DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRIMIDINE-BASED DERIVATIVES AS ANTITUMOR AGENTS

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In this paper we made a contentious effort to afford heterocyclic compounds with interesting biological activities. The reaction of guanidine with either activated methylene groups, arylhydrazono derivatives, dicyanopropene derivatives, malononitrile dimer or arylhydrazononitrile derivatives afforded diaminopyrimidine derivatives, aryldiazenyl pyrimidine derivatives, fused pyridopyrimidine derivatives and pyrimidopyridazine derivatives respectively. Also the reaction of guanidine with phenylhydrazono carbonyl compounds produced phenyldiazenyl pyrimidine derivatives. The latter products were directed toward the reaction with either aetic anhydride or ethylcyanoacetate to form acetamidopyrimidine derivatives and cyanoacetamidopyrimidine derivatives respectively. The latter products underwent cyclization via reaction with either activated methylene groups or activated methylene carbonyl compounds afforded pyridopyrimidine derivatives.

The structures of the newly synthesized compounds were established using IR, 1H NMR, 13C NMR and mass spectrometry and their antitumor activity was investigated. Some of these compounds showed promising inhibitory effects on the three different cell lines.

INTRODUCTION

Heterocyclic organic compounds containing pyrimidine nucleus has a widespread importance as one of the most moiety contained in biologically active compounds such as vitamin B1, and many numerous antineoplastic drugs.1–8 Furthermore, 2,4-diaminopyrimidine derivatives have interested biological properties, like caspase-1 inhibitors effect9 and Aurora a kinase inhibitors activity.10 Also pyridopyrimidine derivatives have a wide biological applications, they are act as topoisomerase I inhibitors,11 antitubercular active agents12 and adenosine kinase inhibitors.13 Additionally, it was found that pyrimidopyridazine has antimicrobial and antitumor activities.14,15

In this article, we are report here the synthesis of some novel pyrimidine derivatives 3a,b, 5a,b,c,d, 15a,b, 16a,b, 17a,b. Also, pyridopyrimidine derivatives 7a,b, 10, 12a,b, 18a,b,c,d, 20a,b,c,d. In addition to, pyrimidopyridazine 13a,b, and the evaluation of their antitumor activities was introduced in Table 1.

MATERIAL AND METHODS

General procedures

The melting points of the synthesized compounds were determined in open capillaries and are uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. 1H NMR and 13C NMR spectra were measured on a
Varian EM 390-200 MHz instrument with CD3SOCD3 as the solvent using TMS as an internal standard material, the chemical shifts were expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

**General procedures for the synthesis of compound:** pyrimidine-2,4,6-triamine (3a) and 2,6-diamino-pyrimidin-4-ol (3b)

Either malononitrile (2a) (0.66 g, 0.01 mol) or ethylcyanocacetate (2b) (1.131 g, 0.01 mol) was added to a solution of guanidine (1) (0.591 g, 0.01 mol) in ethanol (50 mL) containing a catalytic amount of triethylamine (0.5 mL). The reaction mixture, in each case, was heated under reflux for 4h, then poured onto ice-water mixture containing few drops of hydrochloric acid. The solid product, formed was collected by filtration.

**Compound 3a**: Brown crystals from ethanol, yield 74%, 0.926 g, m.p. 248-250 ºC. IR (KBr): υ/cm-1 = 3412-3288 (3NH2), 1660 (C=N), 1649 (C=C). 1H NMR (DMSO-d6) δ = 5.76, 5.87, 6.12 (3s, 6H, D2O-exchangeable, 3NH2), 6.43 (s, 1H, pyrimidine ring). MS (relative intensity) m/z: 125 (M+, 28.2%). Calcd for C10H11N7 (229.24): C, 52.39; H, 4.84; N, 37.18%. Found: C, 52.44; H, 4.22; N, 44.23%.

**Compound 3b**: Faint yellow powder from ethanol, yield 66%, 0.832 g, m.p. 248-250 ºC. IR (KBr): υ/cm-1 = 3412-3288 (3NH2, OH), 1660 (C=N, C=C). 1H NMR (DMSO-d6) δ = 5.52, 5.87, 6.12 (3s, 6H, D2O-exchangeable, 3NH2), 6.43 (s, 1H, pyrimidine ring). MS (relative intensity) m/z: 125 (M+, 28.2%). Calcd for C9H9N5 (159.14): C, 45.31; H, 3.82; N, 44.85%. Found: C, 45.15; H, 3.56; N, 44.23%.

**General procedure for the synthesis of compound:** 5-(phenyldiazenyl)pyrimidine-2,4,6-triamine (5a), 5-(chlorohexa-1,3,5-triynyl)diazenylyl)pyrimidine-2,4,6-triamine (5b), 2,6-diamino-5-(phenyldiazenyl) pyrimidin-4-ol (5c) and 2,6-diamino-5-(chloro-hexa-1,3,5-triynyl)diazenyl)pyrimidin-4-ol (5d)

Either arylhydrazono derivatives (4a), (0.85 g, 0.005 mol), (4b) (1.023 g, 0.005 mol), (4c) (1.085 g, 0.005 mol) or (4d) (1.258 g, 0.005 mol) was added to a solution of compound (1) (0.33 g, 0.005 mol) in ethanol (50 mL) containing a catalytic amount of triethylamine (0.5 ml). The reaction mixture in each case, was heated under reflux for 4h, then poured onto ice-water mixture containing few drops of hydrochloric acid. The solid product, in each case, was formed, collected by filtration.

**Compound 5a**: Pale brown crystals from ethanol, yield 53%, 0.607 g, m.p. 171-173ºC. IR (KBr): υ/cm-1 = 3441-3286 (3NH2), 3048 (CH aromatic), 1656 (C=N). 1H NMR (DMSO-d6) δ = 4.22, 4.63, 4.91 (3s, 6H, D2O-exchangeable, 3NH2), 6.87-7.23 (m, 5H, C6H5). 13C NMR: δ = 122.3, 125.4, 126.8, 129.2, 131.1, 134.1, 135.3, 137.2, 138.8, 140.5 (pyrimidine C, C6H5 C). Calcd for C10H10N7 (264.67): C, 45.55; H, 3.82; N, 44.18%. Found: C, 45.31; H, 3.56; N, 44.23%.

