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Synthesis and Antibacterial Activity of Novel Quinoxaline-5-Carboxamide Derivatives

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ABSTRACT

*Quinoxaline-based molecules have been disclosed as the antibacterial and antiprotozoal properties. The present paper describes the synthesis and antibacterial activity of sixteen new quinoxaline-5-carboxamide derivatives (**5a-5p**) from commercially available methyl 2, 3-diamino benzoate as staring material. The quinoxaline carboxamides (**5a-5p**) have been screened against four bacterial strains such Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes. In general, it is observed that within the quinoxaline-5-carboxamides **5a–5p**, compounds incorporated with Fluoro substituent phenyl groups, cyclic and aliphatic chain exhibited excellent antibacterial activity while the remaining compounds displayed moderate antibacterial activity.*

Keywords: Quinoxaline-5-carboxamide, Synthesis, Antibacterial activity.

INTRODUCTION

There is a growing need to develop new antibacterial agents in order to overcome the emergence of bacterial resistance to antibiotic therapy. Quinoxaline is a nucleus that displays a wide range of activities and these are an important class of nitrogen containing heterocyclic compounds for new drug development and because of their potentially versatile biological activities, including antimycobacterial agents [1], antimicrobial [2-3], antifungal [4-5], antiviral, anticancer , anti protozoal [6-10] and antipasmodial properties [11-13]. Moreover, quinoxaline and its analogues have been investigated as the catalyst's ligands [14]. In view of the literature regarding antimicrobial potency of quinoxalines and their mode of action that prevent DNA-directed RNA synthesis by virtue of binding to CpG site on DNA, the quinoxaline nucleus is focused on synthesizing newer derivatives to explore potent antimicrobial activities [15-16]. Encouraged by these reported activities and with the aim of searching for new, broad spectrum and more potent antimicrobial compounds which can improve the current chemotherapeutic treatments, sixteen new quinoxaline-5-carboxamide derivatives were synthesized and evaluated. In general, it is

observed that within the quinoxaline-5-carboxamides **5a – 5p**, compounds incorporated with 4-Fluoro, 3-CF_3, 5-Fluoro-2-Methyl, 4-Fluoro-3-Methyl, 2-Hydroxy ethyl, morpholine and morpholine ethyl exhibited excellent antibacterial activity while the remaining compounds displayed moderate antibacterial activity.

MATERIALS AND METHODS

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received. For thin-layer chromatography (TLC) analysis, Merck pre-coated Plates (silica gel 60 F254) were used and spots were visualized with UV light. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. ^{1}H NMR spectra were recorded in Varian MR-500 MHz instrument. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals were reported as s (singlet), d (doublet), dd (doblet of doblet), t (triplet), q (quartet), m (multiplet) and coupling constants in Hz. The mass spectra were recorded on Agilent ion trap MS. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer.

Synthesis of methyl 2, 3-dioxo-1, 2, 3, 4-tetrahydro quinoxaline-5-carboxylate 2: Mixture of compound **1** (10.0 g, 0.06 mol) and oxalic acid monohydrate (10.0 g, 0.092 mol) heated to 140-150^{o}C for 3 h. The reaction mixture was cooled to 40-50^{o}C and recrystallized from methanol (100 mL) gave the pure compound **2** as a pale ash solid. Yield: 11.9 g, 90%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.2 (s, 1H), 11.2 (s, 1H), 7.8 (d, *J* = 8.0 Hz, 1H), 7.6 (d, *J* = 6.5 Hz, 1H), 7.2 (t, *J* = 8.0 Hz, 1H), 3.9 (s, 3H).

Synthesis of 2, 3-dimethoxyquinoxaline-5-carboxylic acid 4: To a solution of compound **2** (10.0 g, 0.045 mol) in chloroform (50 mL), tetrahydrofuran (30 mL) and dimethylformamide (1 mL) was added thionyl chloride (40.0 g, 0.336 mol). The reaction mixture was stirred at reflux temperature for 20 h. The reaction mixture was evaporated under reduced pressure and diluted with water to afford crude compound **3** and proceeded to the next sept without further purification. To a solution of compound **3** (10.0 g, 0.038 mol) in methanol (50 mL) and water (8 mL) was added sodium hydroxide (4.0 g, 0.1 mol). The reaction mixture was stirred at reflux temperature for 16 h. The reaction mixture was evaporated under reduced pressure and isolated with water to afford compound **4** as an off-white solid. Yield: 8.01 g, 88%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.60 (s, 1H), 8.03 (d, *J* = 1.0 Hz, 1H), 7.96 (d, *J* = 1.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 4.09 (s, 3H), 4.08 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 54.23, 54.46, 126.27, 124.49, 128.40, 129.97, 133.82, 136.47, 149.73, 149.90, 166.68; ESI-MS: m/z, 234.8 (M^{+1}).

