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Synthesis and antifungal activity of some new pyrido[2,3-*d*]pyrimidines

Fatin Ismail Hanafy

Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, Cairo, EG-11711, Egypt

Corresponding author at: Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, Cairo, EG-11711, Egypt. Tel.: +2025088247; fax: +2025088247. E-mail address: hsseleem@yahoo.com (F.I. Hanafy).

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ABSTRACT

Some new pyrido[2,3-*d*]pyrimidine derivatives (**3a-c**) were synthesized from 2-amino-5-cyano-6-methoxy-4-(4-methoxyphenyl)pyridine-3-carboxamide. 7-methoxy-5-(4-methoxy phenyl)-4-oxo-2-phenyl-3,4-dihydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (**3b**) and 7-methoxy-5-(4-methoxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (**3c**) are used in synthesizing **7a,b**, then **8a,b**. 7-methoxy-5-(4-methoxyphenyl)-2-methyl-4-oxo-3,4-dihydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (**3a**) and 4-hydrazinyl-7-methoxy-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine-6-carbonitrile (**8b**) were condensed with different carbonyl compounds to produce compounds **4**, **5**, **6** and **9**, **10**, **11**, **12**. 3-Methoxy-1-(4-methoxyphenyl)-6-phenyl-7-hydropyridino[2,3-*d*]1,2,3,4-tetrazolo[1,5-e]pyrimidine-2-carbonitrile (**13**) was synthesized from **8a** or **7a**. Condensation of **8b** with acetophenone to yield **14**, which on further reaction gave **15** then **16**. 4-Hydrazinyl-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyrido[2,3-*d*]pyrimidine-6-carbonitrile (**8a**) also condensed with 4-amino antipyrine giving **17** then **18**. Structures of these compounds have been deduced upon the basis of elemental analysis and spectral data. Significant antifungal activities were observed for some of the synthesized compounds.

1. Introduction

Pyrido[2,3-*d*]pyrimidine and few of its derivatives display potentially useful biological activities [1,2]. They show dihydrofolate reductase inhibition and antitumor [3-5] as well as diuretic properties [6]. Moreover, some of these compounds possess antimicrobial [7,8], antibacterial [9,10], and cytotoxic activities [11,12]. Therefore, a few new pyrido[2,3-*d*]pyrimidine derivatives condensed with 1,2,4-triazole, pyrazole, tetrazole and 1,2,4-triazine rings were synthesized. These new prepared compounds were tested for their antifungal activities.

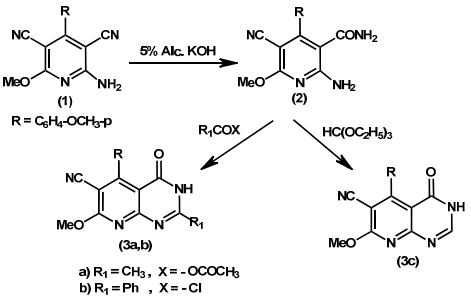
2. Experimental

2.1. Instrumentation

Melting points were determined on a digital Stuart SMP3 and are uncorrected. Infrared spectra were measured on Perkin-Elmer 293 spectrophotometer (γ in cm^{-1}), using KBr disks. ^1H NMR spectra were measured on Gemini-200 spectrometer (200 MHz), using DMSO-d_6 as a solvent and TMS (δ, 0.0 ppm) as internal standard. The mass spectra were recorded on gas chromatographic GCMSqp 1000-ex Shimadzu instrument or HP-MS 5988 mass spectrometer by direct inlet operating at 70 eV. Elemental microanalyses were performed on Perkin Elmer CHN-2400 analyzer or C, H, N manual in micro-analysis center at Cairo University.

2.2. 2-Amino-5-cyano-6-methoxy-4-(4-methoxyphenyl) pyridine-3-carboxamide (2)

To 100 mL of an alcoholic solution of KOH (5%), 0.01 mole of compound **1** was added and the reaction mixture was refluxed for 30 min. After cooling, the reaction mixture was diluted with water and the formed solid was filtered off, washed with water and recrystallized from DMF to give **2** as yellow crystals (Scheme 1). Yield: 82%. M.p.: 250 °C. IR (KBr, ν_{max}, cm^{-1}): 3380 (amide NH_2), 1720 (C=O amide), 1604 (C=C aromatic), 2230 (C≡N). ^1H NMR (DMSO, δ ppm): 2.80-3.02 (6H, s, $-OCH_3$); 6.88 (4H, d, Ar-H); 10.03 (4H, br, NH_2). MS (m/z, %): 298 (M^+, 72.22), 191 (88.89), 147 (100), 121 (61.11), 107(61.11), 76 (55.56), 44 (83.33), 32 (55.56), 26 (61.11). Anal. Calcd. for $C_{15}H_{14}N_4O_3$ (298): C, 60.40; H, 4.98; N, 18.79. Found: C, 60.32; H, 4.90; N, 18.53.