**Compound 5b**: Off white crystals from ethanol, yield 48%, 0.633 g, m.p. 152-154ºC. IR (KBr): υ/cm-1 = 3467-3321 (3NH2), 3054 (CH aromatic), 1652 (C=N). 1H NMR (DMSO-d6) δ = 4.43, 4.68, 5.23 (3s, 6H, D2O-exchangeable, 3NH2), 7.25-7.38 (d.d, 4H, C6H4). 13C NMR: δ = 120.2, 123.8, 126.2, 129.4, 132.5, 134.7, 136.2, 139.7, 140.8, 141.9, 143.1 (pyrimidine C, C6H5 C). Calcd for C10H10ClN7 (263.69): C, 45.55; H, 3.82; N, 37.18%. Found: C, 45.31; H, 3.56; N, 37.44%.

**Compound 5c**: White crystals from ethanol, yield 60%, 0.691 g, m.p. 209-211 ºC. IR (KBr): υ/cm-1 = 3431-3265 (2NH2, OH), 3051 (CH aromatic), 1650 (C=N). 1H NMR (DMSO-d6) δ = 4.27, 5.18 (2s, 4H, D2O-exchangeable, 2NH2), 7.16-7.32 (m, 5H, C6H5). 13C NMR: δ = 121.1, 123.8, 126.6, 130.1, 134.2, 137.7, 140.6 (pyrimidine C, C6H5 C). Calcd for C10H10N4O (230.09): C, 52.17; H, 4.38; N, 36.50%. Found: C, 52.44; H, 4.22; N, 36.24%.

**Compound 5d**: Off White crystals from ethanol, yield 55%, 0.726 g, m.p. 189-190 ºC. IR (KBr): υ/cm-1 = 3389-3177 (2NH2, OH), 3053 (CH aromatic), 1651 (C=N). 1H NMR (DMSO-d6) δ = 4.11, 4.43 (2s, 4H, D2O-exchangeable, 2NH2), 7.42-7.65 (d.d, 4H, C6H4). 13C NMR: δ = 122.3, 124.2, 125.6, 126.6, 130.1, 134.2, 137.7, 140.6 (pyrimidine C, C6H4 C). Calcd for C10H10ClN7 (264.67): C, 45.38; H, 3.43; N, 31.75%. Found: C, 45.15; H, 3.18; N, 31.49%.

**General procedure for the synthesis of compounds:** pyrido[2,3-d]pyrimidine-2,4,7-triamine (7a) and 2,4-diaminopyrido[2,3-d]pyrimidin-7-ol (7b)

To a solution of compound (1) (0.33 g, 0.005 mol) in ethanol (50 mL) containing (0.5 mL) of trimethylamine. Either compound (6a), (0.585 g, 0.005 mol), or (6b) (0.82 g, 0.005 mol) was added. The reaction mixture, in each case, was heated
under reflux for 5 h, then poured onto ice-water mixture containing few drops of hydrochloric acid. The solid product formed was collected by filtration.

Compound 7a: Creamy white crystals from 1,4-dioxane, yield 60%, 0.528 g, m.p. 232-234 °C. IR (KBr): ν/cm−1 = 3324-3198 (3NH2), 1657 (C=N), 1646 (C=C). 1H NMR (DMSO-d6) δ = 4.44, 4.78, 5.86 (3s, 6H, D2O-exchangeable, 3NH2), 6.55-6.73 (m, 2H, pyridine ring). MS (relative intensity) m/z: 176 (M+, 12.8%). Found: C, 47.43; H, 4.74; N, 47.49%.

Compound 7b: White crystals from 1,4-dioxane, yield 55%, 0.487 g, m.p. 218-220 °C. IR (KBr): ν/cm−1 = 3314-3129 (2NH2, OH), 1653 (C=N), 1645 (C=C). 1H NMR (DMSO-d6) δ = 4.33, 4.67 (2s, 4H, D2O-exchangeable, 2NH2), 6.85-6.96 (m, 2H, pyridine ring), 9.76 (s, 1H, D2O-exchangeable, OH). MS (relative intensity) m/z: 178 [M+1]+, 19.1%. Found: C, 43.97; H, 4.74; N, 51.28%. Calcd for C13H10N8 (278.27): C, 56.11; H, 3.62; N, 40.27%.

General procedure for the synthesis of compound: pyrido[2,3-d]pyrimidin-2,4,5,7-tetraamine (10)

The solution of guanidine 1 (0.33 g, 0.005 mol) in ethanol (50 mL) containing (0.5 mL) of piperidine. Compound (9) (0.66 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 8 h, then poured onto ice-water mixture containing few drops of hydrochloric acid. The crude solid product was collected by filtration.

Compound 10: Pale brown crystals from 1,4-dioxane, yield 47%, 0.449 g, m.p. 183-185 °C. IR (KBr): ν/cm−1 = 3277-3134 (4NH2), 1658 (C=C), 1644 (C=C). 1H NMR (DMSO-d6) δ = 4.18, 4.66, 4.92, 5.37 (4s, 8H, D2O-exchangeable, 4NH2), 6.73 (s, 1H, fused pyridine ring). MS (relative intensity) m/z: 191 (M+, 22.4%). Found: C: 49.23; H: 3.74; N, 51.28%. Calcd: C: 49.63; H: 4.93; N, 51.63%.

