Journal of the Korean Chemical Society
2014, *Vol. 58, No. 4*
Printed in the Republic of Korea
http://dx.doi.org/10.5012/jkcs.2014.58.4.366

One Pot Synthesis of Novel Cyanopyridones as an Intermediate of Bioactive Pyrido[2,3-*d*]Pyrimidines

Taslimahemad T. Khatri* and Viresh H. Shah†

Department of Chemistry, KSKV Kachchh University, Mundra road, Bhuj 370 001, Gujarat, India.
**E-mail: khatri_taslim@yahoo.com*
†Department of Chemistry, Saurashtra University, Kalawad road, Rajkot 360 005, Gujarat, India
(Received February 26, 2014; Accepted May 29, 2014)

ABSTRACT. Synthesis, structural characterization, and biological activity studies of novel pyrido[2,3-*d*]pyrimidines (**10a–h**, **11a–h**) are described. Cyclization of cynoacetamides (**4**, **5**) with malonitrile (**7**) and aldehyde (**6a–h**) via Hantzsch pyridine synthesis afforded cyanopyridones (**8a–h**, **9a–h**), which on cyclization with formic acid under microwave conditions led to the final product. All the reactions are significantly faster and the isolated yields are remarkably higher in microwave conditions compared to the conventionally heated reactions. The compounds were tested in vitro for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtillus*, *Staphylococcus aureus*, and *Micrococcus luteus* and antifungal activity against *Trichphyton longifusus*, *Candida albicans*, *Microsporum canis*, *Fusarium solani*. Compounds **10b**, **10e**, **11b** and **11e** exhibited good antibacterial and antifungal activities compared with standards.

Key words: Microwave-assisted synthesis, 2-Cyano-*N*-phenylacetamides, Pyrido[2,3-*d*]pyrimidines, Antibacterial activity, Antifungal activity

INTRODUCTION

In the past few years, heating and driving chemical reactions by microwave energy has been an increasingly popular theme in the scientific community because it increases reaction rates and yields under milder conditions.[1–5] The combination of solvent-free reaction conditions and microwave irradiation leads to large reduction in reaction times, enhancement in conversion and sometimes in selectivity with several advantages of the eco-friendly approach, termed green chemistry.[6,7] Bicyclic nitrogen-containing heterocyclic compounds, such as purines[8–10] quinazolines[11–13] and pyridopyrimidines[14–17] are well-known pharmacophores in drug discovery. Pyrido[2,3-*d*]pyrimidines have been the most thoroughly investigated of the four possible pyrido pyrimidine ring systems and hence, this scaffold is associated with a wide range of biological activities, such as dihydrofolate reductase (DHFR) inhibitory activity, antitumor activity,[18–21] adenosine kinase inhibition[22] and tyrosine kinase inhibition.[23]

Keeping in mind our previous efforts[24] and the biomedical applications of pyrido[2,3-*d*]pyrimidines, with a view to further assess the pharmacological profile of this class of compounds, it was thought worthwhile to synthesize some new congeners of this class. Herein, we report the solvent free approach to synthesis of 4,7-dioxo-5,8-diphenyl-3,4,7,8-tetrahydropyrido[2,3-*d*]pyrimidine-6-carbonitriles (**10**, **11**) under microwave irradiation with high yields. Results from assessment of the antimicrobial activity of these newly synthesized compounds are reported in this study.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC (Kieselgel 60, F_{254}) of 0.5 mm thickness and spots were located by iodine and UV. The microwave-assisted reactions were realized in a QPro-M microwave synthesizer. 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 and ^{13}C NMR were determined in DMSO-d_6 solution on a Bruker Ac 400 MHz FT NMR spectrometer with TMS as internal standard. 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.

General procedure for synthesis of 2-cyano-N-phenylacetamide (4, 5)

10 mmol of aromatic amines (**1**, **2**) and 20mmol of cyanoacetic acid ester (**3**) were refluxed for 8–10 h on oil bath in solvent free condition (under TLC analysis). After com-

pletion of the reaction, the reaction mixture was cooled to room temperature; separated product was filtered, washed with methanol and crystallized from methanol to afford the desired products **4**, **5**.

General procedure for synthesis of 6-amino-1,2-dihydro-4-(aryl)-2-oxo-1-(aryl)pyridine-3,5-dicarbonitriles (8, 9)

10 mmol of 2-cyano-*N*-phenylacetamides (**4**, **5**), 10 mmol of aromatic aldehyde (**6a–h**) and malononitrile (**7**) were dissolved in 20 ml of methanol. The reaction mixture was heated on water bath for 8–16 h using piperidine as catalyst[24] (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 **8**, **9**. The compound **9d** is reported in literature.[25]

6-amino-4-(4-methoxyphenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8a)

White crystalline solid, IR (KBr): υ_{max} 3350 & 3280 (NH_2), 2908 (C=C), 2815 (OCH_3), 2225 (CN), 1708 (CO), 1640 (C=C), 1563, 1213 (C–O), 841 cm^{-1}. MS: *m/z* 342, 311, 300, 265, 251, 235, 164, 158, 150, 142, 107, 77. Anal. Calcd. for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37%. Found: C, 70.12; H, 4.07; N, 16.31%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.56 (s, –NH_2, 2H), 7.56–7.58 (d, *J* = 8.0 Hz, 2CH), 7.44–7.51 (m, 3CH), 7.38–7.41 (d, *J* = 8.4 Hz, 2CH), 6.82–7.84 (d, *J* = 8.4 Hz, 2CH), 2.52 (s, –OCH_3, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.8 (C–ArOMe), 169.4 (C–O), 158.1 (C=O), 157.4 (C–NH_2), 131.7 (C–N), 130.0 (2CH), 128.8 (C), 127.4 (2CH), 126.2 (CH), 125.3 (2CH), 123.1 (2CH), 116.8 (C), 115.3 (CN), 113.4 (CN), 72.1 (C), 55.2 (OCH_3).

6-amino-4-(4-chlorophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8b)

White crystalline solid, IR (KBr): υ_{max} 3410 & 3315 (NH_2), 2968 (C=C), 2287 (CN), 1720 (CO), 1613 (C=C), 1539, 839 cm^{-1}. MS: *m/z* 347, 311, 304, 254, 202, 164, 142, 111, 77. Anal. Calcd. for $C_{19}H_{11}ClN_4O$: C, 65.81; H, 3.20; N, 16.16%. Found: C, 65.78; H, 3.17; N, 16.12%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.12 (s, –NH_2, 2H), 7.77–7.80 (d, *J* = 8.4 Hz, 2CH), 7.48–7.51 (d, *J* = 7.8 Hz, 2CH), 7.41–7.43 (t, *J* = 7.8 Hz, CH), 7.28–7.30 (t, *J* = 8.0 Hz, 2CH), 6.99–7.02 (d, *J* = 8.0 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.2 (C–ArCl), 161.4 (CO), 153.5 (C–NH_2), 151.2 (C–Cl), 134.2 (C–N), 134.1 (2CH), 132.2 (C), 126.9 (2CH), 126.4 (CH), 126.2 (2CH), 124.3 (2CH), 117.2 (C), 116.1 (CN), 114.8 (CN), 74.4 (C).

6-amino-4-(3-chlorophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8c)

White crystalline solid, IR (KBr): υ_{max} 3594 & 3267 (NH_2), 2953 (C=C), 2227 (CN), 1715 (CO), 1621 (C=C), 1602, 1549, 808 cm^{-1}. MS: *m/z* 347, 320, 311, 269, 304, 254, 202, 189, 164, 142, 111, 77. Anal. Calcd. for $C_{19}H_{11}ClN_4O$: C, 65.81; H, 3.20; N, 16.16%. Found: C, 65.77; H, 3.18; N, 16.11%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.69 (s, –NH_2, 2H), 7.51–7.54 (t, *J* = 10.4 Hz, CH), 7.39–7.43 (d, *J* = 10.0 Hz, 2CH), 7.36–7.39 (t, *J* = 8.4 Hz, CH), 7.32–7.34 (t, *J* = 8.4 Hz, CH), 7.28 (s, CH), 7.12–7.14 (d, *J* = 8.4 Hz, CH), 7.06–7.09 (d, *J* = 10.0 Hz, 2CH). ^{13}C NMR (DMSO- d_6, 100 MHz): δ 175.5 (C–ArCl), 165.4 (CO), 157.2 (C–NH_2), 156.1 (C–Cl), 136.5 (C), 135.4 (C–N), 133.3 (CH), 132.1 (CH), 128.4 (CH), 128.0 (CH), 127.4 (CH), 126.8 (2CH), 125.5 (2CH), 119.3 (C), 117.6 (CN), 114.2 (CN), 76.1 (C).

