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Synthesis of Benzo[*g*]quinoxaline-5,10-dione Based Pyridine Derivatives and their Antimycobacterial Activity

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ABSTRACT

Thirteen new compounds belonging to series 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[*g*]quinoxalin-7-yl)-4-(*substituted*)phenylpyridine-3-carbonitrile (6a-m) were synthesized by multistep synthetic scheme. The newly synthesized compounds were screened for their antimycobacterial activity by L.J. Slope (Conventional) Method. Compound 6h with 4-$N(CH_3)_2$ group at phenyl ring of above mentioned position has been reported as most active antimycobacterial compound and compound 6k with 4-CH_3 substitution at phenyl above mentioned position has been reported as the least active antimycobacterial compound.

Keywords: Quinoxaline, Benzo[*g*]quinoxaline-5,10-dione, Pyridine, Antimycobacterial.

INTRODUCTION

Quinoxaline and pyridine derivatives constitute the important part of various antibiotics *viz*. hinomycin, levomycin and actinoleutin that inhibit the growth various bacteria and are found to be cytotoxic against various transplantable tumours[1-7]. Quinoxaline derivatives also exhibited a wide range of biological activities *viz.* antifungal[8-10], antibacterial[11-12], antitubercular[8-9,12-15].

Tuberculosis (TB) is a major health problem worldwide now-a-days. In year 2014, there were an estimated 9.6 million new TB cases were reported and 1.5 million deaths were caused due to TB worldwide[16]. Quinoxaline ring is also a an important part of antileprotic drug clofazimine (CZM) which is also widely indicated for treatment of multidrug resistant tuberculosis. In *Mycobacterium tuberculosis*, CZM is reduced by NADH-dehydrogenase (NDH-2) to release reactive oxygen[17] and CZM also act as competitor with menaquinone (MK-4), which acts as a key factor for its reduction by NDH-2[18]. First line antitubercular drug isoniazid (INH) and second line antitubercular drug ethionamide (Etm) are derivatives of six membered nitrogen containing

pyridine ring. Isoniazid and Ethionamide, after bioactivation by mycobacterial catalase-peroxidase enzyme complex, inhibit synthesis of mycolic acids[19-23], that are important components of mycobacterial cell wall.

EXPERIMENTAL

Chemistry

Melting points were measured in open capillary tubes and are uncorrected. All the Fourier-Transform Infra Red (FT-IR) spectra were recorded on Shimdazu FT-8400 Spectrophotometer using KBr pallets. The ^{1}H- NMR spectra were recorded on Bruker-Spectrospin DCX NMR spectrometer using DMSO-d_6 and $CDCl_3$ as solvents and tetramethylsilane (TMS) as an internal standard (Chemical shifts expressed in δ/ppm The purity of the compounds was checked by thin layer chromatography (TLC) on Merck Silica Gel 60F_{254} precoated sheets using Toluene : Ethylacetate : Formic acid (5:4:1) solution as mobile phase.

Synthesis of 2,3-Dichloronaphthalene (1)

2,3-dihydroxynaphthalene (5 gm) was dissolved in phosphorusoxychloride (90 mL) in parts at 0°-5°C with occasionally stirring and refluxed for about 4 h till the appearance of clear dark pinkish solution. This solution was cooled and poured into ice-cold water. A dark pinkish solid appeared and was filtered, dried and recrystallized with dimethylformaide (DMF). Pinkish crystalline solid; Yield 85%; R_f 0.72; mp 185°C. IR (ν_{max}, KBr, cm^{-1}): 756 (*ortho*-disubstituted phenyl ring), 856 (*meta*-disubstituted phenyl ring), 1160 (aryl C-Cl), 1384 (aromatic C=C); ^{1}H-NMR ($CDCl_3$ 300 MHz) δ : 8.16-8.20 (q, J=5-6 Hz, A_2B_2 pattern, 2H, Ar-H), 7.74-7.79 (m, J=4.5-6 Hz, 2H, Ar-H), 7.50-7.53 (m, J=1-4 Hz, 2H, Ar-H)