General procedure for the synthesis of compounds: 6-(phenyl diazenyl)pyrido[2,3-d]pyrimidine-2,4,5,7-tetraamine (12a), 6-((chloro hexa-1,3,5-triynyl) diazenyl pyrido[2,3-d]pyrimidine-2,4,5,7-tetraamine (12b), 4,5-diamino-7-imino-1-phenyl-1,7-di-hydro pyrimidino[4,5-c]pyrazidine-3-carbonitrile (13a) and 4,5-diamino-1-(chloro hexa-1,3,5-triynyl)7-imino-1,7-dihydro pyridazino[3,4-d]pyrimidine-3-carbonitrile (13b)

The reactions began, either compound 11a (1.181 g, 0.005 mol), 11b (1.353 g, 0.005 mol), 11c (1.181 g, 0.005 mol) or 11d (1.353 g, 0.005 mol) was added to a solution of guanidine 1 (0.33 g, 0.005 mol) in 50 mL of ethanol containing dimethylformamide (5.0 mL) and triethylamine (1.0 mL) as a catalyst. The reaction mixture was heated under reflux for 6 h for compounds 12a,b, and 9 h for compounds 13a,b till ammonia odor disappeared. The formation of the solid products were cooled and poured onto ice containing a few drops of HCl and filtered out.

Compound 12a: Off-white crystals from ethanol, yield 47%, 0.693 g, m.p. 114-116 °C. IR (KBr): ν/cm−1 = 3322-3184 (4NH2), 3051 (CH aromatic), 1653 (C=N), 1646 (C=C). 1H NMR (DMSO-d6) δ = 4.25, 4.78, 5.12, 5.66 (4s, 8H, D2O-exchangeable, 4NH2), 7.14-7.33 (m, 5H, C6H5). 13C NMR: δ = 117.3, 120.8, 122.7, 125.1, 127.2, 128.3, 130.5, 131.8, 133.6, 135.7, 136.9, 138.5, 140.4 (pyrimidine C, pyridine C, C6H5 C). MS (relative intensity) m/z: 295 (M+, 17.5%). Found: C, 52.87; H, 4.44; N, 42.69%. Calcd: C, 52.63; H, 4.21; N, 42.96%.

Compound 12b: Pale brown crystals from ethanol, yield 55%, 0.907 g, m.p. 138-140 °C. IR (KBr): ν/cm−1 = 3282-3155 (4NH2), 3053 (CH aromatic), 1658 (C=N), 1649 (C=C). 1H NMR (DMSO-d6) δ = 4.17, 4.54, 4.83, 5.25 (4s, 8H, D2O-exchangeable, 4NH2), 7.39-7.58 (d.d, 4H, C6H4). 13C NMR: δ = 119.8, 121.6, 122.9, 124.7, 126.2, 127.2, 130.8, 132.5, 135.3, 137.3, 139.2, 140.1, 142.3 (pyrimidine C, pyridine C, C6H4 C). MS (relative intensity) m/z: 329 (M+, 13.4%). Calcd for C13H12ClN9 (329.75): C, 47.35; H, 3.67; N, 38.23%. Found: C, 47.61; H, 3.90; N, 38.02%.

Compound 13a: Yellow crystals from ethanol, yield 51%, 0.709 g, m.p. 250-252 °C. IR (KBr): ν/cm−1 = 3377-3134 (2NH2, NH), 3047 (CH aromatic), 2221 (CN), 1656 (C=N), 1644 (C=C). 1H NMR (DMSO-d6) δ = 4.34, 4.81 (2s, 4H, D2O-exchangeable, 2NH2), 7.22-7.47 (m, 5H, C6H5). 13C NMR: δ = 115.7 (CN), 116.8, 118.9, 121.2, 123.6, 125.7, 127.6, 130.3, 132.5, 134.7, 136.5, 139.4 (pyrimidine C, pyrazidine C, C6H5 C). MS (relative intensity) m/z: 278 (M+, 13.7%). Calcd for C13H11ClN9 (278.73): C, 52.61; H, 3.62; N, 40.27%. Found: C, 52.67; H, 3.33; N, 40.55%.

Compound 13b: Yellow crystals from ethanol, yield 43%, 0.672 g, m.p. 200-202 °C. IR (KBr): ν/cm−1 = 3334-3121 (2NH2, NH), 3055 (CH aromatic), 2223 (CN), 1654 (C=N), 1641 (C=C). 1H NMR (DMSO-d6) δ = 4.56, 5.34 (2s, 4H, D2O-exchangeable, 2NH2), 7.41-7.58 (d.d, 4H, C6H5), 10.52 (s, 1H, D2O-exchangeable, NH). 13C NMR:
δ = 117.2 (CN), 118.8, 119.7, 121.4, 122.8, 124.6, 128.3, 130.1, 132.7, 135.8, 137.9, 139.7 (pyrimidine C, pyridazine C, C6H4 C). MS (relative intensity) m/z: 312 (M+, 10.2%). Caled for C13H10N6O (312.72): C, 52.93; H, 3.23; N, 31.81%. Found: C, 52.46; H, 3.08; N, 31.76%.

General procedure for the synthesis of compounds: 5-(phenyldiazenyl)pyrimidin-2-amine (15a) and 4,6-dimethyl-5-(phenyldiazenyl)pyrimidin-2-amine (15b)

A mixture of guanidine (0.33 g, 0.005 mol) in 50 mL of ethanol was treated with an equimolar amount of either compounds 14a (0.088 g, 0.005 mol) or 14b (1.021 g, 0.005 mol). The clear solution was heated under reflux for 6 h, concentrated, poured onto ice water and neutralized with dil. HCl. The solid obtained was filtered off, washed with cold water, ethanol and dried.

Compound 15a: Yellow crystals from ethanol, yield 54%, 0.537 g, m.p. 155-157 ºC. IR (KBr): υ/cm-1= 3378-3226 (NH), 3049 (CH aromatic), 2965 (CH2), 2223 (CN), 1669 (C=O), 1654 (C=N). 1H NMR (DMSO) δ = 1.77 (s, 3H, CH3), 6.44-6.53 (s, 2H, pyridine ring). 7.22-7.38 (m, 5H, C6H5). MS (relative intensity) m/z: 228 (M+, 20.0%). Calcld for C10H9N5 (269.30): C, 58.64; H, 4.95; N, 29.18%. Found: C, 58.82; H, 4.50; N, 30.05%.