Scheme 1

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2.3. 7-Methoxy-5-(4-methoxyphenyl)-2-substituted-4-oxo-3-hydrpyridino[2,3-d] pyrimidine-6-carbonitrile (3a-c)

A mixture of **2** (0.01 mole) and acetic anhydride, benzoyl chloride or triethyl orthoformate (0.01 mole) was fused at 150 °C for 2 hours. After cooling, the formed solid was washed with ethanol and recrystallized from the proper solvent; **3a** from ethanol and **3b**,**c** from DMF (Scheme 1).

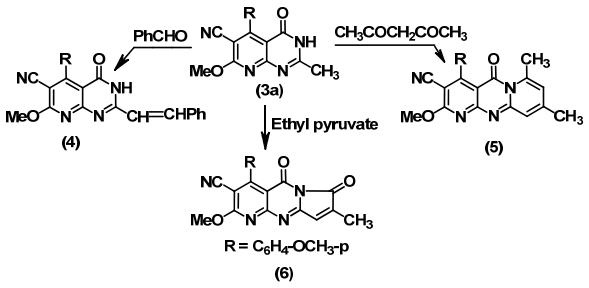
7-methoxy-5-(4-methoxyphenyl)-2-methyl-4-oxo-3,4-dihydro pyrido[2,3-d]pyrimidine-6-carbonitrile (**3a**): Reddish brown crystals. Yield: 73%. M.p.: 270 °C. IR (KBr, ν_{max}, cm^{-1}): 3050 (C-H aromatic), 2980 (C-H aliphatic), 1650 (C=O), 1604 (C=C aromatic), 2240 (C≡N). ^{1}H NMR (DMSO, δ ppm): 1.8 (3H, s, CH_3), 2.73-3.01 (6H, s, OCH_3), 6.72 (4H, d, Ar-H), 11.50 (1H, br, NH). Anal. Calcd. for $C_{17}H_{14}N_4O_3$ (322): C, 63.35; H, 4.35; N,17.39. Found: C, 63.05; H, 4.29; N, 17.41.

7-methoxy-5-(4-methoxyphenyl)-4-oxo-2-phenyl-3,4-dihydro pyrido[2,3-d]pyrimidine-6-carbonitrile (**3b**): Yellow crystals. Yield: 58%. M.p.: 285 °C. IR (KBr, ν_{max}, cm^{-1}): 3020 (C-H aromatic), 2920 (C-H aliphatic), 1690 (C=O), 1590 (C=C aromatic), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.90-3.03 (6H, s, OCH_3), 6.93-7.85 (9H, m, Ar-H), 11.20 (1H, br, NH). Anal. Calcd. for $C_{22}H_{16}N_4O_3$ (384): C, 68.74; H, 4.17; N, 14.58. Found: C, 68.69; H, 4.02; N, 14.53.

7-methoxy-5-(4-methoxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-d] pyrimidine-6-carbonitrile (**3c**): Yellow crystals. Yield: 65%. M.p.: 292 °C. IR (KBr, ν_{max}, cm^{-1}): 3040 (C-H aromatic), 2950 (C-H aliphatic), 1650 (C=O), 1600 (C=C aromatic), 2210 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.75-3.05 (6H, s, OCH_3), 7.05-7.73 (5H, m, Ar-H), 10.80 (1H, br, NH). MS (m/z, %): 322 (M^+, 46.0), 215 (66.5), 107 (100), 108 (39.3), 80 (62.3), 76 (76.5), 65 (26.2), 32 (20.8), 15 (35.1). Anal. Calcd. for $C_{16}H_{12}N_4O_3$ (308): C, 62.34; H, 3.90; N, 18.18. Found: C, 62.25; H, 3.82; N, 18.09.

2.4. 2-((1E)-2-phenylvinyl)-7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydro pyridino[2,3-d]pyrimidine-6-carbonitrile (4)

A mixture of **3a** (0.01 mole) and benzaldehyde (0.012 mole) in ethanol (30 mL) was refluxed for one hour. After cooling, the solid obtained was filtered off and recrystallized from ethanol to give **4** as yellow crystals (Scheme 2). Yield: 55%. M.p.: 230 °C. IR (KBr, ν_{max}, cm^{-1}): 3044 (C-H aromatic), 2978 (C-H aliphatic), 1690 (C=O), 1632 (C=C), 2240 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.73-3.10 (6H, s, OCH_3), 3.5 (2H, d, CH=CH-Ph), 7.2 (9H, m, Ar-H), 12.05 (1H, br, NH). Anal. Calcd. for $C_{24}H_{18}N_4O_3$ (410): C, 70.24; H, 4.39; N,13.66. Found: C, 70.19; H, 4.32; N, 13.58.