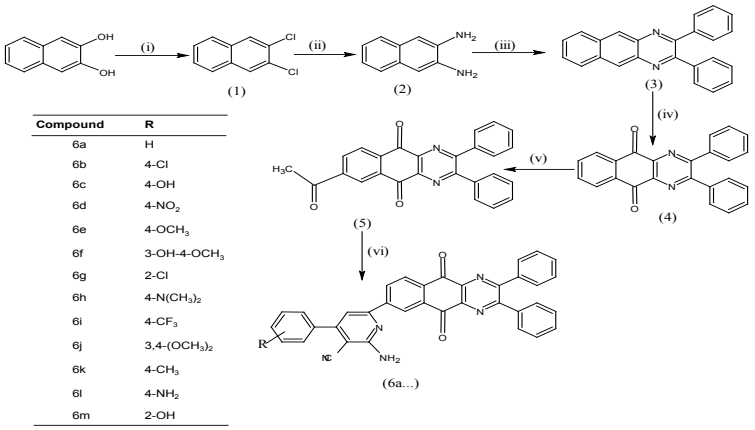
Synthesis of 2,3-Diaminonaphthalene (2)

Compound 1 (2 gm) was dissolved in absolute ethanol. Ammonium chloride (1.5 gm), dil. HCl (in catalytic amount) and excess of strong ammonium solution were added to it and refluxed for 6 h on water bath till appearing of permanent dark greenish colour. After completion of reaction, solution was cooled and kept in deep freezer for overnight.

Greenish crystals were filtered, dried and recrystallized with methanol. Dark greenish crystals; Yield 75%; R_f 0.89, R_f 0.82; mp 150°C; IR (ν_{max}, KBr, cm^{-1}): 758, 854, 1473 (aromatic =C-N), 1500 (aromatic C=C), 1681 (N-H), 3049 (N-H); ^{1}H-NMR ($CDCl_3$ 500 MHz): 7.45-7.46 (dd, J=3.5-4 Hz, 2H, Ar-H), 7.12-7.14 (dd, J=3-3.5 Hz, 2H, Ar-H), 7.09 (s, Ar-H, 2H), 7.05 (s, Ar-NH_2,4H).

2,3-Diphenylbenzo[*g*]quinoxaline (3)

Equimolar quantities of compound 2 (0.001 mol) and benzil (0.001 mol) were dissolved in ethanol (30 mL) and refluxed for 2 h. After completion of reaction, reaction mixture was cooled, kept for overnight, yellow crystalline solid was filtered, dried and recrystallized with ethanol : methanol (1:1)



Compound	R
6a	H
6b	4-Cl
6c	4-OH
6d	4-NO_2
6e	4-OCH_3
6f	3-OH-4-OCH_3
6g	2-Cl
6h	4-$N(CH_3)_2$
6i	4-CF_3
6j	3,4-$(OCH_3)_2$
6k	4-CH_3
6l	4-NH_2
6m	2-OH

Reagents & Conditions : (i) $POCl_3$, Reflux for 4 h (ii) NH_4Cl, NH_3 in excess, EtOH, Reflux at water-bath for 6 h (iii) Benzil, EtOH, Reflux for 2 h (iv) CrO_3/AcOH, Reflux for 2 h (v) CH_3COCl, CCl_4, $AlCl_3$, Reflux for 6 h (vi) Malononitrile, R-C_6H_4-CHO, CH_3OONH_4, NH_3, EtOH, dil. HCl, Reflux for 48-50 h

Fig. 1: Reaction Scheme

mixture. Yellow crystalline solid; Yield 72%; R_f 0.83; mp 170°C; IR (ν_{max},KBr, cm^{-1}) : 725, 785, 820, 875, 1475, 1510 (aromatic C=C), 1660 (quinoxaline C=N); ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.73-8.77 (m, J=2.5-4 Hz, 10H, Ar-H), 8.28 (s, 2H, Ar-H), 8.03-8.07 (m, 4H, J=2.5-4 Hz, Ar-H).