Compound 15b: Yellowish brown crystals from ethanol, yield 47%, 0.534 g, m.p. 155-157 ºC. IR (KBr): υ/cm-1= 3355-3248 (NH2), 3055 (CH aromatic), 2984 (CH3), 2221 (CN), 1681 (C=O), 1655 (C=N). 1H NMR (DMSO) δ = 1.69, 1.97 (2s, 6H, CH3), 4.53 (s, 2H, D2O-exchangeable, NH2), 7.16-7.38 (m, 5H, C6H5). MS (relative intensity) m/z: 269 [M+1]1, 21.4%. Caled for C14H15N5O (266.26): C, 56.97; H, 4.60; N, 29.03%. Found: C, 56.89; H, 4.60; N, 30.05%.

General procedure for the synthesis of compounds: N-(5-(phenyldiazenyl)pyrimidin-2-yl)acetamide (16a) and N-(4,6-dimethyl-5-(phenyldiazenyl)pyrimidin-2-yl)acacetamide (16b)

To a solution of either 5-(phenyldiazenyl)pyrimidin-2-amine (15a) (0.398 g, 0.002 mol) or 4,6-dimethyl-5-(phenyldiazenyl)pyrimidin-2-amine (15b) (0.454 g, 0.002 mol) in 50 mL of ethanol was treated with an equimolar amount of formamide (10 mL) or ethyl cyanoacetate (0.226 g, 0.002 mol). The reaction mixture was then refluxed for 4 h, cooled, then poured onto ice-water and the formed precipitate product in each case was filtered out.

Compound 16a: Off-white crystals from ethanol, yield 60%, 0.289 g, m.p. 179-181 ºC. IR (KBr): υ/cm-1= 3264 (NH), 3052 (CH aromatic), 2990 (CH3), 1681 (C=O), 1655 (C=N). 1H NMR (DMSO) δ = 1.77 (s, 3H, CH3), 6.44-6.53 (s, 2H, pyridine ring). 7.22-7.43 (m, 5H, C6H5). 10.12 (1s, 1H, D2O-exchangeable, NH). MS (relative intensity) m/z: 241 (M+, 23.4%). Caled for C13H11N2O (241.25): C, 63.42; H, 6.57; N, 29.31%. Found: C, 63.22; H, 6.58; N, 30.11%.

Compound 16b: Yellowish brown crystals from ethanol, yield 55%, 0.296 g, m.p. 227-229 ºC. IR (KBr): υ/cm-1= 3232 (NH), 3046 (CH aromatic), 2984 (CH3), 1676 (C=O), 1652 (C=N), 1643 (C=C). 1H NMR (DMSO) δ = 1.63, 1.77, 1.85 (3s, 9H, 3CH3), 7.33-7.54 (m, 5H, C6H5). 10.43 (s, 1H, D2O-exchangeable, NH). MS (relative intensity) m/z: 269 (M+, 21.1%). Caled for C14H12N2O (266.26): C, 62.44; H, 5.61; N, 26.01%. Found: C, 62.17; H, 5.35; N, 25.73%.
General procedure for the synthesis of compounds: 4,6-diamino-2-oxo-1-(5-(phenyl diazzenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (18a), 4,6-diamino-1-(4,6-dimethyl-5-(phenyl diazzenyl)pyrimidin-2-yl)-2,1,2-dihydropyridine-3-carbonitrile (18b), 4-amino-6-hydroxy-2-oxo-1-(5-(phenyl diazzenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (18c) and 4-amino-1-(4,6-dimethyl-5-(phenyl diazzenyl)pyrimidin-2-yl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (18d)

To a solution of either compound 17a (0.266 g, 0.001 mol) or 17b (0.294 g, 0.001 mol) in 1,4-dioxane (50 mL) containing trimethylamine (0.5 mL) either malononitrile (2a) (0.066 g, 0.001 mol) or ethyl cyanoacetate (2b) (0.113 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 6 h, then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound 18a: Brown crystals from ethanol, yield 61%, 0.202 g, m.p. 207-209 °C. IR (KBr): ν/cm⁻¹= 3376-3233 (2NH₂), 3056 (CH aromatic), υ/cm-1= 3344-3276 (2NH₂), 3049 (CH aromatic), yield 61%, 0.202 g, m.p. 207-209 ºC. IR (KBr): υ/cm-1= 3344-3276 (2NH₂), 3049 (CH aromatic), 2227 (CN), 1672 (C=O), 1642 (C=O). MS (relative intensity) m/z: 360 (M⁺, 22.6%). Calcd for C₁₈H₁₅N₇O₂ (361.36): C, 59.83; H, 4.18; N, 27.13%. Found: C, 59.66; H, 4.36; N, 27.41%.

Compound 18b: Brown crystals from ethanol, yield 63%, 0.227 g, m.p. 197-199 °C. IR (KBr): ν/cm⁻¹= 3433-3322 (OH, NH₂), 3056 (CH aromatic), 2990, 2982 (2CH₃), 2227 (CN), 1672 (C=O), 1655 (C=N), 1643 (C=C). ¹HNMR (DMSO) δ = 4.22-4.56 (2s, 4H, D₂O-exchangeable, OH) . ¹³C NMR: δ = 18.3, 21.6 (2CH₃), 115.6 (C=O), 117.3, 119.2, 120.6, 122.9, 123.7, 124.5, 127.1, 129.6, 131.4, 133.2, 134.8, 136.7, 137.3, 138.6 (pyrimidine C, pyridine C, C₆H₅ C), 163.4 (C=O). MS (relative intensity) m/z: 361 (M⁺, 22.6%). Calcd for C₁₈H₁₅N₇O₂ (361.36): C, 59.83; H, 4.18; N, 27.13%. Found: C, 59.66; H, 4.36; N, 27.41%.

