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

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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-methoxyphenyl)-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-hydrazone-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-hydroxypyrido[2,3-d]1,2,3-tetrazolo[1,5-e]pyrimidine-2-carbonitrile (13) was synthesized from 8a or 7a. Condensation of 8b with acetyl phenone to yield 14, which on further reaction gave 15 then 16. 4-Hydrazone-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyrido[2,3-d]pyrimidine-6-carbonitrile (8a) also condensated 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 condensated with 1,2,4-triazole, pyrazole, tetrazole and 1,2,4-triazine rings were synthesized. These new 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 (μ in cm−1), using KBr disks. 1H NMR spectra were measured on Gemini-200 spectrometer (200 MHz), using DMSO-d6 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∞, 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, -OCH3); 6.88 (4H, d, Ar-H); 10.03 (4H, br, NH). 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 C17H14N4O4: C, 60.40; H, 4.90; N, 18.79. Found: C, 60.32; H, 4.80; N, 18.53.

Scheme 1
2.3. 7-Methoxy-5-(4-methoxyphenyl)-2-substituted-4-oxo-3-hydropyridino[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 from DMF [Scheme 1].

7-methoxy-5-(4-methoxyphenyl)-2-methyl-4-oxo-3,4-dihydropyridino[2,3-d]pyrimidine-6-carbonitrile (3a): Reddish brown crystals. Yield: 73%. M.p.: 270 °C. IR (KBr, υmax cm⁻¹): 2860 (C=H aromatic), 1670 (C=C aromatic), 3240 (N-H). Η NMR (DMSO, δ ppm): 1.83 (3H, s, CH₃), 7.30 (6H, s, Ar-H). Anal. Calcd. for C₁₅H₁₂N₂O₃ (308): C, 62.60; H, 4.02; N, 14.53.

7-methoxy-5-(4-methoxyphenyl)-2-methyl-4-oxo-3,4-dihydropyridino[2,3-d] pyrimidine-6-carbonitrile (3b): Yellow crystals. Yield: 80%. M.p.: 285 °C. IR (KBr, υmax cm⁻¹): 3020 (C=H aromatic), 2920 (C=H aromatic), 1690 (C=O), 1590 (C=C aromatic), 2230 (C=N). Η NMR (DMSO, δ ppm): 2.00-3.05 (6H, s, OCH₃), 6.93-7.50 (9H, m, Ar-H). Anal. Calcd. for C₂₀H₁₆N₂O₃ (386): C, 63.85; H, 4.35; N, 11.79. Found: C, 63.05; H, 4.29; N, 17.41.

7-methoxy-5-(4-methoxyphenyl)-2-methyl-4-oxo-3,4-dihydropyridino[2,3-d] pyrimidine-6-carbonitrile (3c): Yellow crystals. Yield: 65%. M.p.: 292 °C. IR (KBr, υmax cm⁻¹): 3040 (C=H aromatic), 1650 (C=O), 1650 (C=C aromatic), 2210 (C=N). Η NMR (DMSO, δ ppm): 2.75-3.50 (6H, s, OCH₃), 7.05-7.73 (5H, m, Ar-H). 10.80 (1H, br, NH). MS (m/z, %): 322 [M⁺, 40], 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 C₂₁H₁₇N₂O₃ (308): C, 62.34; H, 3.90; N, 18.18. Found: C, 62.55; H, 3.82; N, 18.09.

2.4. 1-(1H-2-phenylvinyl)-7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydropyridino[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].

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].

2.6. 2-Methoxy-4-(4-methoxyphenyl)-8-methyl-5,7-dioxo-6-hydropyridino[2,3-d]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, υmax cm⁻¹): 2980 (aliphatic C-H), 1720 (C=O), 1604 (C=C aromatic), 2230 (C=N). Η NMR (DMSO, δ ppm): 2.03-3.20 (9H, d, CH₂, OCH₃), 7.3 (5H, m, Ar-H). Anal. Calcd. for C₂₀H₁₉N₂O₄ (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 POCI₅ (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-phenylpyrroldino[2,3-d]pyrimidine-6-carbonitrile (7a): Yield: 70%. M.p.: 185 °C. IR (KBr, υmax cm⁻¹): 3020 (aromatic C-H), 2980 (aliphatic C-H), 1604 (C=C aromatic), 2230 (C=N). Η NMR (DMSO, δ ppm): 2.3-3.1 (6H, s, OCH₃), 6.89-7.20 (9H, m, Ar-H). Anal. Calcd. for C₂₁H₁₇ClN₂O₂ (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)pyrroldino[2,3-d]pyrimidine-6-carbonitrile (7b): Yield: 75%. M.p.: 172 °C. IR (KBr, υmax cm⁻¹): 3040 (aromatic C-H), 2950 (aliphatic C-H), 1600 (C=C aromatic), 2210 (C=N). Η NMR (DMSO, δ ppm): 2.8-3.5 (6H, s, OCH₃), 7.20-7.77 (5H, m, Ar-H). Anal. Calcd. for C₂₁H₁₇ClN₂O₂ (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.