General experimental procedure for the preparation of novel Quinoxaline-5-Carboxamide derivatives 5a-5p: To a stirred solution of compound **4** (1.0 g, 0.0042 mol) in Dichloromethane (10 ml) and triethylamine (0.86 g, 0.0085 mol) was added pivaloyl chloride (0.62 g, 0.0052 mol) at 0^{o}C and stirred for 1.0 h under nitrogen atmosphere followed by slow addition of corresponding amines (0.0051 mol) and stirred for additional 1 h. The reaction medium was poured into water and extracted with dichloromethane. 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 80 to 97%.

Synthesis of 2, 3-dimethoxy-N-phenylquinoxaline-5-carboxamide (5a): Yield: 84%; IR (KBr): υ_{max} 3235.0, 3044.09, 1666.2, 1608.34, 1596.77, 1585.2, 1555.31, 1519.63, 1479.13, 1409.71, 1366.32, 1243.86, 1228.43, 1165.76, 1154.19, 1068.37, 1022.09, 1004.73, 978.69, 780.06, 759.81, 742.46, 690.39, 581.43, 488.86 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.91 (s, 1H), 8.66 (d, *J* = 6.5.0 Hz, 1H), 7.96 (d, *J* = 7.0 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 2 H), 7.15 (t, *J* = 7.5 Hz, 1H), 4.3 (s, 3H), 4.2 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 54.46, 54.91, 120.34, 124.10, 126.72, 127.18, 129.06, 130.74, 130.80, 133.77, 137.38, 138.47, 149.27, 149.33, 163.21; ESI-MS: m/z, 309.8 (M+1).

Synthesis of N-(4-fluorophenyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5b): Yield: 80%; IR (KBr): υ_{max} 3233.07, 3191.61, 3067.23, 2952.48, 2942.84, 2360.44, 2341.16, 1667.16, 1613.16, 1586.16, 1555.31, 1508.08, 1475.28, 1405.85, 1365.35, 1268.93, 1244.83, 1230.36, 1213.01, 1160.94, 1025.94, 1005.7, 981.59, 856.23, 830.20, 754.98, 743.42, 512.97, 476.03 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 11.92 (s, 1H), 8.64 (d, *J* = 4.8 Hz, 1H), 7.9 (d, *J* = 5.6 Hz, 1H), 7.7 (m, 2H), 7.65 (t, *J* = 6.4 Hz, 1H), 7.07 (t, *J* = 6.4 Hz, 2H), 4.29 (s, 3H), 4.2 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 54.46, 54.81, 115.53, 121.82, 121.88, 126.68, 130.64, 130.85, 133.68, 134.48, 137.34, 149.22, 149.31, 158.20, 160.13, 163.13; ESI-MS: m/z, 327.9 (M^{+1}).

Synthesis of 2, 3-dimethoxy-N-(3-(trifluoromethyl) phenyl) quinoxaline-5-carboxamide (5c): Yield: 82%; IR (KBr): υ_{max} 3185.83, 3090.37, 1671.02, 1604.48, 1586.16, 1556.27, 1521.56, 1481.06, 1446.35, 1431.89, 1409.71, 1367.26, 1334.50, 1294.0, 1235.18, 1182.15, 1162.87, 1118.51, 1069.33, 1006.66, 997.98, 982.55, 917.95, 898.66, 788.74, 762.70, 728.96, 693.28, 676.89, 586.25, 487.90, 454.15, 431.977 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.17 (br, 1H), 8.67 (d, *J* = 8.0 Hz, 1H), 8.07 (s, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.68 (t, *J* = 6.4 Hz, 1H), 7.51 (t, *J* = 6.4 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 4.3 (s, 3H), 4.2 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 54.56, 54.85, 117.01, 117.04, 120.61, 120.64, 123.21, 126.87, 129.63, 130.89, 131.24, 133.80, 137.50, 139.00, 149.45, 149.49, 163.56; ESI-MS: m/z, 377.7 (M^{+1}).