6-amino-4-(2-chlorophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8d)

White crystalline solid, IR (KBr): υ_{max} 3339 & 3281 (NH_2), 3001 (C=C), 2270 (CN), 1680 (CO), 1676 (C=C), 1512, 780 cm^{-1}. MS: *m/z* 347, 311, 304, 254, 209, 202, 164, 142, 111, 77. Anal. Calcd. for $C_{19}H_{11}ClN_4O$: C, 65.81; H, 3.20; N, 16.16%. Found: C, 65.79; H, 3.16; N, 16.13%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.87 (s, –NH_2, 2H), 7.62–7.58 (t, *J* = 11.2 Hz, 2CH), 7.52–7.55 (d, *J* = 11.2, 2CH), 7.30–7.27 (m, 3CH), 7.22–7.18 (d, *J* = 9.6, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.0 (C–ArCl), 158.2 (CO), 155.2 (C–NH_2), 152.8 (C–Cl), 137.4 (CH), 136.2 (C), 135.2 (CH), 132.6 (C–N), 131.1 (CH), 130.0 (2CH), 129.2 (2CH), 129.0 (CH), 126.2 (CH), 118.9 (C), 117.1 (CN), 115.7 (CN), 72.1(C).

6-amino-4-(4-bromophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8e)

White crystalline solid, IR (KBr): υ_{max} 3384 & 3260 (NH_2), 2921 (C=C), 2261 (CN), 1712 (CO), 1653 (C=C), 1551, 841 cm^{-1}. MS: *m/z* 391, 373, 347, 321, 312, 164, 154, 142, 77. Anal. Calcd. for $C_{19}H_{11}BrN_4O$: C, 58.33; H, 2.83; N, 14.32%. Found: C, 58.28; H, 2.78; N, 14.27%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.91 (s, –NH_2, 2H), 7.89–7.92 (d, *J* = 8.0 Hz, 2CH), 7.69–7.73 (d, *J* = 8.0 Hz, 2CH), 7.31–7.33 (t, *J* = 8.4 Hz, CH), 7.22–7.25 (t, *J* = 8.4 Hz, 2CH), 7.19–7.22 (d, *J* = 8.4 Hz, 2CH). ^{13}C NMR (DMSO- d_6, 100 MHz): δ 170.6 (C–ArBr), 162.1 (CO), 155.8 (C–NH_2), 153.1 (C–Br), 136.6 (2CH), 136.2 (C–N), 130.6

(2CH), 127.5 (C), 126.8 (2CH), 126.1 (CH), 126.0 (2CH), 118.5 (C), 116.7 (CN), 114.2 (CN), 73.0 (C).

6-amino-4-(4-nitrophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8f)

Yellow crystalline solid, IR (KBr): υ_{max} 3410 & 3313 (–NH_2), 2893 (C=C), 2312 (CN), 1680 (CO), 1620 (C=C), 1555 (–NO_2), 1456, 1350 (NO_2), 839 cm^{-1}. MS: *m/z* 357, 341, 315, 311, 280, 235, 164, 122, 77. Anal. Calcd. for $C_{19}H_{11}N_5O_3$: C, 63.86; H, 3.10; N, 19.60%. Found: C, 63.81; H, 3.07; N, 19.57%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.84 (s, –NH_2, 2H), 8.32–8.35 (d, *J* = 10.4 Hz, 2CH), 7.70–7.73 (d, *J* = 10.0 Hz, 2CH), 7.48–7.51 (d, *J* = 8.4 Hz, 2CH), 7.41–7.44 (t, *J* = 8.4 Hz, 2CH), 7.19–7.22 (t, *J* = 8.4 Hz, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.4 (C–$ArNO_2$), 160.5 (CO), 158.2 (C–NH_2), 154.6 (C–NO_2), 140.1 (2CH), 139.2 (2CH), 132.7 (C-N), 126.4 (C), 126.1 (2CH), 125.4 (CH), 125.1 (2CH), 117.9 (C), 115.5 (CN), 114.2 (CN), 72.5 (C).

6-amino-4-(3-nitrophenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (8g)

Yellow crystalline solid, IR (KBr): υ_{max} 3420 & 3250 (NH_2), 2961 (C=C), 2222 (CN), 1721 (CO), 1641 (C=C), 1597, 1550 (NO_2), 1429, 1346 (NO_2), 814 cm^{-1}. MS: *m/z* 357, 331, 311, 280, 238, 235, 164, 122, 77. Anal. Calcd. for $C_{19}H_{11}N_5O_3$: C, 63.86; H, 3.10; N, 19.60%. Found: C, 63.80; H, 3.07; N, 19.55%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.72 (s, –NH_2, 2H), 8.22–8.25 (d, *J* = 11.2 Hz, CH), 8.01 (s, CH), 7.77–7.80 (t, *J* = 10.0 Hz, CH), 7.59–7.61 (d, *J* = 10.0 Hz, CH), 7.42–7.44 (d, *J* = 8.0 Hz, 2CH), 7.38–7.41 (t, *J* = 8.0 Hz, 2CH), 7.19–7.22 (t, *J* = 8.4 Hz, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.0 (C–$ArNO_2$), 166.1 (CO), 158.0 (C–NH_2), 154.0 (C–NO_2), 143.8 (CH), 134.4 (CH), 133.1 (C–N), 132.0 (CH), 129.2 (C), 128.0 (CH), 124.2 (2CH), 123.8 (2CH), 123.2 (CH), 118.7 (C), 116.4 (CN), 115.0 (CN), 74.5 (C).

6-amino-2-oxo-1-phenyl-4-(p-tolyl)-1,2-dihydropyridine-3,5-dicarbonitrile (8h)

White crystalline solid, IR (KBr): υ_{max} 3436 & 3320 (NH_2), 3089 (C=C), 2949 (CH_3), 2307 (CN), 1706 (CO), 1620 (C=C), 1540, 1430, 840 cm^{-1}. MS: *m/z* 326, 311, 284, 249, 164, 91, 77. Anal. Calcd. for $C_{20}H_{14}N_4O$: C, 73.61; H, 4.32; N, 17.17%. Found: C, 73.57; H, 4.28; N, 17.13%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.71 (s, –NH_2, 2H), 7.31–7.33 (d, *J* = 10.4 Hz, 2CH), 7.24–7.26 (d, *J* = 8.0 Hz, 2CH), 7.22–7.24 (d, *J* = 10.4 Hz, 2CH), 7.12–7.17 (m, 3CH), 2.23 (s, CH_3, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.8 (C–ArMe), 164.1 (CO), 155.8 (C–NH_2), 138.2 (C–Me), 137.7 (2CH), 134.6 (C–N), 131.6 (CH), 128.1 (C), 126.0 (2CH), 125.2 (2CH), 124.4 (2CH), 116.0 (C), 114.2 (CN), 114.0 (CN), 75.7 (C), 20.3 (CH_3).

6-amino-1-(4-chlorophenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9a)

White crystalline solid, IR (KBr): υ_{max} 3387 & 3325 (NH2), 3011 (C=C), 2974 (CH_3), 2231 (CN), 1712 (CO), 1610 (C=C), 1540, 1204 (C–O), 1108, 837 cm^{-1}. MS: *m/z* 376, 345, 341, 334, 269, 265, 158, 111, 107. Anal. Calcd. for $C_{20}H_{13}ClN_4O_2$: C, 63.75; H, 3.48; N, 14.87%. Found: C, 63.70; H, 3.46; N, 14.84%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.10 (s, –NH_2, 2H), 7.68–7.70 (d, *J* = 8.4 Hz, 2CH), 7.56–7.58 (d, *J* = 8.0 Hz, 2CH), 7.48–7.51 (d, *J* = 8.0 Hz, 2CH), 7.02–7.05 (d, *J* = 8.4 Hz, 2CH), 2.88 (s, CH_3, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.4 (C–ArCl), 170.2 (C–O), 158.6 (CO), 157.2 (C–NH_2), 138.3 (C–Cl), 137.6 (C), 134.8 (C–N), 130.2 (2CH), 128.0 (2CH), 127.0 (2CH), 125.4 (2CH), 116.4 (C), 115.1 (CN), 114.8 (CN), 75.0 (C), 58.2 (OCH_3).

