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Microwave and conventional synthesis of novel pyrido[2,3-*d*]pyrimidine scaffold as an antimicrobial agent

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Abstract: Different novel 8-(4-fluorophenyl)-2-methyl-4,7-dioxo-5-phenyl 3,4,7,8-tetrahydropyrido[2,3-*d*] pyrimidine-6-carbonitriles (**5a-j**) were prepared by the acidic catalytic cyclization of substituted 6-amino-1-(4-fluorophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5 dicarbonitriles (**4a-j**) under conventional heating as well as in microwave irradiation, both the methods were compared. All the reactions were found to be significantly faster and the isolated yields were remarkably higher in microwave conditions compared to the conventionally heated reactions. All the synthesized compounds were characterized by their spectral study and were tested for their antimicrobial and anti fungal activity. The compounds **5h, 5g** and **5j** exhibited significant activity against *B. subtillus,* compounds **5i, 5e, 5f** and **5h** showed good activity against *M. letus* while compounds **5h, 5g, 5a** and **5c** exhibited significant activity against *C.albicans*, **5h** against *T. longifusus* and **5g**, **5c**, **4d, 4f** and **4g** against *A. niger* . The compounds **5h** and **5g** were found to be active against almost all the species tested when compared with the standards.

Keywords: Microwave irradiation; glacial acetic acid; pyrido[2,3-*d*]pyrimidines; antimicrobial activity

1. Introduction

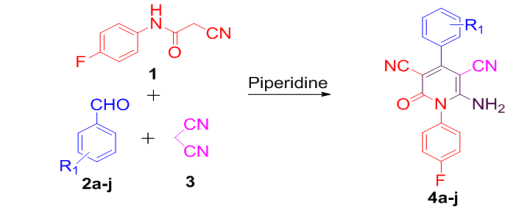
Bicyclic nitrogen-containing heterocyclic compounds, such as purines [1-3], quinazolines [4-8], pyridopyrimidines [9, 10] are well-known pharmacophores in drug discovery. From the past few years, research on pyridopyrimidines revealed that these derivatives bear wide range of biological application such as antibacterial [11-13], antifungal [14] and analgesic and anti-inflammatory [15] activities. In the area of modern crop protection, fluoro agrochemicals are widely employed as herbicides, insecticides and fungicides [16]. Pyrido[2,3-*d*]pyrimidines have been the most thoroughly investigated from the four possible ring systems and hence, this scaffold is associated with a wide range of biological activities, such as HCV NS5A inhibitor

[5] protein tyrosine phosphatase 1B inhibitors, tyrosine kinase c-Met inhibitors [17], CCR4 antagonists [18], potential antiproliferative agents [19] , anti tumor activity [20, 21] and Potent Inhibitor of Cyclin-Dependent Kinase 4 (CDK4) and AMPK-Related Kinase 5 (ARK5) [22]. Mean while, microwave assisted reactions are getting more and more popularity day by day, when they are compared with conventional heating, it can be seen that the microwave conditions accelerated the reaction rates, enhanced the yields and limited the formation of undesired byproducts [23-27]. Moreover it has allowed difficult synthetic transformations to be achieved under milder conditions [28, 29]. The combination of solvent-free reaction conditions and microwave irradiation leads to large reduction in reaction time, enhancement in conversion and sometimes in selectivity with several advantages of the eco-friendly approach, termed green chemistry [30]. The wide range of activity profile of pyrido[2,3-*d*] pyrimidines probed us to synthesize novel analogues. Herein, we report the solvent free synthesis of 8-(4-fluorophenyl)-2-methyl-4,7-dioxo-5-phenyl3,4,7,8-tetrahydropyrido[2,3-*d*] pyrimidine-6-carbonitril (**5a-j**) under conventional heating and microwave irradiation. The many fold importance of these derivatives and their nucleosides and the interest in this area has attracted attention to explore some new pyrido[2,3-*d*]pyrimidine derivatives and their nucleosides with the goal to achieve some novel biological effective compounds.

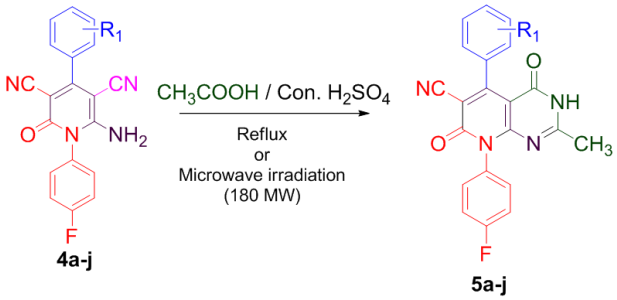
2. Experimental

2.1 *Materials, methods and instruments*

All the reagents were purchased commercially and were used with further purification. Analytical grade solvents from Spectrochem were used. Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC (Mercksilica gel PF_{254} plates) of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GCMS-QP-2010 model using Direct Injection Probe technique. ^{1}H-NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer data are reported in order: multiplicity (bs, broad singlet; s, singlet; d, doublet; t, triplet; m, multiplet). ^{13}C-NMR was determined in DMSO-d_6 solution on a Bruker Ac 100 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.