Synthesis of N-(5-fluoro-2-methylphenyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5d): Yield: 84%; IR (KBr): υ_{max} 3262.0, 3096.15, 1734.68, 1662.34, 1603.52, 1586.16, 1528.31, 1487.81, 1430.92, 1409.71, 1367.28, 1280.50, 1245.79, 1188.90, 1170.58, 1073.19, 1064.51, 1006.66, 981.59, 861.06, 814.77, 780.06, 730.88, 589.14, 453.19 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 11.3 (br, 1H), 8.64 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.22 (t, *J* = 7.0 Hz, 1H), 6.9 (d, *J* = 3.0 Hz, 1H), 4.21 (s, 3H), 4.16 (s, 3H), 2.32 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 17.77, 54.54, 54.72, 54.87, 112.88, 112.92, 113.07, 127.23, 130.92, 131.33, 131.76, 134.07, 136.96, 137.53, 149.62, 160.16, 162.09,163.93; ESI-MS: m/z, 341.7 (M^{+1}).

Synthesis of N-(4-fluoro-3-methylphenyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5e): Yield: 81%; IR (KBr): υ_{max} 3227.29, 3197.40, 3082.65, 2945.73, 1665.23, 1609.31, 1587.13, 1551.45, 1521.56, 1505.17, 1476.24, 1411.64, 1365.35, 1325.82, 1285.32, 1255.43, 1247.72, 1231.33, 1182.15, 1165.7, 1128.15, 1109.83, 1071.21, 1025.94, 1007.62, 981.59, 902.52, 888.05, 873.59, 799.35, 781.99, 759.81, 743.42, 722.21, 687.48, 676.89, 627.71, 570.82, 548.64, 530.32, 480.18, 440.65 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 11.85 (s, 1H), 8.64 (d, *J* = 1.5 Hz, 1H), 7.97 (d, *J* = 1.2 Hz, 1H), 7.65 (t, *J* = 8.0 Hz), 7.39 (d, *J* = 4.0 Hz), 7.0 (t, *J* = 9.0 Hz, 1H), 4.3 (s, 3H), 4.2 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 14.78, 54.49, 54.83, 115.07, 119.11, 123.58, 125.29, 126.77, 127.07, 130.83, 130.70, 133.76, 137.41, 149.28, 149.35, 156.90, 158.83, 163.19; ESI-MS: m/z, 341.8 (M^{+1}).

Synthesis of N-benzyl-2, 3-dimethoxyquinoxaline-5-carboxamide (5f): Yield: 86%; IR (KBr): υ_{max} 3246.57, 3071.08, 2940.91, 1954.5, 1905.33, 1655.59, 1606.41, 1584.24, 1541.81, 1518.67, 1478.17, 1428.99, 1408.75, 1367.28, 1325.82, 1282.43, 1276.65, 1244.83, 1192.76, 1185.04, 1078.98, 1026.91, 1005.7, 982.55, 829.24, 806.09, 779.10, 759.81, 700.03, 675.92, 627.71, 582.39, 539.97, 525.50, 489.83, 446.44 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6): δ 9.85 (s, 1H), 8.20 (d, *J* = 7.5 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 2H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.0 Hz, 1H), 4.62 (d, *J* = 5.5 Hz, 2H), 4.04 (s, 3H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 44.48, 53.94, 54.22, 126.43, 126.76, 127.54, 128.49, 128.61, 130.19, 133.85, 137.12, 138.31, 148.96, 149.01, 164.82; ESI-MS: m/z, 323.8 (M^{+1}).

Synthesis of 2, 3-dimethoxy-N-(4-methoxybenzyl) quinoxaline-5-carboxamide (5g): Yield: 85%; IR (KBr): υ_{max} 3271.64, 3068.19, 3007.44, 2937.06, 2830.99, 1740.44, 1657.52, 1645.95, 1609.31, 1585.2, 1536.99, 1513.85, 1471.42, 1405.85, 1397.17, 1369.21, 1323.89, 1301.72, 1242.90, 1179.26, 1172.51,

1148.4, 1033.66, 986.41, 949.77, 829.24, 760.78, 637.35, 627.71, 564.07, 522.61, 493.68, 485.00, 445.47 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6): δ 9.81 (br, 1H), 8.22 (d, *J* = 7.5 Hz, 1H), 7.89 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 4.54 (d, *J* = 5.5 Hz, 2H), 4.04 (s, 3H), 3.73 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 43.92, 54.10, 55.28, 114.02, 126.55, 126.92, 129.79, 130.25, 130.30, 130.48, 133.96, 137.24, 149.08, 149.12, 159.10, 164.85; ESI-MS: m/z, 353.9 (M^{+1}).