2.8. 4-Hydrainzo-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-Hydrainzo-7-methoxy-5-(4-methoxyphenyl)pyrroldino[2,3-d]pyrimidine-6-carbonitrile (8b): Yield: 63%. M.p.: 235 °C. IR (KBr,
85%.	 M.p.	 235°C.	 IR	 (KBr,
mole)	in	acetic	acid	(20%;	30	mL)	was	refluxed	for	one	hour.

2.9. 4-((1E)-1-aza-2-phenylvinyl)aminol-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, νmax 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). 1H NMR (DMSO, δ ppm): 2.3-2.8 (6H, s, -OCH3), 6.8-8.0 (15H, m, Ar-H, CH=N), 11.9 (1H, br, NH). Anal. Calcd. for C16H14N6O2 (322): C, 59.63; H, 4.35; N, 26.09. Found: C, 59.59; H, 4.35; N, 26.12.

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

Compound 9, was cyclized by oxidation with FeCl3 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, νmax cm⁻¹): 3030 (aromatic C-H), 2970 (aliphatic C-H), 1600 (C=C and C=N), 2220 (C≡N). 1H NMR (DMSO, δ ppm): 2.3-2.9 (6H, s, -OCH3), 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 (64.63). Anal. Calcd. for C29H20N6O2 (486): C, 71.60; H, 4.53; N, 17.28. Found: C, 71.62; H, 4.60; N, 17.33.

2.11. 4-(3,5-dimethylpyrazolyl)-7-methoxy-5-(4-methoxy phenyl)-2-phenylpyridino [2,3,d]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, νmax cm⁻¹): 3030 (aromatic C-H), 2970 (aliphatic C-H), 1595 (C=C and C=N), 2220 (C≡N). 1H NMR (DMSO, δ ppm): 2.3 (6H, s, -CH3), 3.02 (6H, s, -OCH3), 7.8-8.01 (10H, m, H-4[pyrazolo]). 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 C28H22N6O2 (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-aminocan-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, νmax cm⁻¹): 2980 (aliphatic C-H), 1740 (C=O), 1604 (C=C and C=N), 2230 (C≡N). 1H NMR (DMSO, δ ppm): 2.1 (3H, s, -CH3), 2.7 (3H, t, -CH2 of the ester), 3.8 (6H, s, -OCH3), 4.2 (2H, q, -CH2 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 C30H24N8O2 (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]1,2,4-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 1 mole of NaN3 (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 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, νmax cm⁻¹): 2960 (aliphatic C-H), 1600 (C=C and C=N), 2230 (C≡N). 1H NMR (DMSO, δ ppm): 3.19 (6H, s, -OCH3), 6.9-7.5 (9H, m, Ar-H). Anal. Calcd. for C30H24N8O2 (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)aminol-7-methoxy-5-(4-methoxy phenyl) pyridino[2,3,d]pyrimidine-6-carbonitrile (14)

An equimolar mixture of 8b and acetoephone 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, νmax cm⁻¹): 3260-3120 (NH), 3050 (aromatic C-H), 2990 (aliphatic C-H), 1640-1600 (C=C and C=N), 2220 (C≡N). 1H NMR (DMSO, δ ppm):

