (t, J = 8.5 Hz, 2H), 7.50 (d, J = 8.5 Hz, 4H), 7.15 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 3.43 (q, J = 6.5 Hz, 2H), 2.77 (t, J = 7.5 Hz, 2H), 1.48 (s, 6H); ESI-MS: m/z, 499.1 $(M+H)^+$.

(E)-4-chloro-N-(4-((1-(2-(3-bromobenxylidene) hydrazinyl)-2-methyl-1-oxopropan-2-yl)oxy)phenethyl)-benzamide (6i)

White solid; Yield: 84%; M.p: 182-183 ^oC; IR (KBr): υ_{max} 3307, 3248, 3196, 3108, 3055, 3048, 3034, 3015, 3007, 2989, 2983, 2955, 2930, 2889, 2857, 2492, 2461, 1674, 1659, 1645, 1619, 1612, 1607, 1595, 1577, 1547, 1521, 1509, 1495, 1489, 1474, 1471, 1455, 1450, 1439, 1428, 1404, 1397, 1392, 1377, 1371, 1361, 1351, 1343, 1335, 1315, 1293, 1281, 1259, 1231, 1207, 1183, 1166, 1153, 1122, 1115, 1104, 1089, 1080, 1068, 1036, 1030, 1024, 1014, 1001 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.72 (s, 1H), 8.63 (t, J = 5.5 Hz, 1H), 7.85 (s, 1H), 7.82 (d, J = 2.0 Hz, 2H), 7.65-7.61 (m, 2H), 7.50 (d, J = 6.5 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 3.40 (q, J = 6.5 Hz, 2H), 2.78 (t, J = 8.0 Hz, 2H), 1.49 (s, 6H); ESI-MS: m/z, 540.0 $(M+H)^+$.

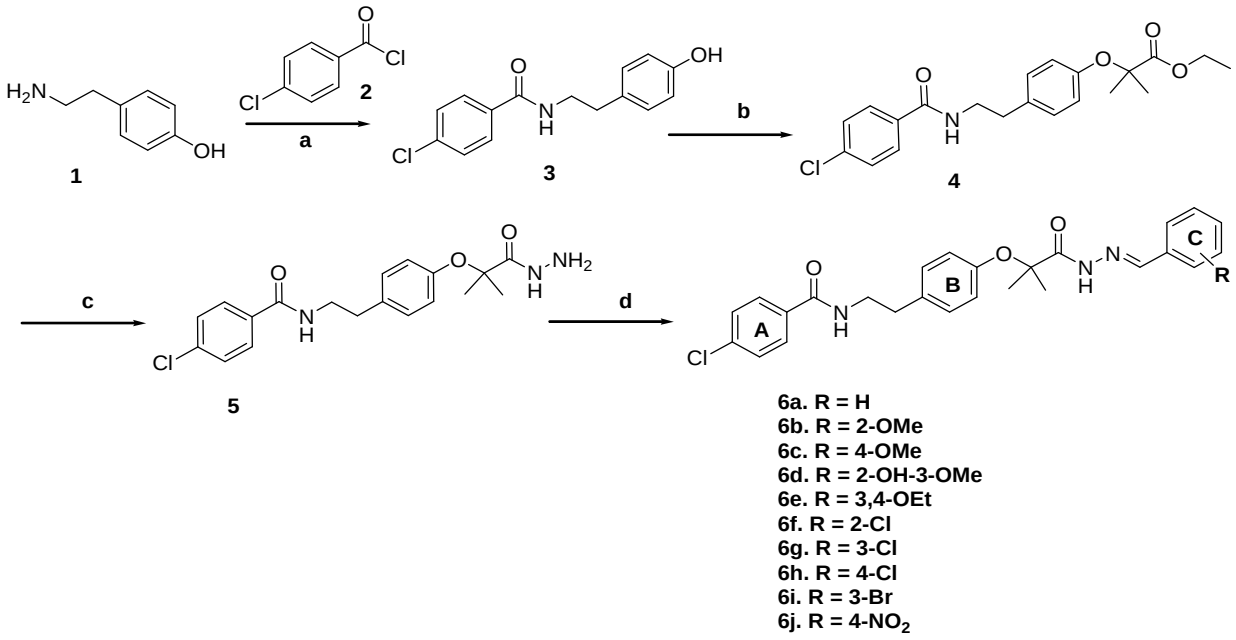
(E)-4-chloro-N-(4-((2-methyl-1-(2-(4-nitrobenzylidene) hydrazinyl)-1-oxopropan-2-yl)oxy) phenethyl)benzamide (6j)