Synthesis of 2, 3-dimethoxy-N-phenethylquinoxaline-5-carboxamide (5h): Yield: 83%; IR (KBr): υ_{max} 3299.61, 2993.94, 1644.02, 1603.52, 1585.20, 1518.67, 1475.28, 1456.96, 1405.85, 1364.39, 1284.36, 1240.0, 1180.22, 1163.83, 1028.84, 1005.7, 981.59, 766.56, 725.10, 488.86 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 10.01 (br, 1H), 8.61 (d, *J* = 6.5 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.30 (m, 5H), 4.1 (s, 3H), 3.8 (d, *J* = 6.5 Hz, 2H), 3.6 (s, 3H), 3.0 (d, *J* = 5.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 35.99, 41.22, 53.98, 54.37, 126.45, 126.65, 127.04, 128.54, 128.93, 130.36, 130.44, 134.09, 137.35, 139.69, 149.12, 149.22, 165.42; ESI-MS: m/z, 337.8 (M^{+1}).

Synthesis of 2, 3-dimethoxy-N-(2-methoxyphenethyl) quinoxaline-5-carboxamide (5i): Yield: 87%; IR (KBr): υ_{max} 3248.5, 3062.41, 1650.77, 1603.52, 1586.16, 1544.70, 1517.70, 1479.13, 1430.92, 1406.82, 1368.25, 1282.43, 1242.90, 1119.48, 1172.51, 1157.08, 1048.12, 1023.05, 994.12, 977.73, 758.85, 747.28, 653.75, 627.71, 573.71, 489.83, 477.29 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.9(br, 1H), 8.6 (d, *J* = 6.5 Hz, 1H), 7.9 (d, *J* = 1.5 Hz, 1H), 7.6 (d, *J* = 7.5 Hz, 1H), 7.2 (t, *J* = 7.5 Hz, 2H), 6.8 (d, *J* = 3.0 Hz, 2H), 4.16 (s, 3H), 3.85 (q, *J* = 5.5 Hz, 2H), 3.74 (s, 3H), 3.66 (s, 3H), 3.0 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 30.60, 39.68, 54.02, 54.34, 54.15, 110.13, 120.5, 126.61, 127.32, 127.74, 127.81, 130.18, 130.35, 134.06, 137.30, 149.05, 157.68, 165.34; ESI-MS: m/z, 368.0 (M^{+1}).

Synthesis of N-(2-hydroxyethyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5j): Yield: 90%; IR (KBr): υ_{max} 3407.6, 3220.54, 3069.16, 2940.91, 1648.84, 1601.59, 1585.2, 1557.24, 1520.6, 1477.21, 1408.75, 1368.25, 1289.18, 1249.65, 1195.65, 1186.97, 1170.58, 1059.69, 1032.69, 1019.19, 1019.19, 974.84, 904.45, 856.23, 832.11, 782.95, 765.60, 752.10, 705.81, 673.03, 628.68, 556.36, 533.22, 494.65, 482.11, 446.44 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 10.32 (br, 1H), 8.52 (d, *J* = 6.0 Hz, 1H), 7.84 (d, *J* = 6.0 Hz, 1H), 7.56 (t, *J* = 6.2 Hz, 1H), 4.21 (s, 6H), 3.82 (m, 2H), 3.74 (m, 2H), 2.91 (br, 1H); ^{13}C NMR ($CDCl_3$): δ 41.26, 54.37, 54.76, 62.18, 126.39, 126.56, 130.16, 130.35, 130.83, 133.99, 137.09, 149.15, 166.32; ESI-MS: m/z, 277.8 (M^{+1}).