Synthesis of 2,3-Diphenylbenzo[*g*]quinoxaline-5,10-dione (4)

Compound 3 (1.2 gm) was dissolved in glacial acetic acid (30 mL) and chromium trioxide (1.1 gm) in 12 mL of glacial acetic acid : water (1:1) solution was added to it. This solution was refluxed at 80°C for 2 h and then poured into ice-chilled water (1500 mL). Resulting solid was filtered, dried, recrystallized with methanol. White solid; Yield 65%; R_f 0.78; mp 162°C; IR (ν_{max}, KBr, cm^{-1}): 718, 795, 875, 1450, 1594 (>C=O, cyclic, conjugated), 1676; ^{1}H-NMR ($CDCl_3$ 500 MHz) δ: 8.13-8.15 (d, 2H, J=8.5 Hz, Ar-H), 7.85-7.88 (t, J=2.5-10.5 Hz, 4H, Ar-H), 7.81-7.82 (d, J=8 Hz, 2H, Ar-H), 7.50-7.53 (t, J=7.5 Hz, 6H, Ar-H); Elemental Anal. Found (Calcd.) for $C_{24}H_{14}N_2O_2$: Found: C, 79.42 (79.55); H 3.91 (3.89); N 7.68 (7.73); O 8.95 (8.83).

Synthesis of 7-Acetyl-2,3-diphenylbenzo[g] quinoxaline-5,10-dione (5)

Anhydrous aluminium trichloride (1 gm) was dissolved in carbon tetrachloride (30 mL) and acetyl chloride (2 mL) was added to it under cold conditions (0°-5°C). Compound 4 (120 mg) was added and stirred at room temperature for 6 h. Yellow crystalline solid was filtered, dried and recrystallized with chloroform.

Yellow crystalline solid; Yield 48%; R_f 0.74; mp 225°C; IR (ν_{max}, KBr, cm^{-1}): 725, 775, 810, 875, 1450, 1500, 1593 (>C=O, cyclic, conjugated), 1672 (C=N, quinoxaline), 1974 (>C=O, aliphatic), 3063 (C-H, sp^3, CH_3); ^{1}H-NMR ($CDCl_3$ 500 MHz) δ: 7.97-7.98 (d, J=8 Hz, 4H, Ar-H), 7.65-7.68 (t, J=7.5 Hz, 3H, Ar-H), 7.50-7.53 (t, 6H, J=7.5-8 Hz, Ar-H), 7.26 (s, 3H, CH_3CO); MS-ESI: 404.14 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{26}H_{16}N_2O_3$: C 77.23 (77.22); H 3.98 (3.99); N 6.97 (6.93); O 11.82 (11.87).

Synthesis of 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[g]quinoxalin-7-yl)-4-(substituted) phenylpyridine-3-carbonitrile (6a-m)

Equimolar quantities of compound 5 (0.01 mol), appropriate benzaldehyde (0.01 mol), ammonium acetate (2 gm) and malononitrile (0.01 mol) and were dissolved in ethanol (30 mL) and was refluxed under anhydrous conditions for 48-50 h till the permanent appearance of crystalline solid. Solid was filtered, dried and recrystallized with chloroform.

2-Amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[g]quinoxalin-7-yl)-4-phenyl pyridine-3-carbonitrile (6a)

Yellow crystalline solid; Yield 74%; R_f 0.84; mp 230°C; IR (ν_{max}, KBr, cm^{-1}): 719, 756, 820, 875, 1296 (C=N, pyridine), 1384 (C-N), 1608 (>C=O, cyclic, conjugated), 1642 (N-H, primary amine, bend), 1697 (C=N, quinoxaline), 2358 (C-N, nitrile), 3417 (N-H, primary amine, str.); ^{1}H-NMR ($CDCl_3$ 500 MHz) δ: 12.47 (s, 1H, Ar-H), 11.91 (s, 2H, Ar-H), 11.73 (bs, 2H, Ar-NH_2), 9.03-9.04 (d, J=5 Hz, 1H, Ar-H), 8.01-8.02 (d, 2H, J=8 Hz, Ar-H), 7.75-7.79 (t, J=8-12 Hz, 3H, Ar-H), 7.59-7.63 (t, J=8.5-9.5 Hz, 4H, Ar-H), 7.12-7.30 (m, J=7.5-14.5 Hz, 6H, Ar-H); MS-ESI: 555.05 (m/z, M^+); Elemental Anal. Found (Calcd.)for $C_{36}H_{21}N_5O_2$: C, 77.80 (77.83); H, 3.82 (3.81); N, 12.60 (12.61); O, 5.76 (5.76).