Scheme 2

2.5. 2-Methoxy-4-(4-methoxyphenyl)-7,9-dimethyl-5-oxo-6-hydropyridino[2,3-d] pyridino[1,2-a]pyrimidine-3-carbonitrile (5)

A mixture of **3a** (0.01 mole) and the corresponding β-dicarbonyl derivative; acetyl acetone, (0.012 mole) in ethanol (30 mL) was refluxed for one hour. After cooling, the solid obtained was filtered off and recrystallized from ethanol to give **5** as yellow crystals (Scheme 2). Yield: 65%. M.p.: 290 °C. IR (KBr, ν_{max}, cm^{-1}): 3035 (C-H aromatic), 2950 (C-H aliphatic), 1710 (C=O), 1645 (C=C), 2237 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.03 (6H, s, $-CH_3$), 2.7-3.0 (6H, s, $-OCH_3$), 6.8 (6H, m, Ar-H, H-8, H-10). MS (m/z, %): 386 (M^+, 11.8), 278 (30.5), 250 (42.5), 171 (100), 115 (32.6), 107 (30.9), 79 (13.8), 30 (17.1), 26 (15.8). Anal. Calcd. for $C_{22}H_{18}N_4O_3$ (386): C, 68.39; H, 4.66; N, 14.51. Found: C, 68.41; H, 4.52; N, 14.60.

2.6. 2-Methoxy-4-(4-methoxyphenyl)-8-methyl-5,7-dioxo-6-hydropyridino[2,3-d]3-pyridino[1,2-a]pyrimidine-3-carbonitrile (6)

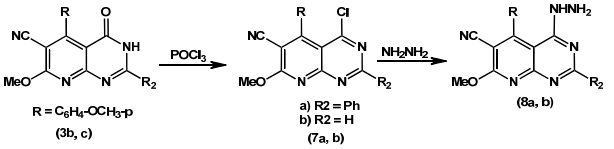
A mixture of **3a** (0.01 mole) and ethyl pyruvate (0.012 mole) in ethanol (30 mL) was refluxed for one hour. After cooling, the solid obtained was filtered off and recrystallized from ethanol to give **6** as pale brown crystals (Scheme 2). Yield: 65%. M.p.: 275 °C. IR (KBr, ν_{max}, cm^{-1}): 2980 (aliphatic C-H), 1720 (C=O), 1604 (C=C aromatic), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.03-3.20 (9H, d, $-CH_3$, $-OCH_3$), 7.3 (5H, m, Ar-H, H-9). Anal. Calcd for $C_{20}H_{14}N_4O_4$ (374): C, 64.17; H, 3.74; N, 14.97. Found: C, 64.09; H, 3.69; N, 15.03.

2.7. 4-Chloro-7-methoxy-5-(4-methoxyphenyl)-2-substituted pyridino[2,3-d]pyrimidine-6-carbonitrile (7a,b)

A mixture of **3b** or **3c** (5 g) and $POCl_3$ (25 mL) was refluxed for 3 hours. The reaction mixture was cooled and poured on 200 g ice water. The formed solid was filtered off, washed with water and recrystallized from ethanol to give **7a** and **7b** as yellow crystals (Scheme 3).

4-chloro-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyrido [2,3-d]pyrimidine-6-carbonitrile (**7a**): Yield: 70%. M.p.: 185 °C. IR (KBr, ν_{max}, cm^{-1}): 3020 (aromatic C-H), 2980 (aliphatic C-H), 1604 (C=C aromatic), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.3-3.1 (6H, s, $-OCH_3$), 6.80-7.20 (9H, m, Ar-H). Anal. Calcd. for $C_{22}H_{15}N_4O_2Cl$ (402.5): C, 65.59; H, 3.73; N,13.91; Cl, 8.82. Found: C, 65.62; H, 3.70; N, 13.89; Cl, 8.70.

4-chloro-7-methoxy-5-(4-methoxyphenyl)pyrido[2,3-d] pyrimidine-6-carbonitrile (**7b**): Yield: 75%. M.p.: 172 °C. IR (KBr, ν_{max}, cm^{-1}): 3040 (aromatic C-H), 2950 (aliphatic C-H), 1600 (C=C aromatic), 2210 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.8-3.5 (6H, s, $-OCH_3$), 7.20-7.77 (5H, m, Ar-H). Anal. Calcd. for $C_{16}H_{11}N_4O_2Cl$ (326.5): C, 58.81; H, 3.37; N, 17.15; Cl, 10.87. Found: C, 59.03; H, 3.40; N, 17.18; Cl, 10.90.