Synthesis of N-(cyclohexylmethyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5k): Yield: 86%; IR (KBr): υ_{max} 3334.32, 3082.65, 2915.84, 2849.31, 1644.02, 1603.52, 1586.16, 1546.63, 1518.67, 1473.36, 1457.92, 1444.42, 1431.89, 1406.82, 1382.71, 1366.32, 1324.86, 1288.22, 1242.9, 1194.69, 1194.69, 1174.44, 1028.84, 997.98, 987.37, 903.48, 819.59, 767.53, 752.10, 670.14, 632.53, 551.54, 525.50, 488.88 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.94 (br, 1H), 8.59 (d, *J* = 6.5 Hz, 1H), 7.92 (d, *J* = 6.5 Hz, 1H), 7.6 (t, *J* = 7.5 Hz, 1H), 4.18 (s, 6H), 3.44 (t, *J* = 6.5 Hz, 2H), 1.89-1.62 (m, 6H), 1.25-1.17 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 25.77, 26.33, 31.14, 38.16, 46.25, 54.31, 126.56, 127.28, 130.12, 133.95, 137.30, 149.10, 149.12, 165.26; ESI-MS: m/z, 329.7 (M^{+1}).

Synthesis of N-(cycloheptylmethyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5l): Yield: 89%; IR (KBr): υ_{max} 3339.14, 3018.05, 2921.63, 2850.27, 1643.05, 1602.56, 1585.20, 1547.59, 1405.85, 1365.35, 1287.25, 1241.93, 1195.65, 1173.47, 1032.69, 1024.98, 993.16, 766.56, 754.99, 632.53, 488.86 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.93 (br, 1H), 8.6 (d, *J* = 0.5 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 4.18(s, 3H), 4.17(2, 3H), 3.43(t, *J* = 6.5 Hz, 2H), 1.89-1.53 (m, 13H); ^{13}C NMR ($CDCl_3$): δ 26.18, 28.28, 32.47, 39.89, 46.54, 54.34, 54.39, 126.60, 127.31, 130.17, 130.43, 133.99, 137.34, 149.15, 165.33; ESI-MS: m/z, 344.0 (M^{+1}).

Synthesis of (2, 3-dimethoxyquinoxalin-5-yl) (piperidin-1-yl) methanone (5m): Yield: 82%; IR (KBr): υ_{max} 3236.93, 3075.90, 2979.48, 2942.84, 2858.95, 1628.59, 1586.16, 1524.48, 1476.24, 1439.60, 1362.46,

1285.32, 1246.75, 1228.43, 1189.86, 1177.33, 1122.36, 1024.02, 1018.23, 978.69, 878.41, 782.95, 760.78, 735.71, 679.78, 643.14, 549.61, 485.97, 466.68 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.76 (d, *J* = 5.6 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 6.0 Hz, 1H), 4.05 (s, 3H), 4.01 (s, 3H), 3.8-3.5 (m, 2H), 3.04 (m, 2H), 1.60 (m, 4H), 1.39-1.30 (m, 2H); ^{13}C NMR (DMSO d_6): δ 23.96, 25.32, 25.94, 41.69, 47.29, 53.73, 53.95, 124.03, 126.22, 126.63, 132.79, 134.73, 136.31, 149.54, 149.91, 166.53; ESI-MS: m/z, 301.9 (M^{+1}).

Synthesis of (2, 3-dimethoxyquinoxalin-5-yl) (morpholino) methanone (5n): Yield: 87%; IR (KBr): υ_{max} 3230.15, 3014.19, 2990.09, 2958.27, 2891.74, 2853.17, 1632.45, 1610.27, 1590.02, 1522.52, 1476.24, 1359.57, 1329.68, 1281.47, 1243.86, 1235.18, 1194.69, 1171.54, 1110.8, 1046.19, 1018.23, 979.66, 753.06, 583.36, 491.75, 464.76 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6): δ 7.78 (d, *J* = 8.5 Hz, 1H), 7.57 (t, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 7.0 Hz, 1H), 4.05 (s, 3H), 4.03 (s, 3H), 3.74 (m, 4H), 3.45 (m, 2H), 3.04 (m, 2H) ; ^{13}C NMR (DMSO d_6): δ 38.99, 41.54, 46.78, 53.86, 66.06, 66.27, 124.48, 126.62, 126.68, 132.81, 133.38, 136.28, 149.76, 150.01, 167.01; ESI-MS: m/z, 303.90 (M^{+1}).