6-amino-1,4-bis(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9b)

White crystalline solid, IR (KBr): υ_{max} 3495 & 3331 (NH_2), 3101 (C=C), 2239 (CN), 1715 (CO), 1641 (C=C), 1515, 842 cm^{-1}. MS: *m/z* 381,364, 345, 338, 303, 269, 158, 111. Anal. Calcd. for $C_{19}H_{10}Cl_2N_4O$: C, 59.86; H, 2.64; N, 14.70%. Found: C, 59.81; H, 2.63; N, 14.66%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.00 (s, –NH_2, 2H), 7.59–7.63 (d, *J* = 10.4 Hz, 2CH), 7.45–7.48 (d, *J* = 8.4 Hz, 2CH), 7.40–7.42 (d, *J* = 8.4 Hz, 2CH), 7.26–7.29 (d, *J* = 10.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.6 (C–ArCl), 169.8 (CO) 156.8 (C–NH_2), 140.0 (C–Cl), 136.1 (C–N), 136.0 (C–Cl), 135.4 (2CH), 134.6 (2CH), 134.0 (C), 129.0 (2CH), 128.4 (2CH), 117.2 (C), 115.1 (CN), 114.7 (CN), 75.1 (C).

6-amino-4-(3-chlorophenyl)-1-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9c)

White crystalline solid, IR (KBr): υ_{max} 3412 & 3325 (NH_2), 3008 (C=C), 2301 (CN), 1720 (CO), 1646 (C=C), 1550, 812 cm^{-1}. MS: *m/z* 381, 364, 338, 303, 158, 111. Anal. Calcd. for $C_{19}H_{10}Cl_2N_4O$: C, 59.86; H, 2.64; N, 14.70%. Found: C, 59.83; H, 2.62; N, 14.64%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.88 (s, –NH_2, 2H), 7.59–7.61 (d, *J* = 7.8 Hz, 2CH), 7.50–7.52 (d, *J* = 7.8 Hz, 2CH), 7.39–7.41 (d, *J* = 8.4 Hz, CH), 7.34–7.38 (t, *J* = 8.4 Hz, CH), 7.28 (s, CH), 7.10–7.13 (d, *J* = 8.4 Hz, CH). ^{13}C NMR

(DMSO-d_6, 100 MHz): δ 177.8 (C–ArCl), 170.4 (CO), 160.2 (C–NH_2), 148.4 (C–Cl), 135.4 (2CH), 134.8 (C–Cl), 132.2 (C–N), 130.8 (2CH), 129.6 (CH), 129.0 (CH), 128.6 (C), 128.0 (CH), 127.7 (CH), 116.5 (C), 115.2 (CN), 115.0 (CN), 75.4 (C).

6-amino-4-(4-bromophenyl)-1-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9e)

White crystalline solid, IR (KBr): υ_{max} 3462 & 3345 (NH_2), 3014 (C=C), 2312 (CN), 1714 (CO), 1640 (C=C), 1561, 838 cm^{-1}. MS: *m/z* 426, 389, 372, 345, 329, 312, 269, 158, 155, 111. Anal. Calcd. for $C_{19}H_{10}BrClN_4O$: C, 53.61; H, 2.37; N, 13.16%. Found: C, 53.58; H, 2.35; N, 13.14%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.95 (s, –NH_2, 2H), 7.71–7.73 (d, *J* = 8.4 Hz, 2CH), 7.52–7.56 (d, *J* = 10.4 Hz, 2CH), 7.44–7.46 (d, *J* = 10.4 Hz, 2CH), 7.29–7.32 (d, *J* = 8.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.0 (C–ArBr), 162.5 (CO), 156.7 (C–NH_2), 155.8 (C–Br), 136.4 (C–Cl), 135.2 (2CH), 133.6 (2CH), 131.4 (C–N), 130.2 (C), 129.6 (2CH), 128.8 (2CH), 119.2 (C), 116.4 (CN), 115.8 (CN), 72.7 (C).

6-amino-1-(4-chlorophenyl)-4-(4-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9f)

Yellow crystalline solid, IR (KBr): υ_{max} 3436 & 3354 (NH_2), 3011 (C=C), 2249 (CN), 1713 (CO), 1634 (C=C), 1558, 1343, 848 cm^{-1}. MS: *m/z* 392, 356, 345, 340, 329, 280, 269, 158, 122, 111. Anal. Calcd. for $C_{19}H_{10}ClN_5O_3$: C, 58.25; H, 2.57; N, 17.88%. Found: C, 58.22; H, 2.54; N, 17.82%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.75 (s, –NH_2, 2H), 8.28–8.31 (d, *J* = 8.0 Hz, 2CH), 7.70–7.73 (d, *J* = 8.0 Hz, 2CH), 7.55–7.58 (d, *J* = 8.4 Hz, 2CH), 7.41–7.44 (d, *J* = 8.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.6 (C–$ArNO_2$), 162.2 (CO), 155.2 (C–NH_2), 155.0 (C–NO_2), 149.2 (2CH), 135.2 (C–Cl), 133.8 (C–N), 131.5 (C), 130.6 (2CH), 128.4 (2CH), 127.0 (2CH), 117.5 (C), 115.2 (CN), 115.0 (CN), 77.1 (C).

6-amino-1-(4-chlorophenyl)-4-(3-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (9g)

Yellow crystalline solid, IR (KBr): υ_{max} 3446 & 3349 (NH_2), 3082 (C=C), 2280 (CN), 1704 (CO), 1614 (C=C), 1559, 754 cm^{-1}. MS: *m/z* 392, 356, 345, 340, 280, 158, 122, 111. Anal. Calcd. for $C_{19}H_{10}ClN_5O_3$: C, 58.25; H, 2.57; N, 17.88%. Found: C, 58.21; H, 2.55; N, 17.83%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.04 (s, –NH_2, 2H), 8.19–8.22 (d, *J* =11.2 Hz, CH), 7.99 (s, CH), 7.74–7.78. (t, *J* = 11.2 Hz, CH), 7.64–7.67 (d, *J* = 11.2 Hz, CH), 7.48–7.51 (d, *J* = 8.4 Hz, 2CH), 7.37–7.40 (d, *J* = 8.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.4 (C–$ArNO_2$), 165.6 (CO), 158.2 (C–NH_2), 156.7 (C–NO_2), 148.0 (CH), 137.2 (CH), 136.4 (C–Cl), 133.3 (C–N), 132.6 (CH), 130.2 (2CH), 129.8 (C), 129.2 (CH), 128.6 (2CH), 117.9 (C), 116.6 (CN), 114.4 (CN), 75.0 (C).

6-amino-1-(4-chlorophenyl)-2-oxo-4-(p-tolyl)-1,2-dihydropyridine-3,5-dicarbonitrile (9h)

White crystalline solid, IR (KBr): υ_{max} 3434 & 3347 (NH_2), 3109 (C=C), 2984 (CH_3), 2307 (CN), 1715 (CO), 1640 (C=C), 1555, 1419, 839 cm^{-1}. MS: *m/z* 360, 345, 325, 309, 269, 233, 158, 111, 91. Anal. Calcd. for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53%. Found: C, 66.54; H, 3.60; N, 15.50%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.88 (s, –NH_2, 2H), 7.44–7.48 (d, *J* = 8.4 Hz, 2CH), 7.38–7.41 (d, *J* = 8.0 Hz, 2CH), 7.30–7.34 (d, *J* = 8.4 Hz, 2CH), 7.12–7.17 (d, *J* = 8.0 Hz, 2CH), 2.34 (s, CH_3, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.5 (C–ArMe), 165.4 (CO), 158.6 (C–NH_2), 135.4 (C–Cl), 134.4 (2CH), 134.0 (2CH), 132.8 (C–Me), 130.4 (C–N), 128.0 (C), 127.7 (2CH), 127.5 (2CH), 116.2 (C), 115.5 (CN), 115.0 (CN), 76.3 (C), 22.9 (CH_3).

General procedure for synthesis of 4,7-dioxo-8-(aryl)-5-(substituted phenyl)-3,4,7,8-tetrahydropyrido[2,3-*d*] pyrimidine-6-carbonitrile (10, 11)

10 mmol of 6-amino-1,2-dihydro-4-(aryl)-2-oxo-1-(aryl) pyridine-3,5-dicarbonitriles (**8**, **9**) was dissolved in 20 ml of formic acid which was used as self solvent. Catalytic amount of conc. Sulphuric acid was added to promote the reaction. The reaction mixture was irradiated at 100 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 **10**, **11**.