Scheme 1. Synthesis of 2-cyno-*N*-aryl acetamides



Scheme 2. Synthesis of pyrido[2,3-*d*] pyrimidines

2.2 *General Procedure for Synthesis of 6-Amino-1-(4-fluorophenyl)-2-oxo-4-phenyl-1,2 dihydro pyridine-3,5 dicarbonitrile (4a-i).*

A mixture of 10mmol of compound 2-cyano-N-(4-fluorophenyl) acetamide (**1**), 10 mmol of appropriate aromatic aldehydes (**2a-j**) and 10 mmol of malononitrile (**3**) were refluxed for 15-16h in 20ml of methanol under catalytic condition (piperidine). The reaction mixture was kept at room temperature for 2-4 hour. The solid product obtained was isolated and recrystallized from ethanol **(4a-4j)**.

2.2a *6-amino-1-(4-fluorophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile* **(4a)** Yield: 75%; MP: 238-240^{0}C; IR (ν_{max}, cm^{-1}): 3382, 3317 (N-H str. of Pri. Amine), 3200 (C-H str.), 2202 (C≡N str. Nitrile group), 1610 (C=O str.), 1452 (C=C- str.), 1250 (C-F str.), 830 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 7.13 - 7.50 (9H, m), 7.92(1H, s, NH_2); ^{13}C NMR(200MHz, DMSO-d_6): 74.5, 114.3, 114.8, 127.6, 128.9, 131.8, 158.4, 165.7, 171.6; MS, *m/z* (%) = 330, 284, 234, 203, 186, 158, 142; Elemental Analysis for $C_{19}H_{11}FN_4O$; Cal: C, 69.09; H, 3.36; N, 16.96%; Found: C, 68.95; H, 3.32; N, 16.90%.

2.2b *6-amino-1-(4-fluorophenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5- dicarbonitrile* **(4b)** Yield: 80%; MP: 240-244^{0}C; IR (ν_{max}, cm^{-1}): 3384, 3317 (N-H str. of Pri. Amine), 3201 (C-H str.), 2209 (C≡N str. Nitrile group), 1608 (C=O str.), 1455 (C=C- str.), 1254 (C-F str.), 1172(C-O-C str.), 830 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 3.85 (3H, s, OCH_3), 7.11 - 7.14 (2H, d, J = 12Hz), 7.41 – 7.52 (6H, m), 7.90(1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 54.9, 70.1, 113.8, 114.3, 122.6, 126.3, 128.9, 156.3, 157.4, 165.9, 170.7; MS, *m/z* (%) = 360, 344, 330, 315, 265, 203, 186, 158, 142, 107; Elemental Analysis for $C_{20}H_{13}FN_4O_2$; Cal: C, 66.66; H, 3.64; N, 15.55%; Found: C, 66.56; H, 3.54; N, 15.45%.

2.2c *6-amino-1-(4-fluorophenyl)-4-(2-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4c)** Yield: 75%; MP: 260-262^{0}C; MS: *m/z* 360; IR (ν_{max}, cm^{-1}): 3384, 3317 (N-H str. of Pri. Amine), 3201 (C-H str.), 2209 (C≡N str. Nitrile group), 1608 (C=O str.), 1455 (C=C str.), 1254 (C-F str.), 1172 (C-O-C str.), 833 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 3.82 (3H, s, OCH_3), 7.20 – 7.62 (8H, m), 7.90(1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 53.2, 70.1, 114.4, 114.9, 118.3, 126.9, 127.9, 157.1, 166.3, 170.7; MS, *m/z* (%) = 360, 344, 330, 315, 265, 203, 186, 158, 142, 107; Elemental Analysis for $C_{20}H_{13}FN_4O_2$; Cal: C, 66.66; H, 3.64; N, 15.55%; Found: C, 66.58; H, 3.54; N, 15.50%.

2.2d *6-amino-1-(4-fluorophenyl)-4-(4-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4d)** Yield: 70%; MP: 266-268^{0}C; MS: *m/z* 375; IR (ν_{max}, cm^{-1}): 3380, 3311 (N-H str. of Pri. Amine), 3201 (C-H str.), 2201 (C≡N str. Nitrile group), 1606 (C=O str.), 1540 (N=O Str.), 1459 (C=C str.), 1258 (C-F str.), 830 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 7.25 – 7.52 (8H, m), 7.90(1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 73.2, 116.3, 120.4, 123.7, 133.2, 134.9, 151.1, 153.4, 160.1, 173.4; MS, *m/z* (%) = 375, 359, 330, 280, 203, 186, 158,142,123; Elemental Analysis for $C_{19}H_{10}FN_5O_3$; Cal: C, 60.80; H, 2.69; N, 18.66%; Found: C, 60.75; H, 2.63; N, 18.60%.