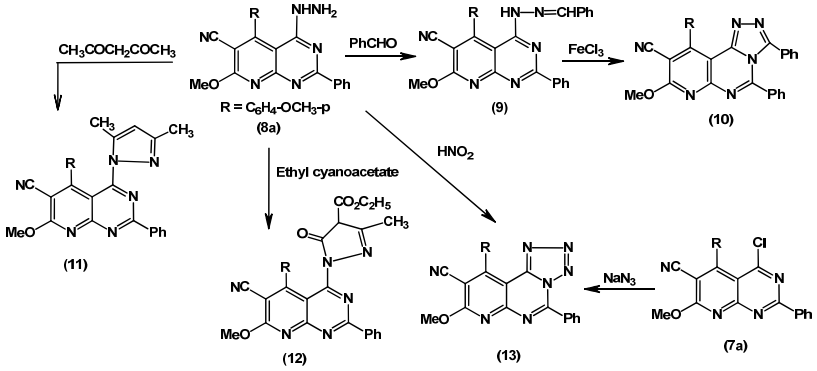
Scheme 3

2.8. 4-Hydrazino-7-methoxy-5-(4-methoxyphenyl)-2-substituted pyridino[2,3-d] pyrimidine-6-carbonitrile (8a,b)

A mixture of **7a** or **7b** (0.01 mole) and hydrazine hydrate (98%; 0.05 mole) in 50 mL ethanol was refluxed for 3 hours. After cooling, the formed solid was filtered off and recrystallized from the proper solvent to give **8a** (ethanol) and **8b** (DMF) as colorless crystals (Scheme 3).

4-Hydrazinyl-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyrido [2,3-d]pyrimidine-6-carbonitrile (**8a**): Yield: 55%. M.p.: 220 °C. IR (KBr, ν_{max}, cm^{-1}): 3020 (aromatic C-H), 2980 (aliphatic C-H), 1604 (C=C aromatic), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.3-2.9 (6H, s, $-OCH_3$), 7.3 (9H, m, Ar-H), 11.9 (3H, br, NH-NH_2). Anal. Calcd. for $C_{22}H_{18}N_6O_2$ (398): C, 66.33; H, 4.52; N, 21.11. Found: C, 66.36; H, 4.50; N, 21.20.

4-Hydrazinyl-7-methoxy-5-(4-methoxyphenyl)pyrido[2,3-d] pyrimidine-6-carbonitrile (**8b**): Yield: 63%. M.p.: 235 °C. IR (KBr,



Scheme 4

ν_{max}, cm^{-1}): 3030 (aromatic C-H), 2910 (aliphatic C-H), 1580 (C=C aromatic), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.7-3.1 (6H, s, -OCH$_3$), 7.05-7.63 (5H, m, Ar-H), 11.7 (3H, br, NH-NH$_2$). Anal. Calcd. for $C_{16}H_{14}N_6O_2$ (322): C, 59.63; H, 4.35; N, 26.09, Found: C, 59.59; H, 4.33; N, 26.12.

2.9. 4-[((1E)-1-aza-2-phenylvinyl)amino]-7-methoxy-5-(4-methoxyphenyl)-2-phenyl pyridino[2,3-d]pyrimidine-6-carbonitrile (9)

A mixture of **8a** (0.01 mole) and the benzaldehyde (0.012 mole) in acetic acid (20%; 30 mL) was refluxed for one hour. The solid formed was filtered off and recrystallized from the ethanol to give **9** as pale yellow crystals (Scheme 4). Yield: 85%. M.p.: 235 °C. IR (KBr, ν_{max}, cm^{-1}): 3480(NH), 3020 (aromatic C-H), 2980 (aliphatic C-H), 2230 (C≡N), 1660 (δ NH), 1610-1590 (C=C and C=N). ^{1}H NMR (DMSO, δ ppm): 2.3-2.8 (6H, s, -OCH$_3$), 6.8-8.0 (15H, m, Ar-H, CH=N), 11.9 (1H, br, NH). Anal. Calcd. for $C_{29}H_{22}N_6O_2$ (486): C, 71.60; H, 4.53; N, 17.28. Found: C, 71.62; H, 4.60; N, 17.33.

2.10. 3-methoxy-1-(4-Methoxyphenyl)-6,8-diphenyl-7-hydropyridino[2,3-d]1,2,4-triazolo[4,5e] pyrimidine-2-carbonitrile (10)

Compound **9**, was cyclized by oxidation with $FeCl_3$ on refluxing in ethanol for 6 hours. The formed solid was filtered off and recrystallized from ethanol to give **10** as colorless crystals (Scheme 4). Yield: 65%. M.p.: 290 °C. IR (KBr, ν_{max}, cm^{-1}): 3030 (aromatic C-H), 2970 (aliphatic C-H), 1600 (C=C and C=N), 2220 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.3-2.9 (6H, s, -OCH$_3$), 6.8-8.1 (14H, m, Ar-H). MS (m/z, %): 484 (M$^+$, 54.51), 377 (33.4), 270 (23.26), 116 (75.97), 107 (100), 77 (52.27), 32 (64.43). Anal. Calcd. for $C_{29}H_{20}N_6O_2$ (486): C, 71.90; H, 4.13; N, 17.36. Found: C, 71.87; H, 4.20; N, 17.40.