5-(4-methoxyphenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10a)

White solid, IR (KBr): υ_{max} 3269 (NH), 2927 (C=C), 2366 (CN), 1771 (CO–NH), 1659 (CO–N), 1603 (C=C), 1164 (C–O), 1102, 1036, 836 cm^{-1}. MS: *m/z* 370, 355, 339, 327, 293, 278, 252, 224, 186, 143, 77. Anal. Calcd. for $C_{21}H_{14}N_4O_3$: C, 68.10; H, 3.81; N, 15.13%. Found: C, 67.80; H, 3.36; N, 15.05%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.28 (s, –$NH_{pyrimidine}$, 1H), 8.20 (s, =$CH_{pyrimidine}$, 1H), 8.01–8.03 (d, *J* = 11.6 Hz, 2CH), 7.65–7.68 (d, *J* = 10.4 Hz, 2CH), 7.33–7.39 (t, *J* = 10.8 Hz, 2CH), 7.10–7.17 (m, 3CH), 3.867 (s, OCH_3, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.5 (C–ArOMe), 172.2 (CO–NH), 168.2 (C–OMe),

160.8 (CO–N), 155.4 (C=N), 150.8 (=C–N=), 133.2 (C–N), 132.8 (2CH), 130.4 (2CH), 129.4 (C), 128.0 (CH), 126.7 (2CH), 118.4 (2CH), 115.8 (C–CN), 115.0 (CN), 105.2 (C–CONH), 56.7 (OCH_3).

5-(4-chlorophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10b)

White crystalline solid, IR (KBr): υ_{max} 3198 (NH), 2363 (CN), 1672 (CO), 1588 (C=C), 1556 (C=N), 1144 (C–N), 752 cm^{-1}. MS: *m/z* 374, 359, 339, 331, 313, 305, 297, 269, 258, 228, 210, 196, 186, 143, 127, 111, 77. Anal. Calcd. for $C_{20}H_{11}ClN_4O_2$: C, 64.09; H, 2.96 N, 14.95%. Found: C, 63.92; H, 2.85; N, 14.84%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.19 (s, $-NH_{pyrimidine}$, 1H), 7.99 (s, $=CH_{pyrimidine}$, 1H), 7.80–7.83 (d, *J* = 8.4 Hz, 2CH), 7.54–7.63 (m, 3CH), 7.50–7.53 (m, 2CH), 7.37–7.39 (m, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.4 (C–ArCl), 170.0 (CO–NH), 166.6 (CO–N), 155.8 (C=N), 153.4 (=C–N=), 144.2 (C–Cl), 134.0 (2CH), 132.7 (C–N), 131.5 (C), 130.3 (2CH), 129.4 (CH), 127.4 (2CH), 126.8 (2CH), 115.0 (C–CN), 114.6 (CN), 106.8 (C–CONH).

5-(3-chlorophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10c)

White crystalline solid, IR (KBr): υ_{max} 3218 (NH), 2213 (CN), 1689 (CO–NH), 1682 (CO–N), 1508 (C=C), 1500 (C=N), 1201 (C–N), 788 cm^{-1}. MS: *m/z* 374, 359, 339, 331, 305, 258, 210, 196, 186, 143, 111, 77. Anal. Calcd. for $C_{20}H_{11}ClN_4O_2$: C, 64.09; H, 2.96 N, 14.95%. Found: C, 63.89; H, 2.90; N, 14.87%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.56 (s, $-NH_{pyrimidine}$, 1H), 8.02 (s, $=CH_{pyrimidine}$, 1H), 7.54–7.55 (d, *J* = 8.0 Hz, 2CH), 7.43–7.46 (m, 3CH), 7.37–7.39 (d, *J* = 8.0 Hz, 2CH), 7.34 (s, CH), 7.30–7.32 (t, *J* = 7.6 Hz, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.8 (C–ArCl), 173.3 (CO–NH), 164.5 (CO–N), 156.8 (C=N), 153.0 (=C–N=), 145.4 (C–Cl), 134.4 (CH), 133.0 (C–N), 132.8 (CH), 132.4 (CH), 130.7 (C), 129.6 (2CH), 128.8 (CH), 127.0 (CH), 126.2 (2CH), 116.4 (C–CN), 115.8 (CN), 105.5 (C–CONH).

5-(2-chlorophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10d)

White crystalline solid, IR (KBr): υ_{max} 3233 (NH), 2323 (CN), 1652 (CO–NH), 1603 (CO–N), 1587 (C=C), 1511 (C=N), 734 cm^{-1}. MS: *m/z* 374, 359, 339, 331, 313, 269, 258, 186, 143, 111, 77. Anal. Calcd. for $C_{20}H_{11}ClN_4O_2$: C, 64.09; H, 2.96 N, 14.95%. Found: C, 63.99; H, 2.88; N, 14.90%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.06 (s, $-NH_{pyrimidine}$, 1H), 7.88 (s, $=CH_{pyrimidine}$, 1H), 7.71–7.73 (d, *J* = 11.6 Hz, 2CH), 7.57–7.61 (m, 3CH), 7.44–7.45 (t, 2CH), 7.12–7.14 (d, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 178.6 (C–ArCl), 174.2 (CO–NH), 162.8 (CO–N), 158.2 (C=N), 151.4 (=C–N=), 146.2 (C–Cl), 135.2 (CH), 134.8 (CH), 133.2 (C–N), 131.8 (C), 130.4 (CH), 129.2 (2CH), 129.0 (CH), 128.4 (2CH), 126.6 (CH), 115.1 (C–CN), 114.7 (CN), 103.4 (C–CONH).

5-(4-bromophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10e)

White crystalline solid, IR (KBr): υ_{max} 3189 (NH), 2311 (CN), 1743 (CO–NH), 1689 (CO–N), 1579 (C=C), 1556 (C=N), 1230 (C–N), 1120, 821 cm^{-1}. MS: *m/z* 418, 375, 347, 339, 263, 186, 154, 77. Anal. Calcd. for $C_{20}H_{11}ClN_4O_2$: Calculated: C, 64.09; H, 2.96 N, 14.95%. Found: C, 63.99; H, 2.88; N, 14.90%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.80 (s, $-NH_{pyrimidine}$, 1H), 8.23 (s, $=CH_{pyrimidine}$, 1H), 7.99–8.02 (d, *J* = 11.6 Hz, 2CH), 7.87–7.88 (d, *J* = 10.4 Hz, 2CH), 7.51–7.53 (d, *J* = 8.4 Hz, 2CH), 7.40–7.47 (m, 3CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 178.4 (C–ArBr), 172.0 (CO–NH), 164.5 (CO–N), 152.1 (C=N), 149.0 (=C–N=), 147.4 (C–Br), 133.8 (2CH), 131.6 (C–N), 130.4 (C), 129.7 (2CH), 128.8 (CH), 126.4 (2CH), 122.3 (2CH), 117.2 (C–CN), 116.0 (CN), 104.8 (C–CONH).

5-(4-nitrophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10f)

Yellow crystalline solid, IR (KBr): υ_{max} 3247 (NH), 2924 (C=C), 2362 (CN), 1694 (CO), 1511 (C=N), 1104 cm^{-1}. MS: *m/z* 385, 370, 342, 316, 308, 295, 266, 239, 143, 122, 77. Anal. Calcd. for $C_{20}H_{11}N_5O_4$: Calculated: C, 62.34; H, 2.88; N, 18.17%. Found: C, 62.24; H, 2.79; N, 18.06%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.38 (s, $-NH_{pyrimidine}$, 1H), 7.78 (s, $=CH_{pyrimidine}$, 1H), 7.60–7.62 (m, 2CH), 7.54–7.60 (m, CH), 7.50–7.53 (dd, *J* = 7.6 Hz, 2CH), 7.38–7.40 (dd, *J* = 7.6 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.4 (C–$ArNO_2$), 170.2 (CO–NH), 163.8 (CO–N), 150.6 (C=N), 149.2 (=C–N=), 148.4 (C–NO_2), 134.0 (2CH), 132.6 (C–N), 131.4 (2CH), 130.8 (2CH), 128.9 (C), 128.3 (CH), 122.8 (2CH), 116.0 (C–CN), 115.7 (CN), 106.5 (C–CONH).