2-Amino-(4-chlorophenyl)-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl} pyridine-3-carbonitrile (6b)

Yellow crystalline solid; Yield 80%; R_f 0.80; mp 232°C; IR (ν_{max}, KBr, cm^{-1}): 723, 760, 819, 875, 1162 (aryl C-Cl), 1284, 1383, 1592, 1645, 1697, 2363, 3404; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.73-8.77 (m, J=2.5-4 Hz, 10H, Ar-H), 8.03-8.07 (m, J=3.0-3.5 Hz, 4H, Ar-H), 7.70-7.78 (q, J=7.5-15 Hz, 1H, Ar-H), 7.36-7.38 (d, J=8.5 Hz, 1H, Ar-H), 7.28-7.30 (d, J=7.5 Hz, 1H, Ar-H), 7.16-7.19 (t, J=8-7.5 Hz, 1H, Ar-H), 6.94-6.96 (d, J=8 Hz, 2H, Ar-NH_2); MS-ESI: 589.08 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{20}ClN_5O_2$: C, 73.27 (73.28); H, 3.43 (3.42); Cl, 5.98 (6.01); N, 11.89 (11.87); O, 5.42 (5.42).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(4-hydroxyphenyl) pyridine-3-carbonitrile (6c)

Yellow crystalline solid; Yield 72%; R_f 0.78; mp 250°C; IR (ν_{max}, KBr, cm^{-1}) : 718, 765, 827, 895, 1256 (phenolic C-O phenolic), 1351, 1402, 1596,

1679, 1683, 2358, 3146, 3367 (O-H phenolic); ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 9.12 (s, 1H, Ar-H), 8.72-8.76 (m, J=2.5-4 Hz, 10H, Ar-H), 8.56-8.58 (d, J=9 Hz, 1H, Ar-H), 8.48-8.49 (d, J=9 Hz, 1H, Ar-H), 7.99-8.11 (t, J=3-5 Hz, 1H, Ar-H), 7.68-7.72 (dd, J=9 Hz, 2H, Ar-H), 7.44-7.49 (dd, J=9 Hz, 2H, Ar-H), 6.98 (s, 2H, Ar-NH_2), 6.86-6.88 (t, J=3-4 Hz, 1H, Ar-OH); MS-ESI: 571.11 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{21}N_5O_3$: C, 75.62 (75.65); H, 3.69 (3.70); N, 12.27 (12.25); O, 8.39 (8.40).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(4-nitrophenyl) pyridine-3-carbonitrile (6d)

Yellow crystalline solid; Yield 68%; mp 210°C; R_f 0.80; IR (ν_{max}, KBr, cm^{-1}): 720, 758, 811, 854, 1284, 1383 (-NO_2, *sym.*), 1410, 1545 (-NO_2, *asym.*), 1594, 1669, 1684, 2359, 3335; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 9.23-9.27 (dd, J=9.5 Hz, 2H, Ar-H), 9.11-9.11 (d, J=3 Hz, 1H, Ar-H), 8.87-8.92 (dd, J=9 Hz, 2H, Ar-H), 8.82-8.86 (m, J=2.5-5 Hz, 10H, Ar-H), 8.76-8.78 (d, J=11 Hz, 1H, Ar-H), 8.68-8.70 (d, J=11 Hz, 1H, Ar-H), 8.59-8.60 (t, J=3-4.5 Hz, 1H, Ar-H), 7.01 (s, 2H, Ar-NH_2); MS-ESI: 600.14 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{20}N_6O_4$: C, 71.98 (71.99); H, 3.34 (3.36); N, 14.02 (13.99); O, 10.65 (10.66).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(4-methoxyphenyl) pyridine-3-carbonitrile (6e)

White crystalline solid; Yield 74%; R_f 0.78; mp 200°C; IR (ν_{max}, KBr, cm^{-1}): 719, 758, 826, 853, 1130 (C-O-C, *sym.*), 1253 (C-O-C, *asym.*), 1340, 1420, 1595, 1645, 1677, 2360, 3315; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.73-8.76 (m, J=2.5-4 Hz, 10H, Ar-H), 8.03-8.07 (m, J=3-4 Hz, 4H, Ar-H), 7.74 (s, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.44-7.46 (d, J=7.5 Hz, 1H, Ar-H), 7.28-7.30 (d, J=7.5 Hz, 1H, Ar-H), 7.13-7.17 (t, 2H, Ar-NH_2), 3.91 (s, 3H, Ar-OCH_3); MS-ESI: 585.13 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{37}H_{23}N_5O_3$: C, 75.90 (75.89); H, 3.95 (3.96); N, 11.97 (11.96); O, (8.18) 8.20.