2.11. 4-(3,5-dimethylpyrazolyl)-7-methoxy-5-(4-methoxy phenyl)-2-phenylpyridino [2,3d] pyrimidine-6-carbonitrile (11)

A mixture of **8a** (0.01 mole) and *β*-dicarbonyl derivative; acetyl acetone (0.012 mole) in ethanol (30 mL) was refluxed for 5 hours. After cooling, the reaction mixture was poured onto cold water and the solid obtained was filtered off and recrystallized from ethanol to give **11** as colorless crystals (Scheme 4). Yield: 55%. M.p.: 190 °C. IR (KBr, ν_{max}, cm^{-1}): 3030 (aromatic C-H), 2970 (aliphatic C-H), 1595 (C=C and C=N), 2220 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.3 (6H, s, -CH$_3$), 3.02 (6H, s, -OCH$_3$), 7.8-8.01 (10H, m, Ar-H, H-4[pyrzolo-]). MS (m/z, %): 462 (M$^+$, 62.50), 376 (68.75), 238 (75.00), 131 (62.50), 129 (62.50), 107 (62.50), 95 (52.30), 76 (100), 32 (75.00). Anal. Calcd. for $C_{27}H_{22}N_6O_2$ (462): C, 70.13; H, 4.76; N, 18.18, Found: C, 70.10; H, 4.52; N, 18.60.

2.12. Ethyl 1-[7-amino-6-cyano-5-(4-methoxyphenyl)-2-phenylpyridino[3,2-e] pyrimidin-4-yl]-3-methyl-5-oxo-2-pyrazoline-4-carboxylate (12)

A mixture of **8a** (0.01 mole) and ethyl cyanoacetate (0.01 mole) in acetic anhydride (20 mL) was refluxed for 3 hours. After cooling, the reaction mixture was poured onto cold water and the solid obtained was filtered off and recrystallized from ethanol to give **12** as a colorless crystals (Scheme 4). Yield: 55%. M.p.: 240 °C. IR (KBr, ν_{max}, cm^{-1}): 2980 (aliphatic C-H), 1740 (C=O), 1604 (C=C and C=N), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.1 (3H, s, -CH$_3$), 2.7 (3H, t, -CH$_3$ of the ester), 3.8 (6H, s, -OCH$_3$), 4.2 (2H, q, -CH$_2$ of the ester), 7.4-8.0 (9H, m, Ar-H). MS (m/z, %): 536 (M$^+$, 45.83), 367 (41.67), 238 (45.83), 168 (41.67), 140 (41.67), 131 (62.50), 129 (45.83), 107 (87.52), 95 (100), 76 (45.83), 45 (70.83), 28 (87.50). Anal. Calcd for $C_{29}H_{24}N_6O_5$ (536): C, 64.93; H, 4.78; N, 15.67. Found: C, 65.02; H, 4.80; N, 15.70.

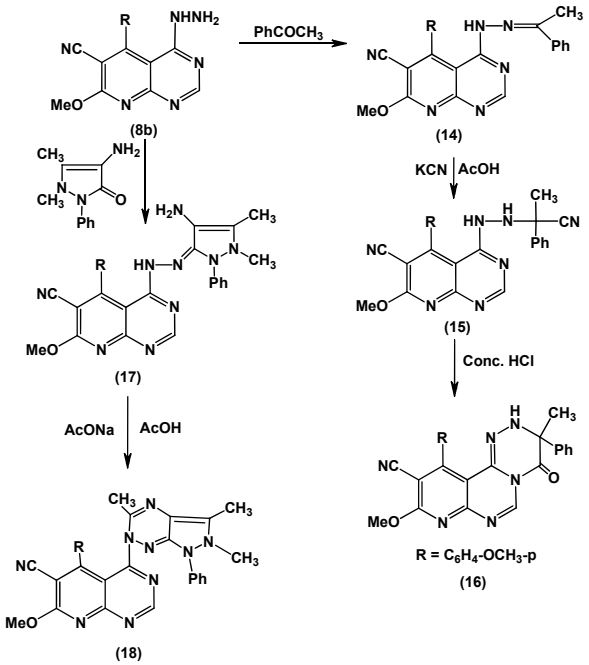
2.13. 3-Methoxy-1-(4-methoxyphenyl)-6-phenyl-7-hydro pyridino[2,3-d]1,2,3,4-tetrazolo[1,5-e]pyrimidine-2-carbonitrile (13)