5-(3-nitrophenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10g)

Yellow crystalline solid, IR (KBr): υ_{max} 3221 (NH), 2952 (C=C), 2212 (CN), 1714 (CO–NH), 1651 (CO–NH), 1581 (C=C), 1104, 788 cm^{-1}. MS: *m/z* 385, 370, 342, 316, 295, 239, 122, 77. Anal. Calcd. for $C_{20}H_{11}N_5O_4$: C, 62.34; H, 2.88; N, 18.17%. Found: C, 62.27; H, 2.74; N, 18.00%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.78 (s, $-NH_{pyrimidine}$, 1H), 7.88 (s, $=CH_{pyrimidine}$, 1H), 7.79–7.81 (d, 2CH), 7.56–7.58 (t, CH),

7.45–7.50 (m, 3CH), 7.34 (s, CH), 7.18–7.20 (d, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.2 (C–$ArNO_2$), 170.6 (CO–NH), 163.2 (CO–N), 155.5 (C=N), 154.2 (=C–N=), 148.0 (C–NO_2), 132.8 (CH), 132.2 (CH), 131.4 (C–N), 130.6 (2CH), 129.9 (2CH), 129.2 (CH), 128.4 (C), 122.2 (CH), 121.0 (CH), 115.5 (C–CN), 114.9 (CN), 109.7 (C–CONH).

5-(4-methylphenyl)-4,7-dioxo-8-phenyl-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (10h)

White solid, IR (KBr): υ_{max} 3301 (NH), 2899 (CH_3), 2310 (CN), 1720 (CO–NH), 1691 (CO–N), 1519 (C=N), 1100, 820 cm^{-1}. MS: *m/z* 354, 329, 311, 286, 277, 263, 186, 91, 77. Anal. Calcd. for $C_{21}H_{14}N_4O_2$: C, 71.18; H, 3.98; N, 15.81%. Found: C, 71.11; H, 3.89; N, 15.78%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.50 (s, –$NH_{pyrimidine}$, 1H), 7.89 (s, =$CH_{pyrimidine}$, 1H), 7.66–7.68 (m, 3CH), 7.54–7.58 (d, *J* = 10.4 Hz, 2CH), 7.51–7.52 (d, *J* = 8.0Hz, 2CH), 7.28–7.30 (d, *J* = 8.0 Hz, 2CH), 2.18 (s, CH_3, 3CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.4 (C–ArMe), 170.8 (CO–NH), 162.4 (CO–N), 153.0 (C=N), 151.8 (=C–N=), 137.2 (C–Me), 133.9 (2CH), 132.0 (C–N), 131.2 (2CH), 130.8 (C), 129.6 (CH), 128.2 (2CH), 126.8 (2CH), 116.6 (C–CN), 115.8 (CN), 104.0 (C–CONH), 22.8 (CH_3).

8-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11a)

White crystalline solid, IR (KBr): υ_{max} 3296 (NH), 3205 (C=C), 2924 (CH_3), 2216 (CN), 1683 (CO–NH), 1626 (CO–N), 1518 (C=N), 1288 (C–O), 1232, 1029, 840 cm^{-1}. MS: *m/z* 404, 389, 378, 362, 334, 293, 267, 252, 223, 186, 127, 111, 93, 75. Anal. Calcd. for $C_{21}H_{13}ClN_4O_3$: C, 62.31; H, 3.24; N, 13.84%. Found: C, 62.22; H, 3.12; N, 13.77%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.66 (s, –$NH_{pyrimidine}$, 1H), 7.52 (s, =$CH_{pyrimidine}$, 1H), 7.29–7.38 (dd, 6CH), 7.01–7.03 (d, *J* = 11.2, 2CH), 3.83 (s, OCH_3, 3CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.5 (C–ArOMe), 170.8 (CO–NH), 169.6 (CO–N), 161.4 (C=N), 156.2 (=C–N=), 147.3 (C–OMe), 144.0 (C–Cl), 133.4 (2CH), 131.3 (C–N), 129.8 (2CH), 128.2 (2CH), 127.9 (C), 123.1 (2CH), 115.8 (C–CN), 115.2 (CN), 107.4 (C–CONH), 55.7 (OCH_3).

5,8-bis(4-chlorophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11b)

White crystalline solid, IR (KBr): υ_{max} 3229 (NH), 2924 (C=C), 2214 (CN), 1641 (CO–N), 1527 (C=C), 1497 (C=N), 1245 (C–N), 1078, 843 cm^{-1}. MS: *m/z* 408, 373, 365, 338, 330, 314, 297, 254, 186, 111. Anal. Calcd. for $C_{20}H_{10}Cl_2N_4O_2$: C, 58.70; H, 2.46; N, 13.69%. Found: C, 58.66; H, 2.39; N, 13.60%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.17 (s, –$NH_{pyrimidine}$, 1H), 8.21 (s, =$CH_{pyrimidine}$, 1H), 7.81–7.82 (d, *J* = 7.2 Hz, 2CH), 7.58–7.60 (d, *J* = 7.2 Hz, 2CH), 7.50–7.52 (d, *J* = 7.6 Hz, 2CH), 7.36–7.38(d, *J* = 6.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.2 (C–ArCl), 172.8 (CO–NH), 165.4 (CO–N), 154.2 (C=N), 152.0 (=C–N=), 144.8 (C–Cl), 143.4 (C–Cl), 133.0 (2CH), 131.5 (C–N), 130.8 (2CH), 128.6 (C), 128.4 (2CH), 126.8 (2CH), 116.2 (C–CN), 115.4 (CN), 105.9 (C–CONH).

5-(3-chlorophenyl)-8-(4-chlorophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11c)

White crystalline solid, IR (KBr): υ_{max} 3189 (NH), 2928 (C=C), 2314 (CN), 1721 (CO–NH), 1664 (CO–N), 1521 (C=N), 1201 (C–N), 1078, 801 cm^{-1}. MS: *m/z* 408, 373, 365, 331, 314, 297, 186, 111. Anal. Calcd. for $C_{20}H_{10}Cl_2N_4O_2$: C, 58.70; H, 2.46; N, 13.69%. Found: C, 58.68; H, 2.37; N, 13.56%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.01 (s, –$NH_{pyrimidine}$, 1H), 8.00 (s, =$CH_{pyrimidine}$, 1H), 7.88–7.89 (d, *J* = 8.0 Hz, 2CH), 7.65–7.62 (d, *J* = 8.0 Hz, 2CH), 7.50–7.57 (m, 3CH), 7.20 (s, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.0 (C–ArCl), 170.7 (CO–NH), 166.2 (CO–N), 155.7 (C=N), 153.8 (=C–N=), 144.2 (C–Cl), 143.8 (C–Cl), 133.0 (2CH), 132.8 (C–N), 131.2 (CH), 130.3 (CH), 128.8 (C), 128.4 (2CH), 127.8 (CH), 126.4 (CH), 115.8 (C–CN), 115.2 (CN), 106.4 (C–CONH).

5-(2-chlorophenyl)-8-(4-chlorophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11d)

White crystalline solid, IR (KBr): υ_{max} 3301 (NH), 2987 (C=C), 2301 (CN), 1689 (CO–NH), 1519 (C=C), 1445 (C=N), 787 cm^{-1}. MS: *m/z* 408, 373, 366, 330, 315, 297, 186, 111. Anal. Calcd. for $C_{20}H_{10}Cl_2N_4O_2$: C, 58.70; H, 2.46; N, 13.69%. Found: C, 58.60; H, 2.37; N, 13.59%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.77 (s, –$NH_{pyrimidine}$, 1H), 7.89 (s, =$CH_{pyrimidine}$, 1H), 7.63–7.65 (d, *J* = 8.4 Hz, 2CH), 7.49–7.51 (d, *J* = 8.2Hz, 2CH), 7.34–7.33 (d, *J* = 7.6 Hz, 2CH), 7.15–7.16 (t, *J* = 7.0 Hz, CH), 7.01 (s, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.8 (C–ArCl), 174.0 (CO–NH), 166.7 (CO–N), 159.4 (C=N), 150.6 (=C–N=), 146.8 (C–Cl), 145.0 (C–Cl), 133.8 (2CH), 133.0 (CH), 132.4 (C–N), 130.4 (CH), 128.6 (C), 128.2 (CH), 128.0 (2CH), 127.4 (CH), 116.8 (C–CN), 115.0 (CN), 104.8 (C–CONH).