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(3-hydroxy-4-methoxyphenyl)pyridine-3-carbonitrile (6f)

Brown crystalline solid; Yield 72%; R_f 0.81; mp 210°C; IR (ν_{max}, KBr, cm^{-1}): 720, 757, 838, 865, 1124 (C-O-C, *sym.*), 1252 (C-O-C, *asym.*), 1295 (C-O, phenolic), 1342, 1424 (C-N), 1611, 1660, 1673, 2360, 3326, 3381 (O-H, phenolic); ^{1}H-NMR ($CDCl_3$ 500 MHz) δ: 7.80-7.81 (d, J=7 Hz, 10H, Ar-H), 7.96-7.97 (d, J=7.5 Hz, 1H, Ar-OH), 7.09 (s, 2H, Ar-NH_2), 7.33-7.36 (t, J=7.5-8 Hz, 3H, Ar-H), 7.48-7.51 (t, J=2.5-7.5 Hz, 2H, Ar-H), 7.63-7.66 (t, J=7-8 Hz, 1H, Ar-H), 7.16-7.20 (t, J=7.5-8.5 Hz, 1H, Ar-H), 3.31 (s, 3H, Ar-OCH_3); MS-ESI: 601.16 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{37}H_{23}N_5O_4$: C, 73.85 (73.87); H, 3.86 (3.85); N, 11.65 (11.64); O, 10.63 (10.64).

2-Amino-4-(2-chlorophenyl)-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl} pyridine-3-carbonitrile (6g)

White crystalline solid; Yield 66%; R_f 0.72; mp 180°C; IR (ν_{max}, KBr, cm^{-1}): 736, 761, 815, 892, 1164 (aryl C-Cl), 1311, 1363, 1591, 1662, 2356, 3305; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.74-8.76 (q, J=2.5-3.5 Hz, 4H, Ar-H), 8.04-8.07 (m, J=3.5-5 Hz, 10H, Ar-H), 7.96 (s, 1H, Ar-H), 7.91-7.93 (t, J=2.5–8.5 Hz, 1H, Ar-H), 7.68-7.71 (d, 2H, Ar-NH_2), 7.51-7.53 (d, J=8.5 Hz, 1H, Ar-H), 7.37-7.39 (d, J=8 Hz, 1H, Ar-H); MS-ESI: 589.14 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{20}ClN_5O_2$: C, 73.28 (73.28); H, 3.41 (3.42); Cl, 6.00 (6.01); N, 11.88 (11.87); O, 5.42 (5.42).

2-Amino-4-[4-(dimethylamino)phenyl]-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g] quinoxalin-7-yl}pyridine-3-carbonitrile (6h)

Lemon crystalline solid, yield 76%; R_f 0.80; mp 185°C; IR (ν_{max}, KBr, cm^{-1}): 723, 759, 819, 837, 1325, 1411, 1593, 1647, 2360, 2958 (C-H, sp^3, CH_3), 3282; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.73-8.77 (m, J=3-4 Hz, 10H, Ar-H), 8.03-8.07 (m, J=3-4 Hz, 4H, Ar-H), 8.28 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.14 (s, 2H, Ar-NH_2), 3.49 (s, 6H, N-CH_3); MS-ESI: 598.31 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{38}H_{26}N_6O_2$: C, 76.22 (76.24); H, 4.37 (4.38); N, 14.05 (14.04); O, 5.34 (5.35).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-[4-(trifluoromethyl) phenyl]pyridine-3-carbonitrile (6i)

Brown crystalline solid; Yield 78%; R_f 0.80; mp 178°C; IR (ν_{max}, KBr, cm^{-1}): 723, 741, 843, 863, 1285 (C-F), 1320, 1420, 1603, 1645, 1675, 2365, 3236; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.73-8.77 (m, J=3-4 Hz, 4H, Ar-H), 8.28 (s, 1H, Ar-H), 8.03-8.07

(m, J=3-4.5 Hz, 10H, Ar-H), 7.73-7.84 (d, J=8.5 zHzHHHHz, 1H, Ar-H), 7.35-7.37 (d, J=8 Hz, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.02-7.03 (d, J=4.5 Hz, 2H, Ar-NH_2); MS-ESI: 623.14 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{37}H_{20}FN_5O_2$: C, 71.26 (71.27); H, 3.24 (3.23); F, 9.12 (9.14); N, 11.24 (11.23); O, 5.13 (5.13).