2.2e *6-amino-1-(4-fluorophenyl)-4-(2-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4e)** Yield: 79%; MP: 255-258^{0}C; MS: *m/z* 375; IR (ν_{max}, cm^{-1}): 3380, 3311 (N-H str. of Pri. Amine), 3204 (C-H str.), 2201 (C≡N str. Nitrile group), 1606 (C=O str.), 1540 (N=O Str.), 1459 (C=C- str.), 1258 (C-F str.), 830 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 7.25 – 7.52 (8H, m), 7.90(1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 73.1, 114.2, 122.6, 126.5, 131.3, 132.9, 152.1, 154.6, 163.9, 170.1; MS, *m/z* (%) = 375, 359, 330, 280, 203, 186, 158,142,123; Elemental Analysis for $C_{19}H_{10}FN_5O_3$; Cal: C, 60.80; H, 2.69; N,

18.66%; Found: C, 60.78; H, 2.65; N, 18.56%.

2.2f *6-amino-1-(4-fluorophenyl)-2-oxo-4-(p-tolyl)-1,2-dihydropyridine-3,5-dicarbonitrile* **(4f)** Yield: 82%; MP: 257-259^{0}C; MS: *m/z* 344; IR (ν_{max}, cm^{-1}): 3385, 3317 (N-H str. of Pri. Amine), 3200 (C-H str.), 2905 (C-H str.), 2202 (C≡N str. Nitrile group), 1610 (C=O str.), 1452 (C=C str.), 1250 (C-F str.), 830 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 1.25 (3H, s, CH_3), 7.15 - 7.28 (2H, d, *J* = 12Hz), 7.38 – 7.70 (6H, m), 8.03 (1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 25.1, 70.1, 114.5, 166.9, 124.4, 127.6, 133.1, 133.9, 135.7, 152.3, 162.8, 173.1; MS, *m/z* (%) = 344, 330, 298, 249, 203, 186, 158, 142, 92; Elemental Analysis for $C_{20}H_{13}FN_4O$; Cal: C, 69.76; H, 3.81; N, 16.27%; Found: C, 69.70; H, 3.71; N, 16.23%.

2.2g *6-amino-1-(4-fluorophenyl)-4-(4-bromophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4g)** Yield: 75%; MP: 249-252^{0}C; MS: *m/z* 408; IR (ν_{max}, cm^{-1}): 3384, 3316 (N-H str. of Pri. Amine), 3207 (C-H str.), 2201 (C≡N str. Nitrile group), 1606 (C=O str.), 1459 (C=C str.), 1258 (C-F str.), 830 (disubstituted), 540(C-Br str.); ^{1}H NMR (400 MHz, DMSO-d_6): 7.03 - 7.15 (2H, d, *J* = 11Hz), 7.20 – 7.41 (6H, m), 7.81 (1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 73.1, 112.7, 120.7, 123.8, 128.1, 129.9, 156.9, 157.7, 160.1, 172.9; MS, *m/z* (%) = 408, 363, 330, 313, 203, 186, 158, 155; Elemental Analysis for $C_{19}H_{10}BrFN_4O$; Cal: C, 55.77; H, 2.46; N, 13.69%; Found: C, 55.70; H, 2.40; N, 13.66%.

2.2h *6-amino-1-(4-fluorophenyl)-4-(3-bromophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4h)** Yield: 72%; MP: 227-230^{0}C; MS: *m/z* 408; IR (ν_{max}, cm^{-1}): 3384, 3316 (N-H str. of Pri. Amine), 3207 (C-H str.), 2201 (C≡N str. Nitrile group), 1606 (C=O str.), 1459 (C=C str.), 1258 (C-F str.), 830 (disubstituted), 540(C-Br str.); ^{1}H NMR (400 MHz, DMSO-d_6): 7.10 – 7.63 (8H, m), 8.04 (1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 73.7, 113.1, 122.5, 123.1, 126.8, 130.1, 155.1, 158.1, 162.9, 170.9; MS, *m/z* (%) = 408, 363, 330, 313, 203, 186, 158, 155; Elemental Analysis for $C_{19}H_{10}BrFN_4O$; Cal: C, 55.77; H, 2.46; N, 13.69%; Found: C, 55.74; H, 2.42; N, 13.64%.

2.2i *6-amino-1-(4-fluorophenyl)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4i)** Yield: 85%; MP: 240-242^{0}C; MS: *m/z* 364; IR (ν_{max}, cm^{-1}): 3388, 3311 (N-H str. of Pri. Amine), 3208 (C-H str.), 2209 (C≡N str. Nitrile group), 1600 (C=O str.), 1450 (C=C- str.), 1252 (C-F str.), 830 (disubstituted), 710(C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): 7.13 - 7.22 (2H, d, *J* = 11Hz), 7.35 – 7.64 (6H, m), 8.10 (1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 67.9, 112.1, 112.9, 123.7, 126.2, 128.9, 134.0, 159.9, 160.1, 171.6; MS, *m/z* (%) =365, 330, 319, 269, 203, 186,158, 117; Elemental Analysis for $C_{19}H_{10}ClFN_4O$; Cal: C, 62.56; H, 2.76; N, 15.36%; Found: C, 62.51; H, 2.70; N, 15.32%.