5-(4-bromophenyl)-8-(4-chlorophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11e)

White crystalline solid, IR (KBr): υ_{max} 3222 (NH), 2936 (C=C), 2214 (CN), 1711 (CO–NH), 1641(CO–N), 1501 (C=N), 1225 (C–N), 851cm^{-1}. MS: *m/z* 453, 417, 408, 381,

373, 346, 340, 303, 297, 186, 155, 111. Anal. Calcd. for $C_{20}H_{10}BrClN_4O_2$: C, 52.95; H, 2.22; N, 12.35%. Found: C, 52.87; H, 2.11; N, 12.27%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.16 (s, –$NH_{pyrimidine}$, 1H), 8.04 (s, =$CH_{pyrimidine}$, 1H), 7.91–7.93 (d, J = 8.0 Hz, 2CH), 7.76–7.78 (d, J = 8.2 Hz, 2CH), 7.61–7.63 (d, J = 7.8 Hz, 2CH), 7.43–7.45 (d, J = 7.4 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.8 (C–ArBr), 172.4 (CO–NH), 165.0 (CO–N), 155.6 (C=N), 153.2 (=C–N=), 148.2 (C–Br), 144.0 (C–Cl), 131.6 (2CH), 131.2 (C–N), 130.4 (2CH), 129.4 (C), 128.2 (2CH), 123.4 (2CH), 116.4 (C–CN), 115.8 (CN), 105.4 (C–CONH).

8-(4-chlorophenyl)-5-(4-nitrophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11f)

Yellow crystalline solid, IR (KBr): υ_{max} 3119 (NH), 2894 (C=C), 2309 (NH), 1709 (CO–NH), 1627 (CO–N), 1481 (C=N), 1154, cm^{-1}. MS: *m/z* 419, 404, 394, 376, 373, 308, 297, 263, 194, 186, 153, 122, 111. Anal. Calcd. for $C_{20}H_{10}ClN_5O_4$: C, 57.22; H, 2.40; N, 16.68%. Found: C, 57.12; H, 2.32; N, 16.58%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.86 (s, –$NH_{pyrimidine}$, 1H), 7.88 (s, =$CH_{pyrimidine}$, 1H), 7.67–7.69 (d, J = 7.2 Hz, 2CH), 7.59–7.61 (d, J = 7.2 Hz, 2CH), 7.48–7.50 (d, J = 8.0 Hz, 2CH), 7.44–7.47 (d, J = 8.0 Hz, 2CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.2 (C–$ArNO_2$), 170.8 (CO–NH), 166.0 (CO–N), 157.5 (C=N), 156.6 (=C–N=), 155.2 (C–NO_2), 144.4 (C–Cl), 133.4 (2CH), 132.5 (2CH), 132.0 (C–N), 129.2 (2CH), 128.4 (C), 123.6 (2CH), 115.4 (C–CN), 114.0 (CN), 105.8 (C–CONH).

8-(4-chlorophenyl)-5-(3-nitrophenyl)-4,7-dioxo-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11g)

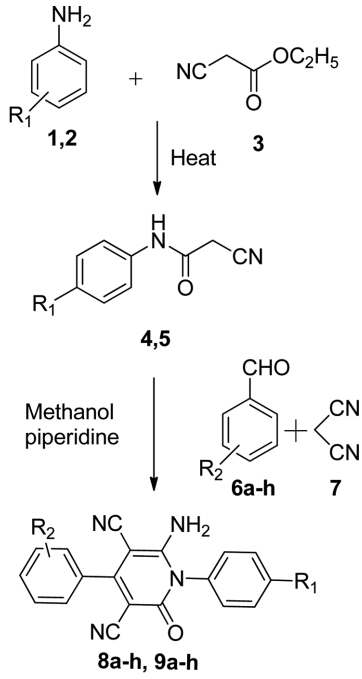
Yellow crystalline solid, IR (KBr): υ_{max} 3233 (NH), 2974 (C=C), 2364 (CN), 1696 (CO–NH), 1618 (CO–N), 1584 (C=N), 1159, 1102, 846 cm^{-1}. MS: *m/z* 419, 404, 393, 376, 373, 349, 308, 297, 262, 195, 186, 153, 122, 111, 93, 75. Anal. Calcd. for $C_{20}H_{10}ClN_5O_4$: C, 57.22; H, 2.40; N, 16.68%. Found: C, 57.14; H, 2.29; N, 16.60%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.60 (s, –$NH_{pyrimidine}$, 1H), 8.14 (s, =$CH_{pyrimidine}$, 1H), 7.64–7.67 (d, J = 8.4 Hz, 2CH), 7.44–7.47 (d, J = 8.8 Hz, 2CH), 7.35–7.39 (t, J = 8.0 Hz, CH), 6.87–6.96 (m, 3CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.0 (C–$ArNO_2$), 172.8 (CO–NH), 166.4 (CO–N), 155.5 (C=N), 154.6 (=C–N=), 153.2 (C–NO_2), 143.4 (C–Cl), 131.2 (CH), 130.8 (2CH), 130.0 (C–N), 129.4 (CH), 129.0 (2CH), 127.6 (C), 122.0 (CH), 121.4 (CH), 116.2 (C–CN), 115.9 (CN), 104.7 (C–CONH).

8-(4-chlorophenyl)-4,7-dioxo-5-(p-tolyl)-3,4,7,8-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (11h)

White crystalline solid, IR (KBr): υ_{max} 3228 (NH), 2971 (C=C), 2228 (CN), 1699 (CO–NH), 1615 (CO–N), 1524 (C=N), 1254 (C–N), 1212, 830 cm^{-1}. MS: *m/z* 388, 373, 353, 345, 338, 318, 310, 297, 277, 186, 111, 91. Anal. Calcd. for $C_{21}H_{13}ClN_4O_2$: C, 64.87; H, 3.37; N, 14.41%. Found: C, 64.78; H, 3.26; N, 14.34%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.10 (s, –$NH_{pyrimidine}$, 1H), 8.11 (s, =$CH_{pyrimidine}$, 1H), 7.69–7.71 (d, J = 8.4 Hz, 2CH), 7.59–7.61 (d, J = 8.4 Hz, 2CH), 7.25–7.28 (d, J = 7.0 Hz, 2CH), 6.88–6.90 (d, J = 7.0 Hz, 2CH), 2.45 (s, CH_3, CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.4 (C–ArMe), 171.4 (CO–NH), 162.8 (CO–N), 154.2 (C=N), 152.6 (=C–N=), 147.6 (C–Cl), 140.3 (C–Me), 132.2 (2CH), 131.5 (2CH), 130.8 (C–N), 129.4 (2CH), 128.8 (C), 126.4 (2CH), 116.6 (C–CN), 115.6 (CN), 105.8 (C–CONH), 23.2 (CH_3).

RESULTS AND DISCUSSION

There are several strategies to prepare pyrido[2,3-*d*]pyrimidines e.g. the 2-amino-3-cyanopyromodones react with formamide and arylidene of different aldehydes or the 2-amino-3-cyano-4,6-disubstituted pyridines reacting with thiourea, formamide and arylisocynate.[23,26] In accordance with our strategy, two 2-cyano-N-phenylacetamides (**4**, **5**) were prepared by refluxing the aromatic amine (**1**, **2**)



R_1 = H, Cl
R_2 = 4-OCH_3, 4-Cl, 3-Cl, 2-Cl, 4-Br, 4-NO_2, 3-NO_2, 4-CH_3

***Scheme* 1.** Synthesis of 6-amino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles.

***Table* 1.** Preparation of 6-amino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**8–9**)

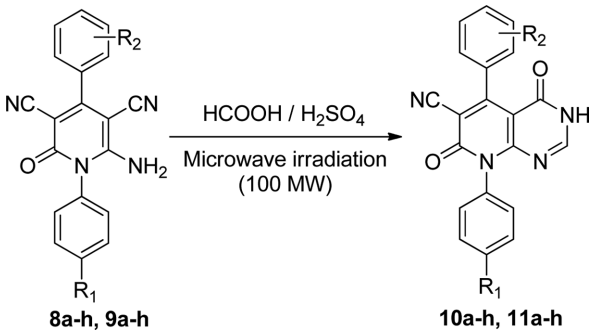
Product	M.P. (°C)	Time (hr)	Yield* (%)
8a	210–212	14	73
8b	274–276	10	72
8c	242–244	11	69
8d	208–210	10	77
8e	294–298	8	78
8f	262–264	16	76
8g	266–268	14	69
8h	214–216	15	78
9a	>300	14	72
9b	>300	11	70
9c	>300	11	69
9e	>300	8	71
9f	>300	10	76
9g	>300	12	79
9h	>300	14	70

*Isolated yield in DMF.

with cyanoacetic acid ester (**3**) under solvent free conditions.[27] The 6-amino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**8**, **9**) were prepared by reacting cyanoacetamides with aldehydes and malononitrile via Hantzsch pyridine synthesis.

The compounds **8**–**9** are useful intermediates for synthesizing various pyridine fused heterocylic compounds. Firstly, compound **4**, 4-methoxy benzaldehyde (**6a**) and malononitrile (**7**) were refluxed in methanol using piperidine as catalyst (*Scheme* 1). After 16 hours the complete conversion of **4** occurred and **8a** was isolated in good yields (*Table* 1).