2-Amino-4-(3,4-dimethoxyphenyl)-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl} pyridine-3-carbonitrile (6j)

White crystalline solid; Yield 66%; R_f 0.75; mp 144°C; IR (ν_{max}, KBr, cm^{-1}): 720, 752, 821, 854, 1125 (C-O-C, *sym.*), 1246 (C-O-C, *asym.*), 1337, 1418, 1608, 1645, 1682, 2357, 3246; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.98 (s, 1H, Ar-H), 8.83-8.94 (m, J=3.5-12 Hz, 10H, Ar-H), 8.11-8.12 (d, J=8 Hz, 1H, Ar-H), 8.01-8.03 (d, J=8 Hz, 1H, Ar-H), 7.92-7.94 (d, J=8.5 Hz, 1H, Ar-H), 7.83 (bs, 2H, Ar-NH_2), 7.24-7.26 (d, J=10 Hz, 1H, Ar-H), 7.21-7.31 (d, J=9.5 Hz, 1H, Ar-H), 6.91-6.93(d, J=10 Hz, 1H, Ar-H), 3.38 (s, 6H, Ar-OCH_3); MS-ESI: 615.19 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{38}H_{25}N_5O_4$: C, 74.16 (74.14); H, 4.07 (4.09); N, 11.38 (11.38); O, 10.39 (10.40).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(4-methylphenyl) pyridine-3-carbonitrile (6k)

Yellow crystalline solid; Yield 70%; R_f 0.70; mp 140°C; IR (ν_{max}, KBr, cm^{-1}): 723, 750, 817, 850, 1328, 1406, 1606, 1650, 2358, 2839 (C-H, sp^3, CH_3), 3209; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.88 (s, 1H, Ar-H), 8.56-8.58 (d, J=10 Hz, 1H, Ar-H), 8.48-8.51 (d, J=10.5 Hz, 1H, Ar-H), 8.42-8.43 (t, J=3.5-4 Hz, 1H, Ar-H), 8.11-8.18 (m, J=2.5-7.5 Hz, 10H, Ar-H), 7.82-7.99 (m, 4H, Ar-H), 6.88 (s, 2H, Ar-NH_2), 3.58-3.59 (t, J=1.5-5 Hz, 3H, CH_3); MS-ESI: 569.17 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{37}H_{23}N_5O_2$: C, 78.03 (78.02); H, 4.06 (4.07); N, 12.30 (12.29); O, 5.60 (5.62).

2-Amino-4-(4-aminophenyl)-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl} pyridine-3-carbonitrile (6l)

Yellow crystalline solid; Yield 74%; R_f 0.84; mp 180°C; IR (ν_{max}, KBr, cm^{-1}): 725, 750, 820, 860, 1325, 1415, 1650 (N-H, primary amine, bend), 1658, 2358, 3240; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 7.24-7.26 (q, J=2.5-3 Hz, 10H, Ar-H), 7.01 (s, 1H, Ar-H), 6.92-6.93 (t, J=2.5-3 Hz, 4H, Ar-H), 6.89-6.91 (d, J=10 Hz, 2H, Ar-H), 6.85 (s, 1H, Ar-H), 6.06 (bs, 4H, Ar-NH_2); MS-ESI: 570.18 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{22}N_6O_2$: C, 75.79 (75.78); H, 3.90 (3.89); N, 14.73 (14.73); O, 5.58 (5.61).