2.2j *6-amino-1-(4-fluorophenyl)-4-(3-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile* **(4j)** Yield: 71%; MP: 221-223^{0}C; MS: *m/z* 364; IR (ν_{max}, cm^{-1}): 3388, 3311 (N-H str. of Pri. Amine), 3208 (C-H str.), 2209 (C≡N str. Nitrile group), 1600 (C=O str.), 1450 (C=C-str.), 1252 (C-F str.), 830 (disubstituted), 710(C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): 7.10 – 7.70 (8H, m), 8.12 (1H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): 70.9, 112.9, 113.1, 124.1, 127.9, 129.1, 134.7, 160.1, 160.9, 173.1; MS, *m/z* (%) =365, 330, 319, 269, 203, 186,158, 117; Elemental Analysis for $C_{19}H_{10}ClFN_4O$; Cal: C, 62.56; H, 2.76; N, 15.36%; Found: C, 62.46; H, 2.56; N, 15.30%.

2.3 *General method for the Synthesis of 8-(4-fluorophenyl)-2-methyl-4,7-dioxo-5-(substituted phenyl)-3,4,7,8-tetrahydro pyrido[2,3-d] pyrimidine-6-carbonitrilee (**5a-i**).*

10 mmol of compound **4a-j** was dissolved in 20 ml of acetic acid which was used as self solvent. Catalytic amount of conc. Sulphuric acid was added to promote the reaction. The reaction mixture was heated on oil bath at reflux temperature; the same reaction mixture was irradiated at 180 MW in microwave under TLC analysis. After completion of the reaction, the reaction mixture was cooled to room temperature; separated product was filtered, washed with methanol and crystallized from DMF to afford the desired products **(5a-j)**.

2.3a *8-(4-fluorophenyl)-2-methyl-4,7-dioxo-5-phenyl3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5a)** Yield: 80%; MP: 338-340^{0}C; MS: *m/z* 372; IR (ν_{max}, cm^{-1}): 3110 (N-H str. of sec. amine), 2905 (C-H str.), 2221 (C≡N str. nitrile group), 1690 (C=O str.), 1591 (C=C- str.), 1479 (C=N-str.) 1246 (C-F str.), 831 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 2.13 (3H, s, CH_3), 7.05 - 7.40 (9H, m), 12.6 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 23.2, 100.0, 113.1, 114.6, 126.9, 128.1, 129.9, 130.4, 151.1, 156.7, 160.1, 164.7, 168.7; MS, *m/z* (%) = 372, 342,323, 315, 301, 277, 232, 248, 208, 174, 143;Elemental Analysis for $C_{21}H_{13}FN_4O_2$:Cal: C, 67.74; H, 3.52; N, 15.05%; Found: C, 67.70; H, 3.48; N, 15.00%.

2.3b *8-(4-fluorophenyl)-5-(4-methoxyphenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5b)** Yield: 71%; MP:>350^{0}C; MS: *m/z* 402; IR (ν_{max}, cm^{-1}): 3068 (N-H str. of sec. amine), 2944 (C-H str.), 2226 (C≡N str. nitrile group), 1684 (C=O str.), 1597 (C=C- str.), 1479 (C=N-str.) 1250 (C-F str.), 1166 (C-O-C str.), 831 (disubstituted);^{1}H NMR (400 MHz, DMSO-d_6): 2.16 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 7.01-7.04 (2H, d, *J*=12.5Hz), 7.29 – 7.39 (6H, m), 12.5 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.1, 52.7, 101.7, 112.1, 116.7, 123.1, 128.9, 133.4, 150.1, 154.7, 155.1, 163.2, 167.7, 171.4; MS, *m/z* (%) = 402, 373, 353, 345, 333, 307, 279, 263, 239, 174, 143;Elemental Analysis for $C_{22}H_{15}FN_4O_3$; Cal: C, 65.67; H, 3.76; N, 13.92%; Found: C, 65.62; H, 3.71; N, 13.82; %.

2.3c *8-(4-fluorophenyl)-5-(2-methoxyphenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5c)** Yield: 82%; MP: 342-345^{0}C; MS: *m/z* 402; IR (ν_{max}, cm^{-1}): 3068 (N-H str. of sec. amine), 2944 (C-H str.), 2226 (C≡N str. nitrile group), 1684 (C=O str.), 1597 (C=C- str.), 1479 (C=N-str.) 1250 (C-F str.), 1166 (C-O-C str.), 831 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 2.16 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 7.12 – 7.62 (8H, m), 12.6 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 22.9, 53.1, 100.8, 110.7, 114.3, 120.1, 126.7, 127.4, 151.2, 153.6, 158.2, 160.1, 166.9, 171.7; MS, *m/z* (%) = 402, 373, 353, 345, 333, 307, 279, 263, 239, 174, 143;Elemental Analysis for $C_{22}H_{15}FN_4O_3$:Cal : C, 65.67; H, 3.76; N, 13.92%; Found: C, 65.60; H, 3.68; N, 13.80%.