White solid; Yield: 84%; M.p: 170-173 ^oC; IR (KBr): υ_{max} 3335, 3303, 3280, 3151, 3088, 2982, 2976, 2970, 2959, 2929, 2893, 2878, 2866, 2858, 2718, 2699, 2476, 2445, 2044, 2030, 1679, 1665, 1650, 1619, 1611, 1603, 1589, 1574, 1567, 1562, 1516, 1507, 1504, 1466, 1458, 1449, 1440, 1399, 1389, 1379, 1372, 1368, 1338, 1323, 1314, 1306, 1297, 1284, 1277, 1255, 1230, 1213, 1208, 1197, 1186, 1169, 1160 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.85 (s, 1H), 8.58 (t, J = 5.5 Hz, 1H), 8.47 (s, 1H), 8.22 (d, J = 9.5 Hz, 2H), 7.86 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 9.0 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.38 (q, J = 6.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 1.44 (s, 6H); ESI-MS: m/z, 507.0 $(M-H)^+$.

Results and Discussion

The synthetic sequence for the preparation of hydrazide-hydrazone derivatives **6a-j** is described in scheme 1. Benzoylation of 4-(2-aminoethyl)phenol **1** with 4-chloro-benzoyl chloride **2** in presence of sodium bicarbonate in tetrahydrofuran:water at 0-5 ^oC for 3 h gave N-(4-hydroxyphenethyl)-4-chlorobenzamide **3** in 93% yield. Esterification of benzamide **3** with ethyl α-bromoisobutyrate in presence of potassium carbonate and catalaytic quantity of tetrabutylamonium bromide in methyl isobutyl ketone:ethanol at 85 ^oC for 30 min yielded ethyl 2-(4-(2-(4-chlorobenzamido) ethyl)phenoxy)-2-methylpropanoate **4**. Reaction of ethyl ester derivative with hydrazine-hydrate in methanol at 45-50°C for 15 h resulted in the key intermediate 4-chloro-N-(4-((1-hydrazinyl-2-methyl-1-oxopropan -2-yl)oxy)phenethyl)benzamide **5** in 85% yield. Condensation of hydrazide **5** with aromatic benzalaldehydes **a-j** afforded hydrazide-hydrazone derivatives **6a-j** in quantitative yields. The structural assignments of the newly synthesized compounds **6a-j** was done by utilizing analytical techniques such as ^{1}HNMR, IR and mass analysis. As a representative example, the spectral analysis of hydrazide-hydrazone **6c** is described as follows: The protons resonating at 11.40 ppm (singlet, 1H), 8.62 ppm (triplet, 1H) and 8.34 ppm (singlet, 1H) corresponds to the groups –CO-NH-N=C-, -CO-NH-CH_2-CH_2- and –CO-NH-N=CH-Ar flanked to aromatic rings A, B and C respectively. The doublets with two proton integration resonating at 7.81 ppm and 7.60 ppm corresponds to the aromatic ring A while the doublets resonating at 7.50 ppm, 7.14 ppm, 7.0 ppm and 6.85 ppm with two proton integration corresponds to the aromatic ring C and B protons respectively. The singlets at 3.80 ppm and 1.48 ppm corresponds to methoxy and methyl groups respectively. The signals at 3.46-3.42 (multiplet) and 2.77 (triplet) correspond to –CH_2-CH_2- groups. (ESI) MS analysis was performed in the positive ion mode, showing peaks at m/z, corresponding to the expected monoisotopic mass of the [M+H]+ ion. The IR spectra of the hydrazo-hydrazide derivatives **6a-j** had a strong characteristic band in the region 1700-1647 cm^{-1} due to the C=O stretching vibration. The N-H stretching vibration of the compounds **6a -j** gave rise to a band at 3473-3180 cm^{-1}. The stretching bands for C=C and C=N groups were observed at 1609-1496 cm^{-1}.