2-Amino-6-{5,10-dioxo-2,3-diphenyl-5H,10H-benzo[g]quinoxalin-7-yl}-4-(2-hydroxyphenyl) pyridine-3-carbonitrile (6m)

Yellow crystalline solid; Yield 72%; R_f 0.86; mp 220°C; IR (ν_{max}, KBr, cm^{-1}): 725, 760, 820, 870, 1280, 1311 (C-O, phenolic), 1382, 1595, 1650, 2358, 3380 (O-H, phenolic), 3406; ^{1}H-NMR (DMSO-d_6 500 MHz) δ: 8.78 (bs, 1H, Ar-H), 8.64-8.66 (d, J=10 Hz, 1H, Ar-H), 8.56-8.59 (t, J=4-10 Hz, 1H, Ar-H), 8.48-8.50 (t, J=4-5 Hz, 1H, Ar-H), 8.22-8.31 (m, J=2.5-8.5 Hz, 10H, Ar-H), 7.12-7.18 (m, J=2.5-10 Hz, 4H, Ar-H), 6.94 (bs, 2H, Ar-NH_2), 6.86-6.87 (d, J=3 Hz, 1H, Ar-OH); MS-ESI: 571.14 (m/z, M^+); Elemental Anal. Found (Calcd.) for $C_{36}H_{21}N_5O_3$: C,

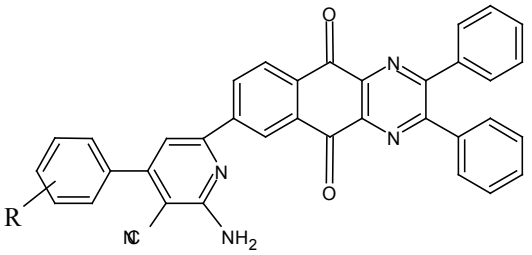


Table 1: Antimycobacterial activity of synthesized compounds against *M. tuberculosis* $H_{37}Rv$ by L. J. Slope Method

Compound	R	MIC (μg/mL)
6a	H	125
6b	4-Cl	62.5
6c	4-OH	100
6d	4-NO_2	500
6e	4-OCH_3	—-
6f	3-OH-4-OCH_3	500
6g	2-Cl	62.5
6h	4-$N(CH_3)_2$	50
6i	4-CF_3	100
6j	3,4-$(OCH_3)_2$	250
6k	4-CH_3	500
6l	4-NH_2	—-
6m	2-OH	—-
Rifampicin	—-	0.25
Isoniazid	—-	0.20

75.64 (75.65); H, 3.69 (3.70); N, 12.27 (12.25); O, 8.39 (8.40).

In-vitro Antimycobacterial Screening

Ten compounds 6a, 6b, 6c, 6d, 6f, 6g, 6h, 6i, 6j and 6k were submitted for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTCC-200) by L.J. Slope (Conventional) Method[24-25]. Each compound was diluted to 2000 ìg/mL concentration (as a stock solution). Inoculum Size for *Mycobacterium tuberculosis* was adjusted to 1 mg/mL. L.J. Medium was used as nutrient medium. Isoniazid (0.20 µg/mL) and rifampicin (0.25 µg/mL) were used as standard drug.

Primary screen

In primary screening, the compounds were diluted to 500 µg/mL, 250 µg/mL and 125 µg/mL concentrations. Compounds found active in this primary screening were further tested in a second set of dilution against *Mycobacterium tuberculosis*.

Secondary screen

The compounds found active in primary screening were further diluted to 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.250 µg/mL, 3.125 µg/mL and 1.5625 µg/mL concentrations.

Reading Result

The highest dilution showing at least 99% inhibition was considered as Minimum Inhibitory Concentration. The inoculum size of test should contain 10^8 organism/mL.

RESULT and DISCUSSIONS

Chemistry

Thirteen new compounds belonging to series 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[*g*]quinoxalin-7-yl)-4-(*substituted*) phenylpyridine-3-carbonitrile (6a-m) were synthesized by multistep synthetic scheme (Figure 1). 2,3-Dihydroxynaphthalene on refluxing with phosphorus oxychloride yielded 2,3-dichloronaphthalene (1). Compound 1 on refluxing with ammonium chloride in excess of ammonia, catalytic amount of dil. HCl and ethanol undergoes amination (*nucleophilic substitution*) & furnished 2,3-diaminonaphthalene (2). Compound 2 on refluxing with equimolar quantity of benzil in ethanol undergoes nucleophilic addition followed by dehydration to yield 2,3-diphenylbenzo[*g*] quinoxaline (3). Compound 3 undergoes aromatic oxidation at C-5 and C-10 on refluxing with equimolar mixture of chromium trioxide and glacial acetic acid in water, till the permanent appearance of dark green colour, furnished 2,3-diphenylbenzo[*g*]quinoxaline-5,10-dione (4). Compound 4 on acetylation reaction with acetylchloride and anhydrous aluminium chloride in carbon tetrachloride yields 7-acetyl-2,3-diphenylbenzo[*g*]quinoxaline-5,10-dione (5). Compound 5 on refluxing with equimolar quantity of substituted aromatic aldehydes, ammonium acetate, malononitrile, ammonia and catalytic amount of dil. HCl undergoes *Heitzch-Pyridine synthesis* to yield 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[*g*]quinoxalin-7-yl)-4-(*substituted*) phenylnicotinonitrile (6a-m). Structure of all newly synthesized thirteen compounds were confirmed by FT-IR, ^{1}H-NMR, MS-ESI spectral data interpretation and their elemental analysis.