Synthesis of 2, 3-dimethoxy-N-(2-morpholinoethyl) quinoxaline-5-carboxamide (5o): Yield: 90%; IR (KBr): υ_{max} 3273.57, 2994.91, 2939.95, 1652.7, 1603.52, 1586.16, 1534.1, 1473.35, 1456.96, 1429.96, 1397.17, 1369.21, 1328.71, 1298.82, 1250.61, 1234.22, 1187.94, 1169.62, 1143.58, 1113.69, 1021.12, 1004.73, 982.55, 942.05, 868.77, 845.633, 779.10, 530.32, 453.19, 408.83 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.96 (s,1H), 8.57 (d, *J* = 7.5 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.62 (t, *J* =8.0 Hz, 1H), 4.2 (s, 3H), 4.19 (s, 3H), 3.7 (m, 6H), 2.68 (t, *J* = 6.5 Hz, 2H), 2.5 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 36.65, 53.70, 54.43, 54.73, 58.05, 66.80, 126.69, 127.24, 130.38, 130.48, 134.17, 137.44, 149.33, 149.36, 165.62; ESI-MS: m/z, 346.8 (M^{+1}).

Synthesis of N-(2-(tert-butyl) phenyl)-2, 3-dimethoxyquinoxaline-5-carboxamide (5p): Yield: 80%; IR (KBr): υ_{max} 3436.4, 3056.14, 2953.06, 1735.83, 1664.12, 1606.90, 1587.59, 1527.18, 1488.76, 1475.47, 1430.29, 1410.67, 1371.39, 1290.71, 1252.44, 1238.25, 1189.90, 1167.34, 1023.87, 976.60,762.48 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.9(s, 1H), 8.62 (d, *J* = 6.5 Hz, 1H), 7.94 (d, *J* = 1.5 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.2 (d, *J* = 7.5 Hz, 2H), 6.81 (d, *J* = 3.0 Hz, 2H), 3.75 (m, 2H), 3.60 (t, *J* = 7.5 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 31.65, 37.2, 55.66, 55.80, 122.34, 123.4, 125.34, 129.20, 130.50, 132.3, 133.90, 132.82, 137.90, 146.20, 163.50; ESI-MS: m/z, 394.0 (M^{+1}).

Biological Assay: The newly synthesized Quinoxaline-5-carboxamide derivatives **5a-5p** were dissolved in dimethylsulphoxide at 25 μg mL^{-1} concentration and tested against two Gram negative strains *viz.*, i) *Escherichia coli* (MTCC 443), *(ii) Pseudomonas aeruginosa* (MTCC 424) and two Gram positive strains *viz.*, (iii) *Staphylococcus aureus* (MTCC 96) strains *iv) Streptococcus pyogenes* (MTCC 442 using agar well diffusion method according to the literature protocol [17-19]. The composition of nutrient agar medium was Yeast extract (5 g), NaCl (10 g), Bactotryptone (10 g), final pH 7.4. After 18 h the exponentially growing cultures of the four bacteria in nutrient broth at 37 ^oC were diluted in sterile broth. From each of these diluted cultures, 1mL was added to 100 mL sterilized and cooled nutrient agar media to give a final bacterial count of 1 x 10^6 cell/ml. The plates were set at room temperature and later dried at 37 ^oC for 20h. Paper discs (6mm, punched from whatmann no 41 paper) were ultraviolet sterilized and used for the assays. Discs were soaked in different concentration of the test solution and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. The plates were incubated at 37 ^oC in an inverted fashion. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicates.

RESULTS AND DISCUSSION

Chemistry: The newly synthesized Quinoxaline-5-carboxamide derivatives **5a – 5p** described in this paper were prepared according to the synthetic **Scheme 1**. Condensation of methyl 2,3-diamino benzoate **1** was carried out using oxalic acid mono hydride at 140^{o}C gave the methyl 2,3-dioxo-1,2,3,4-tetrahydro quinoxaline-5-carboxylate compound **2**, which upon chlorination with thionyl chloride, afforded chloro compound **3**. Hydrolysis of compound **3** was done using NaOH in methanol and water to acid derivative **4**. Compound **4** was reacted with corresponding amines using pivolyl chloride in presence of Et_3N, in DMF to furnish Quinoxaline-5-carboxamide derivatives **5 (a-p)**. The structures of the synthesized compounds were confirmed by ^{1}H NMR, Mass and ^{13} C NMR data. All the aliphatic and aromatic protons were observed at expected regions. The ^{1}H NMR data for the derivatives **5a – 5p** are in agreement with the assigned structures. The mass spectra of compounds showed (M^{+1}) peaks, in agreement with their molecular formula.