Scheme 5

2.15. 4-[2-(1-cyano-1-phenylethyl)hydrazino]-7-methoxy-5-(4-methoxyphenyl) pyridine [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 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, νmax cm⁻¹): 3260-3130 (NH), 3010 (aliphatic C-H), 2950 (aliphatic C-H), 1630-1580 (C=C and C=N), 2260 (C≡N). 1H NMR (DMSO, δ ppm): 2.9 (3H, s, -CH3), 3.1 (6H, s, -OCH3), 7.3-7.9 (10H, m, Ar-H, H-2), 11.9 (2H, br, NH-NH). Anal. Calc'd. for C32H32N2O3 (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-phenoxy-7-hydroxy-10H-pyridino[2,3-d]1,4-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, νmax cm⁻¹): 3250-3100 (NH), 3010 (aliphatic C-H), 2950 (aliphatic C-H), 1640-1600 (C=C and C≡N), 1700 (C=O) 2240 (C≡N). 1H NMR (DMSO, δ ppm): 1.3 (3H, s, -CH3), 2.9 (6H, m, -OCH3), 7.8 (10H, m, Ar-H, H-6), 10.3 (1H, s, NH). Anal. Calc'd. for C42H34N2O3 (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)]azamino)methylene}-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, νmax cm⁻¹): 3400-3200 (NH), 3020 (aliphatic C-H), 2910 (C=C and C≡N), 1700 (C=O), 2220 (C≡N). 1H NMR (DMSO, δ ppm): 1.8-2.9 (12H, br, -CH3, -OCH3), 7.3 (10H, m, Ar-H, H-2), 10.3-11.0 (3H, br, NH, NH2). Anal. Calc'd. for C42H34N2O3 (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-pyrazolin-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.: 360 °C. IR (KBr, νmax cm⁻¹): 3030 (aliphatic C-H), 2960 (aliphatic C-H), 1650-1630 (C=C and C≡N), 2220 (C≡N). 1H NMR (DMSO, δ ppm): 1.8-21 (9H, s, -CH3), 3.02 (6H, s, -OCH3), 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 (65,64), 127 (30,30), 107 (51,52), 76 (57,58), 31 (48,48), 26 (72,73). Anal. Calc'd. for C42H34N2O3 (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 pyridino[2,3-d]pyrimidine moiety has gained much attention due to its high biological activity possessed by these compounds [1-12]. 2-amino-6-methoxy-4-(4-methoxyphenyl)pyridine-3,5-dicarboxonitrile (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 pyridino[2,3-d]pyrimidine derivatives 3a-c, respectively (Scheme 1).

Due to the high reactivity of the methyl group in position-2 of compound 3a underwent condensation reactions with aromatic aldehydes such as benzaldehyde, to give 2-((1E)-2-phenylvinyl)-7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydroxy pyridino[2,3-d]pyrimidine-6-carbonitrile (4). Also, a condensation reaction with β-diketones, as acetyl acetonitrile, gave 2-methoxy-4-(4-methoxyphenyl)-7,9-dimethyl-5-oxo-6-hydroxy pyridino[2,3-d]pyrimidine [12,12]-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]pyridino[1,2-d]pyrimidine-3-carbonitrile (6), (Scheme 2).

7-Methoxy-5-(4-methoxyphenyl)-2-phenyl-4-oxo-3-hydroxy pyridino[2,3-d]pyrimidine-6-carbonitrile (3b) and 7-methoxy-5-(4-methoxyphenyl)-4-oxo-3-hydropyridino[2,3-d] pyrimidine-6-carbonitrile (3c), were refluxed with POCl3 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-hydradino-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyridino[2,3-d]pyrimidine-6-carbonitrile (8a) with benzaldehyde, gave 4-(((1E)-1-aza-2-phenylvinyl)amino)-7-methoxy-5-(4-methoxyphenyl)-2-phenylpyridino[2,3-d]pyrimidine-6-carbonitrile (9), which on oxidative cyclization using FeCl3, yielded the fused pyridino[2,3-d]-1,2,4-triazolo[4,5-e]pyrimidine, 10. β-Dicarbonyl reagents such as acetyl acetonitrile, reacted with 8a to give 4-(3,5-dimethyl pyrazolyl)-7-methoxy-5-(4-methoxyphenyl)-2-phenyl pyridino[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-pyrrazoline-4-carboxylate [12] [15]. Reaction of 8a with nitric acid and the reaction of 7a with sodium azide in DMF yielded 3-methoxy-1-[4-methoxyphenyl]-6-phenyl-7-hydroxy-pyridin-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 hydrazine 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-phenyl-7-hydro-10H-pyridino[2,3-d]-1,2,4-triazin[4,3-e]pyrimidine-2-carbonitrile [16] [18]. Also, 8b, was condensed with 4-aminopyrimidine in absolute ethanold in presence of few drops of acetic acid to give 4-[[4-amino-2,3-dimethyl-1-phenyl][3-pyrazolin-5-ylidine] azemethyl]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-pyrazolin-4,5-e]-1,2,4-triazin-6-yl])pyridin[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 were dissolved in DMF, which was used as a control to get 1 mg/mL solution. The inhibition zones of microbial growth 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.](image-url)

References