The preliminary experimentations showed that when **8a** was reacted with formic acid in the presence of a catalytic amount of sulfuric acid (H_2SO_4) afforded **10a** in poor yield (30%) under reflux conditions for 23 hours (*Scheme* 2). But when the same reaction was performed under microwave condition, we surprisingly found that the reaction time was reduced dramatically and the yield was improved remarkably (**10a**, *Table* 2).



8a-h, 9a-h **10a-h, 11a-h**

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

***Table* 2.** Comparison of microwave and conventional methods for the synthesis of pyrido[2,3-*d*]pyrimidines (**10**,**11**)

Product	R_1	R_2	Microwave	
			Time† (min)	Yield* (%)
10a	H	4-OCH_3	25	73
10b	H	4-Cl	24	72
10c	H	3-Cl	22	69
10d	H	2-Cl	23	77
10e	H	4-Br	20	78
10f	H	4-NO_2	28	76
10g	H	3-NO_2	28	69
10h	H	4-CH_3	32	78
11a	Cl	4-OCH_3	30	72
11b	Cl	4-Cl	31	70
11c	Cl	3-Cl	25	69
11d	Cl	2-Cl	26	72
11e	Cl	4-Br	24	71
11f	Cl	4-NO_2	29	76
11g	Cl	3-NO_2	30	79
11h	Cl	4-CH_3	33	70

*Isolated yield in DMF.
†Continuous irradiation, The melting points of compounds **10a–h**, **11a–h** are above 300 °C.

To expand the course of this reaction, different starting materials were synthesized. For that we subsequently used two different 2-cyano-*N*-phenylacetamides (**4** and **5)** and eight different aldehydes (**6a**–**h**, with electron donating and withdrawing groups), over all 16 reactions were performed and isolated yield of corresponding **8**–**9** is shown in *Table* 1. Microwave magnetron power was varied for the all the reactions performed, but maximum yield was obtained at lower irradiation (100 MW). There were substantial differences regarding the nature of substrate.

To demonstrate the practicality of the developed microwave protocol, large-scale experiments (30 mmol of **8e** and **9g**) were carried out in the synthesis of **10e** and **11g** using a 250 mL Erlenmeyer flask as the reaction vessel. High yields of **10e** (77%) and **11g** (79%) were afforded under microwave irradiation at 100 MW with exposure times of 30 min.

Biological Activity

The wide range of activity profile of pyrido[2,3-*d*]pyrimidines probes us to test and study the biological activities of some of the synthesized novel analogues. Many antimicrobial agents have been introduced into therapy; however, the field still needs extensive efforts for the development of new antimicrobial agents to overcome the highly resistant strains of microorganisms. The newly synthesized com-

***Table* 3.** Antibacterial activity of synthesized compounds **8a–h**, **9a–h**, **10a–h** and **11a–h**

Product	E.c	P.a	B.s	S.a	M.l	Product	E.c	P.a	B.s	S.a	M.l
8a	12.21	11.23	11.36	12.63	10.23	**10a**	12.37	11.36	11.06	12.66	10.10
8b	15.45	14.65	14.24	11.25	16.52	**10b**	**18.31**	**17.20**	**18.10**	12.10	**16.78**
8c	12.00	13.01	12.76	10.28	11.29	**10c**	13.19	13.75	16.21	11.62	12.15
8d	14.23	11.23	12.43	13.69	10.41	**10d**	14.25	15.23	14.62	14.93	11.32
8e	16.02	14.94	15.15	14.13	15.68	**10e**	**18.33**	**17.74**	**18.15**	15.10	**17.47**
8f	12.39	11.26	13.12	11.78	12.23	**10f**	12.85	12.46	16.29	12.16	12.22
8g	13.25	13.45	13.01	12.49	–	**10g**	13.16	13.29	13.15	12.01	–
8h	11.16	–	10.19	–	–	**10h**	12.81	12.02	–	–	–
9a	13.63	10.05	12.34	10.43	11.25	**11a**	14.18	11.11	12.66	11.13	14.85
9b	15.00	13.16	14.11	11.32	15.29	**11b**	**16.75**	**16.38**	**16.13**	13.10	**16.88**
9c	13.14	15.31	14.11	12.36	10.22	**11c**	13.63	16.19	16.61	13.65	10.66
9d	14.33	12.36	13.65	11.63	11.94	**11d**	14.60	14.43	14.98	13.48	13.87
9e	16.14	15.93	13.49	14.52	16.07	**11e**	**17.15**	**18.10**	14.66	15.64	**17.22**
9f	12.67	11.11	11.16	13.14	12.11	**11f**	12.12	12.60	12.11	14.32	12.41
9g	13.25	13.48	13.46	13.10	–	**11g**	13.30	14.32	14.14	12.45	–
9h	11.00	–	10.05	–	–	**11h**	13.14	12.04	–	–	–
Std. 1*	30.00	17.78	28.70	36.12	34.11	Std. 1[a]	30.00	17.78	28.70	36.12	34.11
Std. 2†	17.00	19.60	18.80	16.45	17.20	Std. 2[b]	17.00	19.60	18.80	16.45	17.20

*Std. 1 = Cefixime (Standard).

†Std. 2 = Chloramphenicol (Standard).

Zone diameter of growth inhibition (mm) after 24 h, <10 mm (–), Concentration 1 mg/mL in DMSO. Microorganisms selected are as follows: E.c, *Escherichia coli*; P.a, *Pseudomonas aeruginosa*; B.s, *Bacillus subtillus*; S.a, *Staphylococcus aureus*; M.l, *Micrococcus luteus.* Values are expressed as mean ± standard deviation of the three replicates.

***Table* 4.** Antifungal activity of synthesized compounds **8a–h**, **9a–h**, **10a–h** and **11a–h**

Product	T.l	C.a	M.c	F.s	Product	T.l	C.a	M.c	F.s
8a	40	40	–	30	**10a**	30	46	–	35
8b	90	56	69	45	**10b**	**94**	66	70	48
8c	35	20	–	–	**10c**	35	32	31	–
8d	25	–	–	–	**10d**	28	–	–	–
8e	88	64	77	54	**10e**	**94**	71	83	55
8f	61	–	–	–	**10f**	59	–	–	–
8g	–	–	–	–	**10g**	–	–	–	–
8h	–	–	–	–	**10h**	–	–	–	–
9a	50	41	–	–	**11a**	63	45	–	–
9b	88	83	70	54	**11b**	**90**	**89**	70	55
9c	40	25	30	–	**11c**	47	34	30	–
9d	25	–	–	–	**11d**	25	–	–	–
9e	91	74	81	63	**11e**	**94**	81	**88**	60
9f	30	–	29	–	**11f**	36	–	–	–
9g	–	–	–	–	**11g**	–	–	–	–
9h	–	–	–	–	**11h**	–	–	–	–
Std*	100	90	90	90	Std*	100	90	90	90

*Std=Miconazole (Standard) Conc. of sample 200 μg/mL in DMSO at 27 °C, <20 mm (–), Incubation period 7 days. Microorganisms selected are as follows: T.l, *Trichphyton longifusus*; C.a, *Candida albicans*; M.c, *Microsporum canis*; F.s, *Fusarium solani*, Values are expressed as mean ± standard deviation of the three replicates.

pounds **8a**–**h** to **11a**–**h** were tested in vitro for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtillus*, *Staphylococcus aureus*, and *Micrococcus luteus* bacteria by the agar well diffusion method.[28] DMSO was used as a control solvent and, chloramphenicol and cefixime as standard drugs.

After 24 h incubation at 37 °C, the zone of inhibition was measured in mm. The results are depicted in *Table* 3. The results showed that almost all compounds were active against the microorganism tested. It is worth noting here that compounds **10b**, **10e**, **11b** and **11e** exhibited significant activity against *E. coli*, *P. aeruginosa*, *B. subtillus* and *M. letus*. The other compounds showed moderate-to-low activity. The structure–activity relationship (SAR) shows that the presence of cyclic amide in pyrimidine ring might have increased the activity especially when R_2 is halogen at para position. The electron donating group at the para position as in case of **8h**, **9h**, **10h** and **11h**, diminishes the activity.