APPLICATIONS

Anti-bacterial activity: The preliminary antibacterial activity results (**Table 1**) revealed that the newly synthesized quinoxaline-5-carboxamide derivatives **5a – 5p**, showed varying pattern of inhibition against the tested microorganisms. Among the series it is observed that compounds **5b, 5c, 5d, 5e, 5j, 5n** and **5o** exhibited excellent antibacterial activity while the compounds **5k, 5l and 5m** displayed equipotent activity and remaining all other compounds shows moderate antibacterial activity against all the tested bacterial strains *viz., Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. In general, it is observed that within the quinoxaline -5-carboxamides **5a – 5p**, compounds incorporated with the following substituents such as 4-Fluoro, 3-CF_3, 5-Fluoro-2-Methyl, 4-Fluoro-3-Methyl, 2-Hydroxy ethyl, morpholine and morpholine ethyl exhibited excellent antibacterial activity while the compounds having the substituent's cyclohexyl methyl, cycloheptyl methyl and piperidin-1yl displayed equipotent activity and remaining all showed moderate antibacterial activity. From the above result it can be inferred that by varying the suitable R in the quinoxaline-5-carboxamide derivative may lead to a promising antibacterial agent.

Scheme 1. Synthesis of novel Quinoxaline-5- carboxamide derivatives **5a – 5p**

Experimental Conditions: a) Oxalic acid monohydride, methanol, 140^{o}C, 3 h; b) $SOCl_2$, DMF, THF, MeOH, reflux temp., 20 h; c) NaOH, MeOH, water, reflux temp., 16 h; d) Pivalolyl chloride, DCM, TEA, R-NH_2, r.t, 1.0 h.

Table-1: Results of Antibacterial Bioassay of Compounds **5a-5p** (Concentration Used 25 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 6a –6k in mm			
5a	Phenyl	18	12	18	12
5b	4-Fluorophenyl	28	21	27	21
5c	3-CF_3-phenyl	27	22	28	21
5d	5-F,2-Me-phenyl	29	24	29	23
5e	4-F,3-Me-phenyl	28	21	27	21
5f	Benzyl	18	15	17	15
5g	4-OMe-Benzyl	19	15	18	14
5h	Phenethyl	18	14	17	12
5i	2-MeO-Phenethyl	17	12	16	14
5j	2-Hydroxy-ethyl	29	24	29	23
5k	Cyclohexyl methyl	25	19	25	19
5l	Cycloheptyl methyl	25	19	25	19
5m	piperidine-1-yl	25	19	25	19
5n	Morpholine	27	22	28	21
5o	Morpholine-ethyl	28	23	27	23
5p	2-tery-Butyl-Phenyl	18	12	18	12
Standard Drug	**Norfloxacin** *(25 µg/mL of DMSO)*	25	19	25	19

CONCLUSIONS

In conclusion, the present paper describes the synthesis and antibacterial activity of sixteen new quinoxaline -5-carboxamide derivatives from commercially available 2, 3-diamino benzoate as staring material in four steps and was screened against four bacterial strains such *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. It is observed that within the Quinoxaline-5-carboxamide **5a-5p**, compounds incorporated with the substituents such as 4-Fluoro, 3-CF_3, 5-Fluoro-2-Methyl, 4-Fluoro-3-Methyl, 2-Hydroxy ethyl, morpholine and ethyl morpholine exhibited excellent antibacterial activity while the remaining compounds displayed moderate antibacterial activity.

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REFERENCES

[1] A. Awad Radwan, M. Wael Abdel-Mageed, *Molecules* **2014**, 19, 2247-2260.
[2] Mónica Vieira, Cátia Pinheiro, Rúben Fernandes, João Paulo Noronha, *Cristina Prudêncio. Microbiological Research.*, **2014**, 169, 287-293.