2.3d *8-(4-fluorophenyl)-2-methyl-5-(4-nitrophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5d)** Yield: 78%; MP: 315-318^{0}C; MS: *m/z* 417; IR (ν_{max}, cm^{-1}): 3120 (N-H str. of sec. amine), 2920 (C-H str.), 2208 (C≡N str. nitrile group), 1692 (C=O str.), 1590 (C=C- str.), 1570 (N=O Str.), 1458 (C=N-str.) 1259 (C-F str.), 831 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 2.18 (3H, s, CH_3), 7.13-7.15 (2H, d, *J*=12Hz), 7.32 – 7.43 (6H, m), 12.2 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.9, 107.3, 113.2, 114.6, 121.7, 126.9, 128.1, 135.4, 142.9, 152.8, 154.4, 156.3, 161.8, 163.7, 170.1; MS, *m/z* (%) = 417, 388, 368, 360, 348, 322, 294, 278, 254, 174, 143; Elemental Analysis for $C_{21}H_{12}FN_5O_4$: Cal: C, 60.43; H, 2.90; N, 16.78%; Found: C, 60.38; H, 2.89; N, 16.72; %.

2.3e *8-(4-fluorophenyl)-2-methyl-5-(2-nitrophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5e)** Yield: 85%; MP: 328-332^{0}C; MS: *m/z* 417; IR (ν_{max}, cm^{-1}): 3120 (N-H str. of sec. amine), 2920 (C-H str.), 2208 (C≡N str. nitrile group), 1692 (C=O str.), 1590 (C=C- str.), 1570 (N=O Str.), 1458 (C=N-str.) 1259 (C-F str.), 831 (disubstituted); ^{1}H NMR (400 MHz, DMSO-d_6): 2.11 (3H, s, CH_3), 7.15– 7.59 (2H, m), 12.8 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 28.3, 103.2, 114.1, 116.9, 122.8, 127.1, 131.1, 133.9, 143.2, 153.6, 154.8, 156.8, 160.7, 166.1, 169.1; MS, *m/z* (%) = 417, 388, 368, 360, 348, 322, 294, 278, 254, 174, 143; Elemental Analysis for $C_{21}H_{12}FN_5O_4$: Cal:C, 60.43; H, 2.90; N, 16.78; %. Found: C, 60.36; H, 2.87; N, 16.70; %.

2.3f *8-(4-fluorophenyl)-2-methyl-4,7-dioxo-5-(p-tolyl)-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5f)** Yield: 80%; MP: 336-340^{0}C; MS: *m/z* 386; IR (ν_{max}, cm^{-1}): 3072 (N-H str. of sec. amine), 2933 (C-H str.), 2228 (C≡N str. nitrile group), 1678 (C=O str.), 1590 (C=C- str.), 1478 (C=N-str.) 1246 (C-F str.), 831 (disubstituted);^{1}H NMR (400 MHz, DMSO-d_6): 2.19 (3H, s, CH_3), 2.28 (3H, s, CH_3), 7.03-7.05 (2H, d, *J*=11.8Hz), 7.26 – 7.52 (6H, m), 12.6 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 20.2, 24.6, 106.3, 114.2, 115.1, 126.9, 127.1, 130.7, 137.6, 137.9, 153.2, 158.4, 161.1, 167.3, 169.9; MS, *m/z* (%) = 386, 357, 337, 329, 317, 291, 263, 247, 223, 174, 143;Elemental Analysis for $C_{22}H_{15}FN_4O_2$: Cal: C, 68.39; H, 3.91; N, 14.50; %.Found: C, 68.32; H, 3.88; N, 14.41; %.

2.3g *5-(4-bromopnenyl)-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile* **(5g)** Yield: 68%; MP: 343-346^{0}C; MS: *m/z* 450; IR (ν_{max}, cm^{-1}): 3098 (N-H str. of sec. amine), 2932 (C-H str.), 2201 (C≡N str. nitrile group), 1684 (C=O str.), 1595 (C=C-str.), 1452 (C=N-str.) 1250 (C-F str.), 831 (disubstituted); 582 (C-Br str.); ^{1}H NMR (400 MHz, DMSO-d_6): 2.08 (3H, s, CH_3), 7.0-7.06 (2H, d, *J*=12.2Hz), 7.20 – 7.48 (6H, m), 12.8 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.9, 105.3, 114.9, 115.8, 120.3, 125.3, 128.9, 130.1, 150.4, 152.6, 160.7, 164.2, 168.7; MS, *m/z* (%) = 450, 420, 401, 393, 379, 355, 326, 310, 286, 174, 143; Elemental Analysis for $C_{21}H_{12}BrFN_4O_2$: Cal: C, 55.89; H, 2.68; N, 12.42; %Found: C, 55.82; H, 2.61; N, 12.41; %.