A solution of **8a** (0.01 mole) in acetic acid (5 mL) and water (1 mL) was treated with a solution of $NaNO_2$ (0.015 mole) in water (2 mL) at 0-5 °C. The solid obtained upon diluting the reaction mixture with 10 mL of water was filtered off and recrystallized from ethanol to give **13** as yellow crystals (Scheme 4). Yield: 65%. M.p.: 235 °C. This compound was also synthesized by refluxing of a mixture of **7a** (0.01 mole) in DMF (30 mL) and sodium azide (0.01 mole) in water (2 mL) for 3 hours, then the reaction mixture was cooled, poured onto cold water and the resulting solid was filtered off (Scheme 4). IR (KBr, ν_{max}, cm^{-1}): 2960 (aliphatic C-H), 1600 (C=C and C=N), 2230 (C≡N). ^{1}H NMR (DMSO, δ ppm): 3.19 (6H, s, -OCH$_3$), 6.9-7.5 (9H, m, Ar-H). Anal. Calcd for $C_{22}H_{15}N_7O_2$ (409): C, 64.55; H, 3.67; N, 23.96. Found: C, 64.49; H, 3.71; N, 23.89.

2.14. 4-[((1E)-1-Aza-2-phenyl prop-1-enyl)amino]-7-methoxy-5-(4-methoxy phenyl) pyridino[2,3-d]pyrimidine-6-carbonitrile (14)

An equimolar mixture of **8b** and acetophenone in ethanol was refluxed for 15 minutes. After cooling, the resulting solid was recrystallized from ethanol to give **14** as colorless crystals (Scheme 5). Yield: 70%. M.p.: 210 °C. IR (KBr, ν_{max}, cm^{-1}): 3260-3120 (NH), 3050 (aromatic C-H), 2990 (aliphatic C-H), 1630-1600 (C=C and C=N), 2220 (C≡N). ^{1}H NMR (DMSO, δ ppm):

2.6-3.0 (9H, d, -CH_3, -OCH_3), 7.1-8.0 (10H, m, Ar-H, H-2), 12.09 (1H, br, NH). Anal. Calcd. for $C_{24}H_{20}N_6O_2$ (424): C, 67.92; H, 4.72; N, 19.18, Found: C, 67.90; H, 4.30; N, 19.72.



Scheme 5

2.15. 4-[2-(1-cyano-1-phenylethyl)hydrazino]-7-methoxy-5-(4-methoxyphenyl) pyridino [2,3-d]pyrimidine-6-carbonitrile (15)

A mixture of **14** (0.01 mole) and KCN (0.01 mole, in 10 mL water) in glacial acetic acid (50 mL) and ethanol (10 mL) was refluxed for 2 hours. After cooling and diluting with cold water, the resulting solid was filtered off and recrystallized from ethanol to give **15** as colorless crystals (Scheme 5). Yield: 50%. M.p.: 221 °C. IR (KBr, ν_{max}, cm^{-1}): 3260-3130 (NH), 3010 (aromatic C-H), 2950 (aliphatic C-H), 1630-1580 (C=C and C=N), 2260 (C≡N). ^{1}H NMR (DMSO, δ ppm): 2.9 (3H, s, -CH_3), 3.1 (6H, s, -OCH_3), 7.3-7.9 (10H, m, Ar-H, H-2), 11.9 (2H, br, NH-NH). Anal. Calcd. for $C_{25}H_{21}N_7O_2$ (451): C, 66.52; H, 4.56; N, 21.73. Found: C, 66.55; H, 4.61; N, 21.70.

2.16. 3-Methoxy-1-(4-methoxyphenyl)-9-methyl-8-oxo-9-phenyl-7-hydro-10H-pyridino[2,3-d]1,2,4-triazino[4,3-e]pyrimidine-2-carbonitrile (16)

Compound **15** (2 g) was refluxed in concentrated HCl (50%; 5 mL) for 4 hours. After cooling and diluting with water, the resulting solid was filtered off washed with water and recrystallized from ethanol to give **16** as colorless crystals (Scheme 5). Yield: 30%. M.p.: >300 °C. IR (KBr, ν_{max}, cm^{-1}): 3250-3100 (NH), 3010 (aromatic C-H), 2950 (aliphatic C-H), 1640-1600 (C=C and C=N), 1700 (C=O) 2240 (C≡N). ^{1}H NMR (DMSO, δ ppm): 1.3 (3H, s, -CH_3), 2.9 (6H, m, -OCH_3), 7.8 (10H, m, Ar-H, H-6), 10.3 (1H, s, NH). Anal. Calcd. for $C_{25}H_{20}N_6O_3$ (452): C, 66.37; H, 4.42; N, 18.58. Found: C, 66.42; H, 4.30; N, 19.06.