General procedure for the synthesis of compounds: 4,6-dimethyl-2-oxo-1-(5-(phenyl diazzenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (20a), 1-(4,6-dimethyl-5-(phenyl diazzenyl)pyrimidin-2-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (20b), 6-hydroxy-4-methyl-2-oxo-1-(5-(phenyl diazzenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (20c) and 1-(4,6-dimethyl-5-(phenyl diazzenyl)pyrimidin-2-yl)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (20d)

To a solution of either compound 17a (0.266 g, 0.001 mol) or 17b (0.294 g, 0.001 mol) in 1,4-dioxane (50 mL) containing trimethylamine (0.5 mL), either acetylacetone (19a) (0.1 g, 1 mmol) or ethyl cyanoacetate (19b) (0.130 g, 1 mmol) was added. The reaction mixture in each case was heated under reflux for 5 h, then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound 20a: Creamy white crystals from ethanol, yield 65%, 0.215 g, m.p. 177-179 °C. IR (KBr): ν/cm⁻¹= 3056 (CH aromatic), 2988 (CH₃), 2229 (CN), 1671 (C=O), 1655 (C=N), 1649 (C=C).
\[ ^1 \text{H} \text{NMR (DMSO)} \delta = 1.65-1.77 \text{ (2s, 6H, 2CH$_3$), 6.33-6.53 \text{ (3s, 3H, pyrimidine ring, pyridine ring).} \]
\[ 7.11-7.28 \text{ (m, 5H, C$_6$H$_5$). 13C NMR: } \delta = 18.9, 24.0 \text{ (2CH$_3$), 119.1} \text{ (CN), 121.2, 122.3, 125.4, 128.2, 130.2, 131.9, 134.7, 142.2, 146.5, 150.5, 151.6, 157.4, 158.5 \text{ (pyrimidine C, pyridine C, C$_6$H$_5$ C), 161.3} \text{ (C=O). MS (relative intensity) m/z: 330 (M+, 31.5%). Caled for C$_{18}$H$_{14}$N$_6$O (330.34): C, 65.44; H, 4.27; N, 25.44%. Found: C, 65.18; H, 4.39; N, 25.72%.} \]

**Compound 20b**: Off white crystals from ethanol, yield 63%, 0.226 g, m.p. 102-104 °C. IR (KBr): \( \nu / \text{cm}^{-1} = 3387-3224 \text{ (OH), 2986 (CH$_3$), 3051} \text{ (CH aromatic), 2223 (CN), 1661} \text{ (C=O), 1643 (C= C). 1H NMR (DMSO) } \delta = 1.65-2.17 \text{ (4s, 12H, 4CH$_3$), 6.48} \text{ (s, 1H, pyridine ring), 7.18-7.43} \text{ (m, 5H, C$_6$H$_5$). 13C NMR: } \delta = 18.2, 19.6, 20.3, 21.5 \text{ (4CH$_3$), 117.2} \text{ (CN), 118.9, 119.6, 120.8, 121.7, 122.4, 124.3, 126.8, 128.2, 130.5, 131.7, 133.6, 135.7, 137.4 \text{ (pyrimidine C, pyridine C, C$_6$H$_5$ C), 160.9} \text{ (C=O). MS (relative intensity) m/z: 358 (M+, 19.2%). Caled for C$_{19}$H$_{16}$N$_6$O$_2$ (360.37): C, 61.44; H, 3.64; N, 25.29%. Found: C, 61.19; H, 3.99; N, 25.72%.} \]

**Compound 20c**: Faint brown crystals from ethanol, yield 55%, 0.182 g, m.p. 102-104 °C. IR (KBr): \( \nu / \text{cm}^{-1} = 3387-3224 \text{ (OH), 2985 (CH$_3$), 3052} \text{ (CH aromatic), 2223 (CN), 1661} \text{ (C=O), 1647} \text{ (C=N), 1642 (C=C). 1H NMR (DMSO) } \delta = 1.86 \text{ (s, 3H, CH$_3$), 6.44-6.65} \text{ (3s, 3H, pyrimidine ring, pyridine ring), 7.13-7.48} \text{ (m, 5H, C$_6$H$_5$), 9.22} \text{ (s, 1H, D$_2$O-exchangeable, OH). 13C NMR: } \delta = 19.5 \text{ (CH$_3$), 115.8} \text{ (CN), 117.6, 118.3, 119.6, 121.1, 123.1, 125.3, 126.8, 130.3, 131.8, 133.2, 134.1, 136.3, 138.2, 140.5 \text{ (pyrimidine C, pyridine C, C$_6$H$_5$ C), 161.4} \text{ (C=O). MS (relative intensity) m/z: 358 (M+, 27.6%). Caled for C$_{20}$H$_{18}$N$_6$O (358.40): C, 67.02; H, 5.06; N, 23.45%. Found: C, 67.22; H, 5.39; N, 23.70%.} \]

**Compound 20d**: Brown crystals from ethanol, yield 66%, 0.182 g, m.p. 102-104 °C. IR (KBr): \( \nu / \text{cm}^{-1} = 3387-3224 \text{ (OH), 2985 (CH$_3$), 3052} \text{ (CH aromatic), 2223 (CN), 1661} \text{ (C=O), 1647} \text{ (C=N), 1642 (C=C). 1H NMR (DMSO) } \delta = 1.65-1.77 \text{ (2s, 6H, 2CH$_3$), 6.33-6.53} \text{ (3s, 3H, pyrimidine ring, pyridine ring).} \]

### Antitumor activity tests

The reagents and chemicals that are used for antitumor activity tests will be described as follow, dimethyl sulfoxide (DMSO), penicillin, streptomycin, doxorubicin and sulforhodamine B (SRB) were provided from Sigma Chemical Co. (USA). L-Glutamine and fetal bovine serum (FBS) were purchased from Gibco Invitrogen Co. (UK). RPMI-1640 medium was from Cambrex (USA).