Scheme-1



Scheme 1: Synthesis of novel hydrazide-hydrazone derivatives **6a – 6j**

Experimental Conditions

a): **2**, tetrahydrofuran, $NaHCO_3$, benzoylchloride, 0-5 ^oC, 3 h; b) ethyl-α-bromoisobutyrate, TBAB, K_2CO_3, MIBK-ethanol, 85 ^oC, 24 h; c) aqueous; hydrazine-hydrate (80%), methanol, 45-50°C, 15 h; d) Aromatic benzaldehydes **a-j,** methanol, 50 °C, 5 h.

Biological activity

Antimicrobial Screening

The antimicrobial activity was determined using disc diffusion method by measuring zone of inhibition in mm [11]. All the compounds, **6a-j** were screened *in-vitro* at a concentration of 250 µg/mL for antibacterial activity against two Gram-positive pathogenic organisms: *Staphylococcus aureus* and *Staphylococcus pyogenes,* two Gram-negative organisms: *Escherichia coli* and *Pseudomonas aeruginosa* (**Table 1**). Standard antibacterial drug ciprofloxacin (250 µg/disc) was also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken. Growth inhibition was calculated with reference to positive control. Hydrazide-hydrazone **(6a – j)** were dissolved in dimethyl sulphoxide at 250 µg/mL concentration. The inhibition zones were measured in millimeters at the end of an incubation period of 48 hours at (35±2) °C. DMSO alone showed no inhibition. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4.

Antibacterial activity

The outcome of the antibacterial results is tabulated in Table-1. Interpretation of antibacterial data from table 1 revealed that, in case of gram positive bacteria: *viz., E.coli* and *P.aeruginosa*: compound **6a** (R = H) showed weak antibacterial activity (zone of inhibition: 17-18 mm), compounds **6b** (R = 2-OMe), **6c** (R = 4-OMe), **6e** (R = 3,4-OEt) showed good antibacterial activity (zone of inhibition: 22-24 mm) while compounds **6d** (R = 2-OH-3-OMe) and **6j** (R = 4-NO_2) exhibited excellent antibacterial activity ((zone of inhibition: 28-30 mm) and the remaining compounds **6f**, **6g**, **6h** and **6i** showed no activity. Against gram negative bacteria: *viz., S.aureus* and *S.pyogenes*: compounds **6d** (R = 2-OH-3-OMe) and **6j** (R = 4-NO_2) exhibited excellent antibacterial activity ((zone of inhibition: 23-24 mm) while compounds **6b** (R = 2-OMe), **6c** (R = 4-OMe), **6e** (R = 3,4-OEt) showed good antibacterial activity (zone of inhibition: 18-20 mm) and compound **6a** (R = H) showed weak antibacterial activity (zone of inhibition: 15-16 mm), and the remaining compounds **6f**, **6g**, **6h** and **6i** showed no activity.

Table 1: Antibacterial activity data of novel hydrazo-hydrazide derivatives (6a-j)

Compound no.	*Gram negative bacteria*		*Gram positive bacteria*	
	E. coli MTCC 443	*P. aeruginosa MTCC 424*	*S.aureus MTCC 96*	*S.poygenes MTCC 442*
	Diameter of Zone of inhibition in mm			
6a (R = H)	18	17	15	16
6b (R = 2-OMe)	25	24	18	19
6c (R = 4-OMe)	24	22	19	18
6d (R = 2-OH-3-OMe)	30	29	24	24
6e (R = 3,4-OEt)	25	24	20	20
6f (R = 2-Cl)	--	--	--	--
6g (R = 3-Cl)	--	--	--	--
6h (R = 4-Cl)	--	--	--	--
6i (R = 3-Br)	--	--	--	--
6j (R = 4-NO_2)	29	28	24	23
SD*	28	27	22	22

* Ciprofloxacin (Conc. 250 µg/mL)

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Conflict of Interest

The authors have reported no conflict of interest.

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