2.17. 4-{[(4-Amino-2,3-dimethyl-1-phenyl](3-pyrazolin-5-ylidene))azamethyl]amino)-7-methoxy-5-(4-methoxy phenyl)pyridino[2,3-d]pyrimidine-6-carbonitrile (17)

A mixture of **8b** (0.01 mole) and 4-aminoantipyrine (0.01 mole) in absolute ethanol (50 mL) and few drops of acetic acid was refluxed for one hour. The reaction mixture was then cooled, filtered off and recrystallized from ethanol to give **17** as pale yellow crystals (Scheme 5). Yield: 40%. M.p.: 135 °C. IR (KBr, ν_{max}, cm^{-1}): 3400-3200 (NH), 3020 (aromatic C-H), 2910 (aliphatic C-H), 1640-1600 (C=C and C=N), 1700 (C=O), 2220 (C≡N). ^{1}H NMR (DMSO, δ ppm): 1.8-2.9 (12H, br, -CH_3, -OCH_3), 7.3 (10H, m, Ar-H, H-2), 10.3-11.02 (3H, br, NH, NH_2). Anal. Calcd. for $C_{27}H_{25}N_9O_2$ (507): C, 63.91; H, 4.93; N, 24.85. Found: C, 64.09; H, 5.07; N, 24.78.

2.18. 7-Methoxy-5-(4-methoxyphenyl)-4-(2,3,5-trimethyl-1-phenyl(3-pyrzolino[4,5-e]1,2,4-triazin-6-yl))pyridino[2,3-d]pyrimidine-6-carbonitrile (18)

Compound **17** (0.01 mole) in glacial acetic acid (50 mL) and fused sodium acetate (10 g), was refluxed for 6 hours. After cooling and diluting with water, the formed solid was filtered off and recrystallized from ethanol to give **18** as yellow crystals (Scheme 5). Yield: 50%. M.p.: 260 °C. IR (KBr, ν_{max}, cm^{-1}): 3030 (aromatic C-H), 2960 (aliphatic C-H), 1650-1630 (C=C and C=N), 2220 (C≡N). ^{1}H NMR (DMSO, δ ppm): 1.8-2.1 (9H, s, -CH_3), 3.02 (6H, s, -OCH_3), 6.7-7.2 (10H, m, Ar-H, H-2). MS (m/z, %): 531 (M^+, 30.30), 291 (33.33), 240 (84.85), 184 (100), 158 (63.64), 127 (30.30), 107 (51.52), 76 (57.58), 31 (48.48), 26 (72.73). Anal. Calcd. for $C_{29}H_{25}N_9O_2$ (531): C, 65.54; H, 4.71; N, 23.73. Found: C, 65.50; H, 4.69; N, 23.67.

3. Results and discussion

3.1. Synthesis

The synthesis of heterocyclic systems containing pyrido[2,3-*d*]pyrimidine moiety has gained much attention due to high biological activity possessed by these compunds [1-12]. 2-amino-6-methoxy-4-(4-methoxyphenyl)pyridine-3,5-dicarbonitrile (**1**) was prepared [13]. Hydrolysis of **1** using alcoholic solution of KOH (5%) gave the amide derivative **2**, which on condensation with acetic anhydride, benzoyl chloride or triethyl orthoformate [14] yielded the pyrido[2,3-*d*]pyrimidine derivatives **3a-c**, respectively (Scheme 1).

Due to the high reactivity of the methyl group in position-2, compound **3a** underwent condensation reactions with aromatic aldehydes such as benzaldehyde, to give 2-((1*E*)-2-phenylvinyl)-7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydro pyridino[2,3-*d*]pyrimidine-6-carbonitrile (**4**). Also, a condensation reaction with β-diketones, as acetyl acetone, gave 2-methoxy-4-(4-methoxyphenyl)-7,9-dimethyl-5-oxo-6-hydro pyridino[2,3-*d*]pyridino [1,2-*a*]pyrimidine-3-carbonitrile (**5**). Also, 3a was condensed with ethyl pyruvate to give 2-methoxy-4-(4-methoxyphenyl)-8-methyl-5,7-dioxo-6-hydropyridino [2,3-*d*]3-pyridino[1,2-*a*]pyrimidine-3-carbonitrile (**6**), (Scheme 2).

7-Methoxy-5-(4-methoxyphenyl)-2-phenyl-4-oxo-3-hydro pyridino[2,3-*d*] pyrimidine-6-carbonitrile (**3b**) and 7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydrpyridino[2,3-*d*] pyrimidine-6-carbonitrile (**3c**), were refluxed with $POCl_3$ to give 4-chloro-7-methoxy-5-(4-methoxyphenyl)-2-substituted pyridino[2,3-*d*]pyrimidine-6-carbonitrile (**7a**,**b**), respectively, which on reaction with hydrazine hydrate yielded the hydrazino-derivatives **8a**,**b**, respectively (Scheme 3).