They grew as monolayers and were routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mmol L$^{-1}$ glutamine and antibiotics (penicillin 100 Um L$^{-1}$ and streptomycin 100 µg L$^{-1}$), at 37 °C in a humidified atmosphere containing 5% CO$_2$. Exponentially growing cells were obtained by plating 1.5 $\times$ 10$^5$ cell mL$^{-1}$ for MCF-7 and SF-268 and 0.75 X 10$^4$ cell mL$^{-1}$ for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

### Tumor cell growth assay

The effects on the in vitro growth of human tumor cell lines were evaluated on the synthesized compounds 3a, b, 20a, b, c, d according to the procedure described by the National Cancer Institute (NCI, USA), that uses the protein-binding dye sulforhodamine B to assess cell growth. Exponentially growing cells in 96-well plates were then exposed for 48 h to five serial concentrations of each compound starting from a maximum concentration of 150 µmolL$^{-1}$. Following this exposure period, adherent cells were fixed, washed and stained. The bound stain was dissolved in dimethylsulfoxide and then the absorbance was measured at 492 nm in a plate reader (Power wave XS, Bio-Tek Instruments, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI$_{50}$) (corresponding to the concentration of the compound that inhibited 50% of the net cell...
growth) was calculated as described elsewhere. Doxorubicin was used as a positive control test and it was examined in the same condition.

RESULTS AND DISCUSSION

Chemistry

All the synthesized compounds are new except compounds 3a, b, 15a, b. The syntheses of the newly heterocyclic compounds, pyridine and pyridazine derivatives in this study are depicted in Schemes 1-3. The establishment of these structures was based on analytical and spectral data.

Guanidine-based functional groups occur in many different chemical reactions aimed to synthesis of heterocyclic compounds, thus the reaction of guanidine (1) with either malononitrile (2a) or ethylcyanoacetate (2b) produced pyrimidine derivatives 3a, b, the structure of compounds 3a, b were verified by analytical and spectral data. Also, guanidine (1) was reacted with arylhydrazone derivatives 4a, b, c, d to form aryl diazenylpyrimidine derivatives 5a, b, c, d. The of compounds 5a, b, c, d were established applying analytical and spectral data, in compound 5a 1HNMR spectrum indicated the presence of three singlets, D2O-exchangeable at δ = 4.22, 4.63, 4.91 ppm which indicate the presence of 3NH2 groups, multiplet at δ = 6.87-7.23 ppm corresponding to 5H of phenyl group. Furthermore, the 13C NMR spectrum revealed eight signals at δ = 117.3, 120.8, 122.7, 125.1, 127.2, 128.3, 130.5, 131.8, 133.6, 135.7, 136.9, 138.5, 140.4 ppm for the pyrimidine ring, pyridine ring and benzene ring. In addition to, the reaction of guanidine (1) with either compounds 6a or 6b afforded pyridopyrimidine derivatives 7a, b, where compounds 8a, b were rolled out as IR spectra indicates the absence of stretching vibration bands of CN groups in compounds 7a, b (Scheme 1).

Furthermore, pyridopyrimidine derivative 10 was produced through the reaction of guanidine (1) with malononitrile dimer (9). The structure of compound 10 was proved using analytical and spectral data, 1HNMR spectrum showed the presence of four singlets, D2O-exchangeable at δ = 4.18, 4.66, 4.92, 5.37 ppm which indicate the presence of 4NH2 groups, singlet at δ = 6.73 ppm corresponding to 1H of fused pyridine ring. In addition to, the reaction of guanidine (1) with either arylhydrazonoderivatives 11a, b or pyridazine derivatives 11c, d were afforded either pyridopyrimidine derivatives 12a, b or pyrimidopyridazine derivatives 13a, b respectively. The structure of compounds 12a, b and 13a, b was confirmed by analytical and spectral data, thus, 1HNMR spectrum of compound 12a showed the presence of four singlets, D2O-exchangeable at δ = 4.25, 4.78, 5.12, 5.66 ppm which indicate the presence of 4NH2 groups, multiplet at δ = 7.14-7.33 ppm corresponding to 5H of benzene ring. The 13C NMR spectrum indicated eleven signals at δ = 117.3, 120.8, 122.7, 125.1, 127.2, 128.3, 130.5, 131.8, 133.6, 135.7, 136.9, 138.5, 140.4 ppm for the pyrimidine ring, pyridine ring and benzene ring. Moreover, 1HNMR spectrum of compound 13a showed the presence of two singlets, D2O-exchangeable at δ = 4.34, 4.81 ppm which indicate the presence of 2NH2 groups, multiplet at δ = 7.22-7.47 ppm corresponding to 5H of benzene ring, singlet, D2O-exchangeable at δ = 10.23 ppm which indicate the presence of NH group. Finally, the reaction of guanidine (1) with phenylhydrazonodicarbonyl derivatives 14a, b yielded pyrimidine derivatives 15a, b, these compounds 15a, b were subjected to acylation afforded compounds 16a, b, the elucidation of these structures was based on analytical and spectral data (Scheme 2). Either compounds 15a or 15b directed toward the reaction with ethylcyanoacetate (2b) to give compounds 17a, b, the structure of these compounds was established by analytical and spectral data. Compounds 17a, b underwent cyclization via the reaction with either malononitrile (2a) or ethylcyanoacetate (2b) affording compounds 18a, b, c, d. The elucidation of the structure of compounds 18a, b, c, d were confirmed, 1HNMR spectrum of compound 18a presented the presence of two singlets, D2O-exchangeable at δ = 4.22-4.56 ppm which indicate the presence of 2NH2 groups, three singlets at δ = 6.11-6.75 ppm which indicate the presence of 3H groups of pyrimidine ring and pyridine ring, multiplet at δ = 7.22-7.51 ppm corresponding to 5H of benzene ring. Moreover, the mass spectrum revealed m/z at 333 [M+1]+, m/z at 332 [M]+ and m/z at 77 [C6H5]+ for the phenyl moiety. Also, compounds 17a, b were pointed to react with either acetylacetone (19a) or ethylacetoacetate (19b) formed pyridopyrimidine derivatives 20a, b, c, d respectively, the structure of compounds 20a, b, c, d were confirmed by analytical and spectral data. 1HNMR spectrum of compound 20a presented the presence of two singlets at δ = 1.65-1.77 ppm which indicate the presence of 2CH3 groups, three singlets at δ = 6.33-6.53 ppm which indicate the presence of 3H groups of pyrimidine ring and pyridine ring, multiplet at δ = 7.11-7.28 ppm corresponding to 5H of benzene ring. The 13C NMR spectrum indicated two signals at δ = 18.9, 24.0 ppm due to the presence of two methyl groups, one signal at δ = 119.1 indicates the presence of nitrile group, thirteen
signals at δ = 121.2, 122.3, 125.4, 128.2, 130.2, 131.9, 134.7, 142.2, 146.5, 150.5, 151.6, 157.4, 158.5 ppm for the pyrimidine ring, pyridine ring and benzene ring and one signal at δ = 161.3 due to the presence of carbonyl group (Scheme 3).