Biological Activity (In-vitro Antimycobacterial Screening)

The ten newly synthesized compounds *viz*. 6a, 6b, 6c, 6d, 6f, 6g, 6h, 6i, 6j and 6k were screened for their antimycobacterial screening against *Mycobacterium tuberculosis* $H_{37}Rv$ by L.J. Slope (Conventional) Method to observe the effect of substitution at phenyl ring attached to C-4 of pyridine ring of 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo[*g*]quinoxalin-7-yl)-4-(*substituted*) phenylpyridine-3-carbonitrile (Compound 6a-m). Isoniazid and Rifampicin were used as standard drugs.

Unsubstituted phenyl ring (6a) on the 4-position of pyridine ring in above mentioned nucleus exhibited moderate antimycobacterial activity. Substitution with electron donating *para*-dimethylamino group (6h) on the phenyl ring present at C-4 of pyridine ring increases antimycobacterial activity and produces the most active antimycobacterial compound of the above mentioned newly synthesized series. Substitution with electron withdrawing 4-Cl group (6b) and 2-Cl (6g) at the phenyl ring also increases the antimycobacterial activity but lesser than that of substitution with *para*-dimethylamino group (6h). Substitution with *para*-hydroxy (6c) and electron withdrawing *para*-trifluoromethyl (6i) at the pheny

ring exhibits moderate activity lesser than that of compounds 6h, 6g and 6b and more than that of compound with unsubstituted phenyl ring (6a). While, substitution with bulky electron withdrawing group *para*-nitro (6d), electron donating *para*-methyl (6k) and bulky electron donating groups 3-OH-4-OCH_3 (6f) and 3,4-dimethoxy (6j) decreases the antimycobacterial activity to great extent and produces the least active compounds of the newly synthesized series.

From the above observations, Structure Activity Relationship (SAR) may be established as "substitution with smaller electron withdrawing and donating groups at the *ortho* and *para* positions of phenyl ring attached to C-4 of pyridine ring, present at 7-position of 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo**[***g***]**quinoxalin-7-yl)-4-**(***substituted***)**phenylpyridine-3-carbonitrile increases antimycobacterial activity, while substitution with bulky electron donating groups at the phenyl ring attached to above mentioned position decreases antimycobacterial activity.**"**

CONCLUSION

Among the newly compounds synthesized compounds of series phenyl ring of 2-amino**-**6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo**[***g***]**quinoxalin-7-yl)-4-(*substituted*)phenylpyridine-3-carbonitrile (6a-m), which were screened for antimycobacterial screening against *Mycobacterium tuberculosis* $H_{37}Rv$ by L. J. Slope (Conventional) Method, compound 6h with 4-$N(CH_3)_2$ at phenyl ring attached at pyridine group of 2-amino-6-(5,10-dioxo-2,3-diphenyl-5,10-dihydrobenzo**[***g***]**quinoxalin-7-yl)-4-**(***substituted***)** phenylpyridine-3-carbonitrile (6a-m) exhibited the maximal potency, while compounds with 4-Cl (6b) and 2-Cl (6g) substitutions at the above mentioned position exhibited better antimycobacterial potency as compared to other compounds. Compounds with 4-OH (6c), 4-CF_3 (6i) and with no substitution (6a) at phenyl ring of the above mentioned position exhibited moderate antimycobacterial activity, while compounds with 3,4-$(OCH_3)_2$ (6j), 4-NO_2 (6d), 3-OH-4-OCH_3 (6f) and 4-CH_3 (6k) showed minimal antimycobacterial potency against *Mycobacterium tuberculosis* $H_{37}Rv$ strain under observation.

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