Compounds **8a**–**h** to **11a**–**h** were also screened in vitro for their antifungal activity against four species using the agar plate technique.[29] 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 shown in *Table* 4 indicated that compounds **10b**, **10e**, **11e** exhibited significant activity against *Trichphyton longifusus*, **11b** against *Candida albicans* and **11e** against *Micrococcus luteus*. It is worth noting that compounds **10b**, **10e**, **11b** and **11e** exhibited significant (maximum) antibacterial and antifungal activities, possibly because of the presence of halogen substitution at the 4-position (para) of the *N*-phenyl substituent, in addition to the cyclic amide group.

CONCLUSION

In summary, a straightforward and effective method to synthesize novel 5-8-diaryl tetrahydro pyrido[2,3-*d*]pyridines has been developed using microwave-assisted synthesis. The substrates were obtained in good yields and in short reaction times. The microwave technique provides a rapid, simple, and effective method to synthesize such compounds that may have the potential application in the field of drug discovery. Moreover the reaction is simple, one pot and also gives excellent yields at larger scales. The compounds **10b**, **10e**, **11b** and **11e** exhibited significant (maximum) antibacterial and antifungal activities, which may develop into the potential class of antimicrobial agents. The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial and antifungal activities. Further studies are in progress in our laboratories and will be reported upon in the future.

Acknowledgments. Publication cost of this paper was supported by the Korean Chemical Society.

REFERENCES

1. Zbancioc, G.; et al. Microwave Assisted Reactions of Some Azaheterocylic Compounds. *Molecule.* **2009**, *14*(1), 403.
2. Bardagi, J. I.; Rossi, R. A. Short Access to 6-Substituted Pyrimidine Derivatives by the S (RN) Mechanism. Synthesis of 6-Substituted Uracils Through a One-pot Procedure. *J Org Chem.* **2010**, *75*(15), 5271.
3. Youssef, M. M.; Amin, M. A. Microwave Assisted Synthesis of Some New Thiazolopyrimidine, Thiazolodipyrimidine and Thiazolopyrimidothiazolopyrimidine Derivatives with Potential Antioxidant and Antimicrobial Activity. *Molecules*. **2012**, *17*(8), 9652.
4. Kanagarajan, V.; et al. A Facile Microwave Assisted Green Chemical Synthesis of Novel Piperidino 2-Thioxoimidazolidin-4-ones and Their in Vitro Microbiological Evaluation. *J. Enzyme. Inhib. Med. Chem*. **2011**, *26*(1), 67.
5. Gitto, R.; et al. Synthesis and Evaluation of Pharmacological Profile of 1-Aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-sulfonamides. *Bioorg. Med. Chem*. **2009**, *17*(10), 3659.
6. Rodriguez, H.; et al. Eco-friendly Methodology to Prepare N-Heterocycles Related to Dihydropyridines: Microwave-assisted Synthesis of Alkyl 4-Arylsubstituted-6-chloro-5-formyl-2-methyl-1,4-dihydropyridine-3-carboxylate and 4-Arylsubstituted-4,7-dihydrofuro[3,4-*b*]pyridine-2,5(1H, 3H)-dione. *Molecules* **2011**, *16*(11), 9620.
7. Martinez, J.; et al. Green Approach & # 8212; Multicomponent Production of Boron & # 8212; Containing Hantzsch and Biginelli Esters. *Int. J. Mol. Sci*. **2013**, *14*(2), 2903.
8. Balatsos, N. A.; et al. Inhibition of Human Poly(A)-specific Ribonuclease (PARN) by Purine Nucleotides: Kinetic Analysis. *J. Enzyme. Inhib. Med. Chem.* **2009**, *24*(2), 516.
9. Raboisson, P.; et al. Design, Synthesis and Structure-activity Relationships of a Series of 9-Substituted Adenine Derivatives as Selective Phosphodiesterase Type-4 Inhibitors. *Eur. J. Med. Chem.* **2003**, *38*(2), 199.
10. Manikowski, A.; et al. Inhibition of Herpes Simplex Virus Thymidine Kinases by 2-Phenylamino-6-oxopurines and Related Compounds: Structure-activity Relationships and Antiherpetic Activity in Vivo. *J. Med. Chem*. **2005**, *48*(11), 3919.
11. Pandey, A.; et al. Identification of Orally Active, Potent, and Selective 4-Piperazinylquinazolines as Antagonists of the Platelet-derived Growth Factor Receptor Tyrosine Kinase Family. *J. Med. Chem.* **2002**, *45*(17), 3772.
12. Antonello, A.; et al. Design, Synthesis, and Biological Evaluation of Prazosin-related Derivatives as Multipotent Compounds. *J. Med. Chem*. **2005**, *48*(1), 28.
13. Bathini, Y.; et al. 2-Aminoquinazoline Inhibitors of Cyclin-dependent Kinases. *Bioorg. Med. Chem. Lett*. **2005**, *15*(17), 3881.
14. Matulenko, M. A.; et al. 5-(3-Bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido[2,3-d]pyrimidin-4-ylamine: Structure-activity Relationships of 7-substituted Heteroaryl Analogs as Non-nucleoside Adenosine Kinase Inhibitors.

Bioorg. Med. Chem. **2005**, *13*(11), 3705.
15. Wu, Z.; et al. Development of Pyridopyrimidines as Potent Akt1/2 Inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18*(4), 1274.
16. Ribble, W.; et al. Discovery and Analysis of 4H-pyridopy-Rimidines, a Class of Selective Bacterial Protein Synthesis Inhibitors. *Antimicrob Agents Chemother.* **2010**, *54*(11), 4648.
17. Guiles, J. W.; et al. Development of 4H-Pyridopyrimidines: A Class of Selective Bacterial Protein Synthesis Inhibitors. *Org. Med. Chem. Lett.* **2012**, *2*(1), 5.
18. Kovacs, J. A.; et al. Potent Antipneumocystis and Antitoxoplasma Activities of Piritrexim, a Lipid-soluble Antifolate. *Antimicrob Agents Chemother.* **1988**, *32*(4), 430.
19. Gangjee, A.; et al. Pneumocystis Carinii and Toxoplasma Gondii Dihydrofolate Reductase Inhibitors and Antitumor Agents: Synthesis and Biological Activities of 2,4-Diamino-5-methyl-6-[(monosubstituted anilino)methyl] pyrido[2,3-*d*]pyrimidines. *J. Med. Chem.* **1999**, *42*(13), 2447.
20. Gangjee, A.; et al. Synthesis and Biological Evaluation of 2,4-Diamino-6-(arylaminomethyl)pyrido[2,3-*d*]pyrimidines as Inhibitors of Pneumocystis Carinii and Toxoplasma Gondii Dihydrofolate Reductase and as Antiopportunistic Infection and Antitumor Agents. *J. Med. Chem.* **2003**, *46*(23), 5074.
21. Lee, C. H.; et al. Discovery of 4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*] pyrimidine, an Orally Active, Non-nucleoside Adenosine Kinase Inhibitor. *J. Med. Chem.* **2001**, *44*(13), 2133.
22. Trumpp-Kallmeyer, S.; et al. Development of a Binding Model to Protein Tyrosine Kinases for Substituted Pyrido[2,3-*d*]pyrimidine Inhibitors. *J. Med. Chem.* **1998**, *41*(11), 1752.
23. El-Gazzar, A. R.; Hafez, H. N. Synthesis of 4-Substituted pyrido[2,3-*d*]pyrimidin-4(1H)-one as Analgesic and Anti-inflammatory Agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*(13), 3392.
24. Kamlesh, K.; Taslimahemad, K.; Praful, P. One Pot Synthesis of Bioactive Novel Cyanopyridones. *J. Korean Chem. Soc.* **2013**, *57*(4), 476.
25. Al-Sehemi, A. G. A Convenient Synthesis and Characterization of 1,2-Dihydrpyridine-2-one, Pyrido[2,3-*d*]pyrimidine and Thieno [3,4-*c*]pyridine derivatives. *Der. Pharma. Chemica.* **2010**, *2*(2), 336.
26. Mont, N.; et al. A Diversity Oriented, Microwave Assisted Synthesis of N-Substituted 2-Hydro-4-amino-pyrido[2,3-*d*]pyrimidin-7(8H)-ones. *Mol. Divers.* **2009**, *13*(1), 39.
27. Wang, K.; et al. Cyanoacetamide Multicomponent Reaction (I): Parallel Synthesis of Cyanoacetamides. *J. Comb. Chem.* **2009**, *11*(5), 920.
28. Linday, E. M. *Practical Introduction to Microbiology*; E & FN spon Ltd: London, U.K., 1962; p 177.
29. Collins, C. H. *Microbiological Methods*; Butterworths: London, U.K., 1967; p 364.