Effect on the growth of human tumor cell lines

The inhibitory effect of compounds 3a,b-20a,b,c,d was evaluated on the in vitro growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) after a continuous exposure for 48h. All of the tested compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner (data not shown). The Table 1 results indicated that hydroxy-pyridopyrimidine derivative 18c showed the highest inhibitory effect against all the three different tumor cell lines with respect to reference standard material (Doxorubicin). On the other hand, cyano-acetamidopyrimidine derivative 17a showed the highest inhibitory effect against all the three tumor cell lines corresponding to the remaining synthesized compounds. Furthermore, diamino-pyridopyrimidine derivative 18a showed moderate inhibitory effects against the three cancer cell lines. The remaining compounds 3a,b, 5a,b,c,d, 7a,b, 10, 12a,b, 13a,b, 15a,b, 16a,b, 17b, 18b,d, and 20a,b,c,d showed a low growth inhibitory effect.

On comparing cyano-acetamidopyrimidine derivatives 17a,b it was found that compound 17a is more effective as antitumor active compound against three different cell lines, it may be due to the presence of free pyrimidine ring with no alkyl groups. On the other hand, pyridopyrimidine derivatives 18a,b,c,d, it was clear that compound 18c act as the most active one against the three different cell lines, it may be due to the presence of hydroxy group on pyridine ring in addition to the presence of free pyrimidine ring from any alkyl groups.

Also, comparing pyrimidine derivatives 3a,b, 5a,b,c,d, 15a,b, 16a,b it was found that compound 3b act as the more effective one, that is may be due to the presence of hydroxy group with absent of arylhydrazenyl group, the remaining compounds 3a, 5a,b,c,d, 15a,b, 16a,b they are low effect against three different cell lines. Furthermore, comparing pyridopyrimidine derivatives 20a,b,c,d, it was found that these compounds they are nearly has the same effect with low activity against the three different cell lines.

Table 1

Effect of compounds 3a,b-20a,b,c,d on the growth of three tumor cell

<table>
<thead>
<tr>
<th>No.</th>
<th>MCF-7 (µmol L⁻¹)</th>
<th>NCI-H460 (µmol L⁻¹)</th>
<th>SF-268 (µmol L⁻¹)</th>
<th>WI-38 (µmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>28.2 ± 7.9</td>
<td>19.8 ± 3.6</td>
<td>23.5 ± 5.7</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b</td>
<td>22.3 ± 8.3</td>
<td>27.5 ± 6.3</td>
<td>25.5 ± 4.6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5a</td>
<td>34.6 ± 5.2</td>
<td>33.1 ± 5.7</td>
<td>40.3 ± 7.5</td>
<td>65 ± 11.7</td>
</tr>
<tr>
<td>5b</td>
<td>42.2 ± 8.2</td>
<td>44.4 ± 7.3</td>
<td>40.1 ± 7.3</td>
<td>na</td>
</tr>
<tr>
<td>5c</td>
<td>47.2 ± 9.2</td>
<td>52.3 ± 8.4</td>
<td>44.5 ± 10.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5d</td>
<td>40.2 ± 8.1</td>
<td>42.1 ± 9.4</td>
<td>41.5 ± 8.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7a</td>
<td>48.0 ± 10.9</td>
<td>42.2 ± 9.7</td>
<td>44.7 ± 9.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7b</td>
<td>42.8 ± 8.3</td>
<td>43.1 ± 7.5</td>
<td>43.9 ± 8.9</td>
<td>na</td>
</tr>
<tr>
<td>10</td>
<td>50.1 ± 8.6</td>
<td>42.2 ± 6.4</td>
<td>46.6 ± 8.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12a</td>
<td>39.1 ± 8.2</td>
<td>35.2 ± 6.4</td>
<td>37.2 ± 8.3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12b</td>
<td>35.5 ± 9.5</td>
<td>39.2 ± 8.1</td>
<td>30.7 ± 6.5</td>
<td>na</td>
</tr>
<tr>
<td>13a</td>
<td>39.1 ± 10.2</td>
<td>37.1 ± 8.2</td>
<td>32.8 ± 9.1</td>
<td>27.1 ± 11.2</td>
</tr>
<tr>
<td>13b</td>
<td>40.6 ± 8.4</td>
<td>41.9 ± 7.2</td>
<td>33.8 ± 8.5</td>
<td>25.4 ± 10.2</td>
</tr>
<tr>
<td>15a</td>
<td>37.2 ± 8.5</td>
<td>28.3 ± 6.4</td>
<td>26.1 ± 8.9</td>
<td>&gt;100</td>
</tr>
<tr>
<td>15b</td>
<td>29.3 ± 7.8</td>
<td>34.3 ± 8.2</td>
<td>31.9 ± 10.2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>16a</td>
<td>29.2 ± 8.8</td>
<td>30.2 ± 8.1</td>
<td>38.1 ± 8.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>16b</td>
<td>28.5 ± 7.9</td>
<td>41.9 ± 5.3</td>
<td>33.7 ± 6.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>17a</td>
<td>1.4 ± 0.06</td>
<td>2.1 ± 0.9</td>
<td>1.1 ± 0.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>17b</td>
<td>25.7 ± 5.5</td>
<td>30.4 ± 6.9</td>
<td>32.8 ± 8.5</td>
<td>na</td>
</tr>
<tr>
<td>18a</td>
<td>3.9 ± 1.1</td>
<td>2.5 ± 1.2</td>
<td>2.9 ± 0.9</td>
<td>&gt;100</td>
</tr>
<tr>
<td>18b</td>
<td>40.1 ± 10.2</td>
<td>43.5 ± 11.7</td>
<td>36.0 ± 8.5</td>
<td>na</td>
</tr>
<tr>
<td>18c</td>
<td>0.05 ± 0.02</td>
<td>0.08 ± 0.5</td>
<td>0.09 ± 0.03</td>
<td>&gt;100</td>
</tr>
<tr>
<td>18d</td>
<td>44.1 ± 12.2</td>
<td>43.4 ± 10.4</td>
<td>34.8 ± 7.9</td>
<td>na</td>
</tr>
<tr>
<td>20a</td>
<td>30.7 ± 7.5</td>
<td>42.4 ± 8.4</td>
<td>30.6 ± 10.8</td>
<td>na</td>
</tr>
<tr>
<td>20b</td>
<td>44.7 ± 8.5</td>
<td>43.4 ± 6.5</td>
<td>29.8 ± 12.5</td>
<td>na</td>
</tr>
<tr>
<td>20c</td>
<td>34.3 ± 6.9</td>
<td>32.1 ± 5.7</td>
<td>38.6 ± 7.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>20d</td>
<td>39.7 ± 8.2</td>
<td>37.4 ± 9.9</td>
<td>32.6 ± 10.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.03 ±0.008</td>
<td>0.07±0.008</td>
<td>0.09±0.007</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Guanidine (1) + \( \text{H}_2\text{C} \quad \text{CN} \) → \( \text{H}_2\text{N} \quad \text{NH}_2 \)