Treatment of 4-hydrazino-7-methoxy-5-(4-methoxy phenyl)-2-phenylpyridino[2,3-*d*] pyrimidne-6-carbonitrile (**8a**) with benzaldehyde, gave 4-[((1*E*)-1-aza-2-phenylvinyl)amino]-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyridino[2,3-*d*] pyrimidine-6-carbonitrile (**9**), which on oxidative cyclization using $FeCl_3$, yielded the fused pyridino[2,3-*d*]1,2,4-triazolo[4,5*e*] pyrimidine, **10**. β-Dicarbonyl reagents such as acetyl acetone, reacted with **8a** to give 4-(3,5-dimethyl pyrazolyl)-7-methoxy-5-(4-methoxyphenyl)-2-phenyl pyridine [2,3-*d*]pyrimidine-6-carbonitrile (**11**), [15]. Moreover, **8a** on refluxing with a mixture of acetic anhydride and ethyl cyanoacetate afforded ethyl-1-[7-amino-6-cyano-5-(4-methoxyphenyl)-2-phenylpyri-

dino[3,2-*e*]pyrimidin-4-yl]-3-methyl-5-oxo-2-pyra-zoline-4-carboxylate (**12**) [15]. Reaction of **8a** with nitrous acid and the reaction of **7a** with sodium azide in DMF yielded 3-methoxy-1-(4-methoxyphenyl)-6-phenyl-7-hydro-pyridino[2,3-*d*]1,2,3,4-tetrazolo[1,5-*e*]pyrimidine-2-carbonitrile (**13**) [16,17] (Scheme 4).

Compound **8b** was condensed with acetophenone in glacial acetic acid to give the hydrazone **14** which underwent addition of HCN in acetic acid-ethanol mixture, giving the cyano hydrazone **15**. Further, acidic hydrolysis of **15** by refluxing in concentrated HCl, led to the formation of 3-methoxy-1-(4-methoxyphenyl)-9-methyl-8-oxo-9-phenyl-7-hydro-10*H*-pyridino[2,3-*d*]1,2,4-triazino[4,3-*e*]pyrimidine-2-carbonitrile (**16**) [18]. Also, **8b**, was condensed with 4-aminoantipyrine in absolute ethanol in presence of few drops of acetic acid to give 4-{[(4-amino-2,3-dimethyl-1-phenyl](3-pyrazolin-5-ylidene)) azamethyl]amino)-7-methoxy-5-(4-methoxyphenyl)pyridine [2,3-*d*]pyrimidine-6-carbonitrile (**17**), which on refluxing with glacial acetic acid-fused sodium acetate, *via* acylation followed by cyclo-condensation, produced 7-methoxy-5-(4-methoxy phenyl)-4-(2,3,5-trimethyl-1-phenyl(3-pyrazolino[4,5-*e*]1,2,4-triazin-6-yl))pyridino[2,3-*d*]pyrimidine-6-carbonitrile (**18**), (Scheme 5).

3.2. Biological screening

A few newly synthesized compounds were screened for their antifungal activities against three types of fungi, *Alternaria alternata, Aspergillus niger*, and *Aspergillus flavipes*, using the disk diffusion method [19-21]. The tested compounds were dissolved in DMF, which was used as a control to get 1 mg/mL solution. The inhibition zones of microbial growth surrounding the filter paper disc (2.5 mm) were measured in millimeters at the end of an incubation period at 30 °C for 3 days. Activity of each compound was compared with that of flucanazole as the standard. The investigation of fungicidal screening data revealed that all the tested compounds showed variable activities towards the investigated fungi used, indicating that the compounds are biologically active due to the presence of different heterocycles and functional groups. Compounds **3a, 13** and **18** showed very high activities, whereas compounds **6**, **10** and **17** showed high activity against them. On the other hand, compounds **3b** and **7a** showed moderate activities against *Alternaria alternata* and *Aspergillus niger*, while compound **11** showed low activity against *Alternaria alternata* and *Aspergillus niger* and moderate activity against *Aspergillus flavipes* (Table 1).

Table 1. Antifungal activities data of some of the prepared compounds.

Compound	Diameter of inhibition zone (mm)*		
	Alternaria alternata	Aspergillus niger	*Aspergillus flavipes*
3a	++++	++++	++++
3b	++	++	+
5	+	++	++
6	+++	+++	+++
7a	++	++	++
10	+++	+++	+++
11	+	+	++
13	++++	++++	++++
16	+++	++	++
17	+++	+++	+++
18	++++	++++	++++
Fluconazole	++++	++++	++++

*Very high activity = ++++ (inhibition zone > 30 mm), High activity = +++ (inhibition zone 21-30 mm), Moderate activity = ++ (inhibition zone 11-20 mm), Low activity = + (inhibition zone 1-10 mm).

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