2.3h *5-(3-bromopnenyl)-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5h)** Yield: 72%; MP:>350^{0}C; MS: *m/z* 450; IR (ν_{max}, cm^{-1}): 3098 (N-H str. of sec. amine), 2932 (C-H str.), 2201 (C≡N str. nitrile group), 1684 (C=O str.), 1595 (C=C- str.), 1452 (C=N-str.) 1250 (C-F str.), 831 (disubstituted), 582 (C-Br str.); ^{1}H NMR (400 MHz, DMSO-d_6): 2.09 (3H, s, CH_3), 7.05 – 7.58 (8H, m), 12.7 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.7, 104.9, 115.6, 121.2, 125.7, 128.3, 129.6, 129.9, 150.8, 154.3, 164.9, 169.3; MS, *m/z* (%) = 450, 420, 401, 393, 379, 355, 326, 310, 286, 174, 143; Elemental Analysis for $C_{21}H_{12}BrFN_4O_2$: Cal: C, 55.89; H, 2.68; N, 12.42; %.Found: C, 55.85; H, 2.60; N, 12.40;%.

2.3i *5-(4-chloropnenyl)-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5i)** Yield: 74%; MP: 350-352^{0}C; MS: *m/z* 406; IR (ν_{max}, cm^{-1}): 3074 (N-H str. of sec. amine), 2938 (C-H str.), 2229 (C≡N str. nitrile group), 1688 (C=O str.), 1585 (C=C-str.), 1483 (C=N-str.) 1257 (C-F str.), 831 (disubstituted); 732 (C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): 2.12 (3H, s, CH_3), 7.06-7.08 (2H, d, *J*=12.8Hz), 7.22 – 7.48(6H, m), 12.4 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 23.9, 105.9, 114.8, 128.6, 128.9, 129.1, 135.1, 150.1, 152.4, 160.2, 160.8, 162.8, 172.7; MS, *m/z* (%)

= 406, 377, 358, 350, 336, 312, 283, 267, 174, 143; Elemental Analysis for $C_{21}H_{12}ClFN_4O_2$: Cal: C, 62.00; H, 2.97; N, 13.77; %. Found: C, 61.82; H, 2.90; N, 13.72; %.

2.3j *5-(3-chloropnenyl)-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d] pyrimidine-6-carbonitrile* **(5j)** Yield: 69%; MP:>350^{0}C; MS: *m/z* 406; IR (ν_{max}, cm^{-1}): 3074 (N-H str. of sec. amine), 2938 (C-H str.), 2229 (C≡N str. nitrile group), 1688 (C=O str.), 1585 (C=C- str.), 1483 (C=N-str.) 1257 (C-F str.), 831 (disubstituted); 732 (C-Cl str.);^{1}H NMR (400 MHz, DMSO-d_6): 2.09 (3H, s, CH_3), 7.01– 7.62 (8H, m), 12.7 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.1, 103.8, 113.6, 128.1, 128.7, 129.7, 133.6, 134.7, 153.9, 154.1, 159.7, 160.1, 166.7, 170.1; MS, *m/z* (%) = 450, 420, 401, 393, 379, 355, 326, 310, 286, 174, 143; Elemental Analysis for $C_{21}H_{12}ClFN_4O_2$:Cal: C, 62.00; H, 2.97; N, 13.77; %.Found: C, 61.92; H, 2.93; N,13.70; %.

Table 1. Preparation of 2-cyno-*N*-arylacetamides

Product	R_1	M.W (gm/mol)	M.P. (^{0}C)	Time (hr)	yield[a] (%)
4a	H	330	238-240	10	75
4b	4-OCH_3	360	240-244	14	80
4c	2-OCH_3	360	260-262	15	75
4d	4-NO_2	375	266-268	16	70
4e	2-NO_2	375	255-258	18	79
4f	4-CH_3	344	257-259	16	82
4g	4-Br	408	249-252	8	75
4h	3-Br	408	227-230	9	72
4i	4-Cl	364	240-242	12	79
4j	3-Cl	364	221-223	12	71

[a]Isolated yield in DMF

Table 2. Comparison of microwave and conventional heating

Code	R_1	M.P.(^{0}C)	Conventional		Microwave		R_f[c]
			Time (hr)	Yield[a] (%)	Time[b] (min)	Yield[a] (%)	
5a	H	338-340	20	41	27	76	0.56
5b	4-OCH_3	>350	24	30	30	79	0.50
5c	2-OCH_3	342-345	24	25	32	77	0.53
5d	4-NO_2	315-318	29	34	29	78	0.45
5e	2-NO_2	328-332	27	34	29	70	0.48
5f	4-CH_3	336-340	30	35	33	80	0.52
5g	4-Br	343-346	18	52	23	74	0.49
5h	3-Br	>350	19	49	24	72	0.52
5i	4-Cl	350-352	21	43	25	71	0.58
5j	3-Cl	>350	21	39	25	69	0.54