[3] A. Mostafa, Hussein. *Bull. Korean Chem. Soc.*, **2011**, 32, 1511.
[4] Dalia Hussein Soliman. *International Journal of Organic Chemistry.*, **2013**, 3, 65-72.
[5] S.Shiv Kumar, A. Khan, A. Ozair, A. Rizwan, A. Khurana, M. Shaquiquzzaman, N. Siddiqui, A. Waquar . *Bull. Korean Chem. Soc.*, **2011**, 7, 2260.
[6] E. Vicente, R. Villar, B. Solano, A. Burguete, S. Ancizu, S. Pérez-Silanes, I. Aldana, A. Monge, *An. R. Acad. Nac. Farm*., **2007**, *73*, 927–945.
[7] G. Aguirre, H. Cerecetto, R. di Maio, M. Gonzalez, M.E.M. Alfaro, A. Jaso, B. Zarranz, M.A. Ortega, I. Aldana, A. Monge-Vega. *Bioorg.Med. Chem. Lett.* **2004**, *14*, 3835–3839.
[8] C. Urquiola, M. Vieites, G. Aguirre, A. Marin, B. Solano, G. Arrambide, P. Noblia, M.L. Lavaggi, M.H. Torre, M. Gonzalez. *Bioorg. Med. Chem.*, **2006,** *14*, 5503–5509.
[9] A. Carta, M. Loriga, G. Paglietti, A. Mattana, P.L. Fiori, P. Mollicotti, L. Sechi, S. Zanetti. *Eur. J. Med. Chem,*. **2004**, *39*, 195–203.
[10] B. Ganley, G. Chowdhury, J. Bhansali, J.S. Daniels, K.S. Gates. *Bioorg. Med. Chem.*, **2001**, *9*, 2395–2401.
[11] B. Ganley, G. Chowdhury, J. Bhansali, J. S. Daniels and K. S. Gates, *Bioorg.Med.Chem.* 2001, 9, 2395- 2401.
[12] D. Benitez, M. Cabrera, P. Hernández, L. Boiani, M. L. Lavaggi, R. Di Maio, G. Yaluff, E. Serna, S. Torres, M. E. Ferreira, N. Vera de Bilbao, E. Torres, S. Pérez-Silanes, B. Solano, E. Moreno, I. Aldana, A. López de Ceráin, H. Cerecetto, M. González and A. Monge, *J.Med.Chem.*, **2011**, 54, 3624-3636.
[13] G. Aguirre, H. Cerecetto, R. Di Maio, M. González, M. E. Alfaro, A. Jaso, B. Zarranz, M. A. Ortega, I. Aldana, A. Monge-Vega. *Bioorg.Med.Chem.Lett.* **2004**,14, 3835-3839.
[14] H.M.A. El-Wahy, *Tetrahedron.*, **2000**, 56, 897.
[15] P. Ramalingam, S. Ganapaty, B. Rao Ch. *Bioorg. Med. Chem.Lett.*, **2010**, 20, 406.
[16] A.S. Khosarkar, D.B. Shinde, *Bioorg. Med. Chem. Lett.*, **2006**, 16, 6181.
[17] Navneet Kumar, Pratima Sharma, Navjeet Kaur, Aastha Pareek. An Efficient synthesis and Biological activity of Quinoxaline-2-Carboxylic acid and its derivatives *Journal of Applicable Chemistry*, 2013, 2 (2):143-149.
[18] N. Rambabu, P. K. Dubey, B. Ram and B. Balram. Synthesis and Antibacterial Activity of 2,3-dihydro-1H-benzo[f]chromen-1-one and 2,3-dihydro-1H-benzo[f]chromen-1-ol Derivatives, *Journal of Applicable Chemistry*,**2013**, 2 (6):1516-1522.
[19] Sandeep Mohanty, G. Sandeep Reddy and Arun Chandra Karmakar. Synthesis of New 5-Substituted –Aminomethylene-Thiazolidine-2,4-dione Derivatives As Potential Antibacterial Agents**.** *Journal of Applicable Chemistry***, 2014, 3 (1): 82-90.**
[20] Mohan R Nadigar, Swamy Sreenivasa, Manojkumar K.E and T Madhu Chakrapani Rao Synthesis and invitro antibacterial activity of some novel Sulfonamide derivatives bearing 1,4-disbstituted-1,2,4-oxadiazole Moiety**,** *Journal of Applicable Chemistry*., **2013**, 2 (4):722-729.