2a, \( X = \text{CN} \)

2b, \( X = \text{COOEt} \)

\( \text{Y} = \text{NH}_2 \)

\( \text{Y} = \text{OH} \)

EtOH, Et\(_3\)N

\( \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{Y} \)

1 + \( \text{C} \quad \text{X} \quad \text{CN} \)

4a, \( X = \text{CN}, \text{Ar} = \text{C}_6\text{H}_5 \)

4b, \( X = \text{CN}, \text{Ar} = 4\text{-Cl-C}_6\text{H}_4 \)

4c, \( X = \text{COOEt}, \text{Ar} = \text{C}_6\text{H}_5 \)

4d, \( X = \text{COOEt}, \text{Ar} = 4\text{-Cl-C}_6\text{H}_4 \)

EtOH, Et\(_3\)N

\( \text{H}_2\text{N} \quad \text{ArHN} \quad \text{N} \quad \text{N} \quad \text{NH}_2 \)

5a, \( Y = \text{NH}_2, \text{Ar} = \text{C}_6\text{H}_5 \)

5b, \( Y = \text{NH}_2, \text{Ar} = 4\text{-Cl-C}_6\text{H}_4 \)

5c, \( Y = \text{OH}, \text{Ar} = \text{C}_6\text{H}_5 \)

5d, \( Y = \text{OH}, \text{Ar} = 4\text{-Cl-C}_6\text{H}_4 \)

1 + \( \text{C} \quad \text{X} \quad \text{CN} \)

6a, \( X = \text{CN} \)

6b, \( X = \text{COOEt} \)

EtOH, Et\(_3\)N

\( \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{Y} \)

7a, 7b, \( Y = \text{NH}_2 \)

7b, \( Y = \text{OH} \)

8a, 8b, \( Y = \text{NH}_2 \)

8b, \( Y = \text{OH} \)

Scheme 1
Scheme 2
Scheme 3

15a,b + C\(\text{H}_2\text{COOC}_2\text{H}_5\) \(\rightarrow\) DMF reflux

17a,b

18a, R,R\(_1\) = H, X = NH\(_2\)
b, R,R\(_1\) = CH\(_3\), X = NH\(_2\)
c, R,R\(_1\) = H, X = OH
d, R,R\(_1\) = CH\(_3\), X = OH

17a,b + \(\text{C}_2\text{H}_4\text{O}_2\text{C}_2\text{H}_5\) \(\rightarrow\) 1,4 Dioxane, Et\(_3\)N

19a, X = CH\(_3\)
b, X = O\(\text{C}_2\text{H}_5\)

20a, R,R\(_1\) = H, X = CH\(_3\)
b, R,R\(_1\) = CH\(_3\), X = CH\(_3\)
c, R,R\(_1\) = H, X = OH
d, R,R\(_1\) = CH\(_3\), X = OH
Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure for 48 h and show means ± SEM of three-independent experiments performed in duplicate.

CONCLUSIONS

In this article the synthesized compounds pyrimidine, pyridopyrimidine and pyrimidopyridazine derivatives were investigated to detect their antitumor activity against three different cell lines comparing to reference standard “doxorubicin”. Among the synthesized compounds, hydroxy-pyridopyrimidine derivative 18c showed the highest inhibitory effect against all the three different tumor cell lines corresponding to reference standard material (Doxorubicin). Also, cyanoacetamido- pyrimidine derivative 17a showed the highest inhibitory effect against all the three tumor cell lines comparing to the remaining synthesized compounds.

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REFERENCES