[a] Isolated yield in DMF, [b] Continuous irradiation, [c] Solvent system : n-Hexane: Ethyl acetate - 6:4

2.4 *Antimicrobial activity*

These days the microbes are getting resistance to the mostly available drugs in the market so it is necessary to find out the new potent class of antimicrobial agents. The lack of development of new antimicrobial drugs is a potential serious threat to public health [31]. The wide range of activity profile of pyrido[2,3-*d*]pyrimidines probed us to test and study the biological activities of some of the synthesized novel analogues. The newly synthesized compounds **4a-j** to **5a-j** were tested in *vitro* for their antibacterial activity against *Escherichia coli, Bacillus subtillus, Staphylococcus aureus*, and *Micrococcus luteus* bacteria at different concentration (25, 50, 100 µg/mL) with by the agar well diffusion method [32], DMSO was used as a control solvent and Ampicillin, chloramphenicol and ciprofloxacin as standard drugs. Each purified compound was dissolved in DMSO, sterilized using filteration and stored at 4 °C. Each agent was then added to molten nutrient agar in the 0 (control), 25, 50 and 100 µg/mL concentration and poured into sterile petridish. The pH of media was adjusted

Table 3. Antibacterial activity of compounds **4a-j** and **5a-j**

No.	E. *coli* Conc. (µg/mL)			B. *subtillus* Conc. (µg/mL)			S. *aureus* Conc. (µg/mL)			M.*letus* Conc. (µg/mL)		
	25	50	100	25	50	100	25	50	100	25	50	100
4a	10	11	12	11	12	14	11	12	14	11	13	14
4b	11	12	13	11	11	13	12	12	13	11	12	14
4c	8	11	12	9	10	10	11	13	14	9	11	12
4d	7	10	11	8	12	11	10	11	11	10	11	11
4e	7	10	10	8	10	12	8	10	12	8	10	13
4f	11	14	14	12	12	13	12	13	14	12	12	13
4g	12	13	15	12	14	15	11	14	16	10	13	14
4h	12	14	14	11	13	13	10	13	13	12	14	14
4i	10	12	12	8	10	13	12	12	13	13	14	15
4j	10	11	11	11	11	12	10	11	11	11	12	12
5a	12	13	14	12	14	14	12	13	15	12	13	16
5b	11	14	16	13	15	16	10	12	14	12	13	15
5c	9	13	14	12	13	14	11	12	15	10	12	14
5d	10	12	14	12	14	14	10	13	13	10	12	14
5e	10	13	15	14	12	15	9	11	12	11	12	16
5f	12	15	16	14	14	16	13	14	15	12	13	16
5g	13	15	18	15	16	19	12	13	17	12	15	15
5h	12	15	16	16	18	20	10	14	14	13	16	16
5i	11	14	15	10	12	13	12	14	14	14	18	20
5j	11	13	14	13	16	18	11	12	13	12	13	15
Amp	15	15	19	18	19	20	13	14	16	14	16	17
Chl	17	22	23	16	19	22	14	19	21	16	18	22
Cip	23	28	28	19	20	22	19	21	21	20	22	24

Zone diameter of growth inhibition (mm) after 24 h,
Amp - Ampicillin, Chl - Chloramphenicol and Cip – Ciprofloxacin

in the range of 7.2-7.4. These were inoculated on nutrient agar plates containing the increase amount of the compounds, incubated at 37 °C up to 24h for the determination of minimum inhibitory concentration (MIC). After 24-h incubation at 37 °C, the zone of inhibition was measured in mm. The results are depicted in Table 3. All the synthesized compounds were also screened in *vitro* for their antifungal activity against three species *Candida albicans*, *Trichphytonlongifusus* and *Aspergillusniger* using the agar plate technique [32] compared with Griseofulvin. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after 7 days. The amount of growth inhibition in each case was calculated as percentage inhibition. The results are shown in Table 4.

3. Results and discussion

3.1. Chemistry

There are several strategies to prepare pyrido[2,3-*d*]pyrimidines e.g. the one pot reaction of 6-amino-1, 2, 3, 4-tetrahydro-2, 4-dioxopyrimidine, proper aldehyde and malononitrile (or ethyl cyanoacetate) or cyliczation of properly substituted pyridine nucleus with phenyl iso(thio)cyanate/formic acid [33]. Even the cyclocondensation of α,β-unsaturated esters, amidine systems and malononitrile (or ethyl cyanoacetate) yields the desired product [34,35].

Table 4. Antifungal activity of compounds **4a-j** and **5a-j**

No.	C. *albicans* Conc. (µg/mL)			T. *longifusus* Conc. (µg/mL)			A. *Niger* Conc. (µg/mL)		
	25	50	100	25	50	100	25	50	100
4a	14	15	15	14	15	16	16	16	18
4b	15	16	16	13	14	15	17	18	19
4c	14	14	15	14	16	17	14	16	18
4d	12	13	14	15	15	17	16	19	20
4e	14	15	16	14	14	16	15	15	17
4f	15	15	18	12	13	14	14	17	20
4g	15	16	17	12	14	16	16	17	20
4h	13	15	16	14	15	15	14	16	18
4i	14	15	16	15	16	18	15	16	17
4j	14	14	14	16	16	17	13	15	16
5a	17	19	19	15	16	18	14	16	17
5b	16	16	17	12	14	16	16	17	19
5c	18	18	19	16	18	19	18	18	20
5d	14	15	16	12	14	16	14	16	19
5e	16	16	16	14	16	16	15	16	17
5f	16	17	18	14	16	17	16	17	17
5g	18	18	20	16	19	22	19	21	24
5h	19	20	22	19	20	23	18	20	21
5i	18	19	20	17	18	20	19	21	23
Gri	21	22	22	20	22	25	23	25	25

Conc. of sample 200 µg/mL in DMSO at 27 °C, Incubation period 7 days.
Gri – Griseofulvin

Here in we have reported the synthesis of pyrido[2,3-*d*]pyrimidine with cyclization of cynopyridones (**4a-j**) with glacial acetic acid under catalytic conditions. The study began with synthesis of required starting material 2-cyano-*N*-(4-fluorophenyl)acetamide (**1**) [36,37] which was refluxed with 2-methoxy benzaldehyde (**2**) and malonitrile (**3)** in basic conditions (scheme 1) to yield **4c**. To set the protocol for the synthesis of pyrido[2,3-*d*]pyrimidines, **4c** was reacted with glacial acetic acid in presence of a catalytic amount of sulfuric acid (H_2SO_4) under reflux conditions for 24 hours (under TLC analysis), afforded **5c** in poor yields (25%) while when the same reaction was carried out in microwave conditions gave higher yields (77%) in just 32 minutes (Table 2).

To expand the course of this reaction and in order to make the compound libraries more diverse, different starting materials were synthesized. For that we subsequently selected compound **1** and ten different aldehydes (**2a-j**), over all ten reactions were performed and isolated yield of corresponding **4a-j** is shown in Table 1.The cyclization reactions of **4a-j** were performed on various magnetron power of microwave and maximum yield was found at 180 MW (Scheme 2). There was a clear improvement in using microwave heating over conventional heating in all of our studied substrates (Table 2). The reaction time for microwave-assisted reactions was up to fifteen times shorter than for comparable reactions under conventional heating. Shorter reaction time, higher isolated yields and more simplified product purification was observed with comparison to conventional heating method. As shown in Table 2, we can see that a series of aldehydes bearing either electron-withdrawing or electron donating groups perform equally well in the reaction.

All the synthesized compounds were characterized by spectroscopic methods and elemental analysis. The final derivatives **5a-j** where confirmed form following observations: IR spectra of each compound showed characteristic CN stretching vibrations near 2220 cm^{-1}, while secondary amine and carbonyl of amide shown around 3200 cm^{-1} and 1670 cm^{-1} respectively. In case of ^{1}H-NMR the chemical shift value of secondary amine proton appeared at 12.7 ppm. The ^{13}C NMR showed methyl group signal around 24 ppm, two peaks of cyclic amide carbonyl groups of pyridine and pyrimidine rings around 166 and 170 ppm respectively. All the synthesized compounds showed [M+] of 100% intensity as the molecular ion peak.

3.2. Antimicrobial activityt

From the antimicrobial study it was found that almost all compounds were active against the microorganism tested. It is worth noting here that compounds **5h, 5g** and **5j** exhibited significant activity against *B. subtillus,* compounds **5i, 5e, 5f** and **5h** showed good activity against *M. letus*. The other compounds showed moderate-to-low activity. The structure–activity relationship (SAR) shows that the presence of cyclic amide group increases the activity especially when phenyl ring (other than *N*-phenyl ring) is substituted with a bromine or chlorine at 3 or 4th position. The results shown in Table 4 indicated that compounds **5h, 5g, 5a** and **5c** exhibited significant activity against C.*albicans*, **5h** against T. *longifusus* and **5g**, **5c**, **4d, 4f** and **4g** against A. *niger*. It is worth noting that compounds **5h** and **5g** exhibited significant (maximum) antibacterial and antifungal activities, possibly because of the presence of bromine substitution at the 3and 4th position of aromatic ring, in addition to the cyclic amide group.

4. Conclusions

In the present article, we have reported the synthesis, spectral studies, antibacterial and antifungal activities of a novel series of

substituted pyrido[2,3-*d*]pyrimidine scaffold. These were characterized by IR, NMR, mass spectrometry study and elemental analyses. The substrates were obtained in good yields and in short reaction times under microwave conditions. Moreover the reactions are simple, one pot and also give excellent yields at larger scales. The compounds **5h** and **5g** exhibited significant (maximum) antibacterial and antifungal activities, which may develop into the potential class of antimicrobial agents.

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