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

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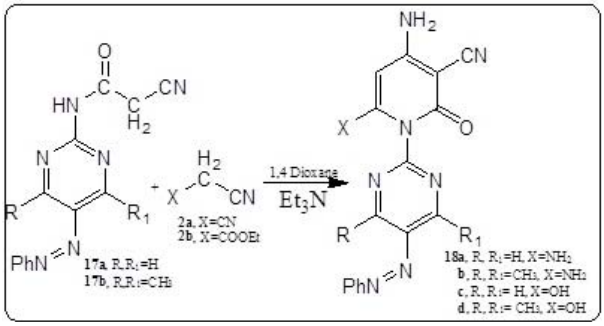
In this paper we made a contentious effort to afford heterocyclic compounds with interesting biological activities. The reaction of guanidine with either activated methylene groups, arylhydrazono derivatives, dicyanopropene derivatives, malononitrile dimer or arylhydarazononitrile derivatives afforded diaminopyrimidine derivatives, aryldiazenyl pyrimidine derivatives, fused pyridopyrimidne 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 acetic 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 pyridopyrimidne derivatives.
The structures of the newly synthesized compounds were established using IR, ^{1}H NMR, ^{13}C 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 B_1, and many numerous antineoplastic drugs.[1–8] Furthermore, 2,4-diaminopyrimidine derivatives have interested biological properties, like caspase-1 inhibitors effect[9] 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 agents[12] 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. ^{1}H NMR and ^{13}C NMR spectra were measured on a

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Varian EM 390-200 M*Hz* instrument with CD_3SOCD_3 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 ethylcyanoacetate (**2b**) (1.131 g, 0.0l mol) was added to a solution of guanidine (**1**) (0.591 g, 0.0l 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 ($3NH_2$), 1660 (C=N), 1649 (C=C). ^{1}H NMR (DMSO-d_6) δ = 5.52, 5.87, 6.12 (3s, 6H, D_2O-exchangeable, $3NH_2$), 6.43 (s, 1H, pyrimidine ring). MS (relative intensity) m/z: 125 (M^+, 28.2%). Calcd for $C_4H_7N_5$ (125.13) : C, 38.39; H, 5.64; N, 55.97%. Found: C, 38.64; H, 5.29; N, 55.69%.

Compound **3b**: Faint yellow powder from ethanol, yield 66%, 0.832 g, m.p. 284-286 ºC. IR (KBr): υ/cm-1 = 3455-3156 ($2NH_2$, OH), 1664 (C=N), 1651 (C=C). ^{1}H NMR (DMSO-d6) δ = 5.76, 6.22 (2s, 4H, D_2O-exchangeable, $2NH_2$), 6.61 (s, 1H, pyrimidine ring), 9.77 (s, 1H, D_2O-exchangeable, OH). MS (relative intensity) m/z: 126 (M+, 19.5%). Calcd for $C_4H_6N_4O$ (126.12) : C, 38.09; H, 4.80; N, 44.42%. Found: C, 37.84; H, 5.03; N, 44.23%.

General procedure for the synthesis of compound: 5-(phenyldiazenyl)pyrimidine-2,4,6-triamine (**5a**)*, 5-((chlorohexa-1,3,5-triynyldiazenyl)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)pyrimidine-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 ($3NH_2$), 3048 (CH aromatic), 1656 (C=N). ^{1}H NMR (DMSO-d_6) δ = 4.22, 4.63, 4.91 (3s, 6H, D_2O-exchangeable, $3NH_2$), 6.87-7.23 (m, 5H, C_6H_5). ^{13}C NMR: δ = 122.3, 125.4, 126.8, 129.2, 131.1, 134.1, 135.3, 137.2, 138.8, 140.5 (pyrimidine C, C_6H_5 C). Calcd for $C_{10}H_{11}N_7$ (229.24): C, 52.39; H, 4.84; N, 42.77%. Found: C, 52.64; H, 4.57; N, 42.99%.

Compound **5b**: Off white crystals from ethanol, yield 48%, 0.633 g, m.p. 152-154ºC. IR (KBr): υ/cm^{-1} = 3467-3321 ($3NH_2$), 3054 (CH aromatic), 1652 (C=N). ^{1}H NMR (DMSO-d_6) δ = 4.43, 4.68, 5.23 (3s, 6H, D_2O-exchangeable, $3NH_2$), 7.25-7.38 (d.d, 4H, C_6H_4). ^{13}C 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, C_6H_4 C). Calcd for $C_{10}H_{10}ClN_7$ (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 ($2NH_2$, OH), 3051 (CH aromatic), 1650 (C=N). ^{1}H NMR (DMSO-d_6) δ = 4.27, 5.18 (2s, 4H, D_2O-exchangeable, $2NH_2$), 7.16-7.32 (m, 5H, C_6H_5), 9.33 (s, 1H, D_2O-exchangeable, OH). ^{13}C NMR: δ = 118.7, 121.3, 123.1, 124.2, 125.6, 126.6, 130.1, 134.2, 137.7, 140.6 (pyrimidine C, C_6H_5 C). Calcd for $C_{10}H_{10}N_6O$ (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 ($2NH_2$, OH), 3053 (CH aromatic), 1651 (C=N). ^{1}H NMR (DMSO-d_6) δ = 4.11, 4.43 (2s, 4H, D_2O-exchangeable, $2NH_2$), 7.42-7.65 (d.d, 4H, C_6H_4), 8.56 (s, 1H, D_2O-exchangeable, OH). ^{13}C NMR: δ = 121.1, 123.5, 124.7, 126.3, 127.9, 129.2, 130.2, 132.4, 135.4, 138.6 (pyrimidine C, C_6H_4 C). Calcd for $C_{10}H_9ClN_6O$ (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 5h, 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 ºC. IR (KBr): υ/cm^{-1} = 3324-3198 (3NH$_2$), 1657 (C=N), 1646 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.44, 4.78, 5.86 (3s, 6H, D$_2$O-exchangeable, 3NH$_2$), 6.55-6.73 (m, 2H, pyridine ring). MS (relative intensity) m/z: 176 (M$^+$, 12.8%). Calcd for $C_7H_8N_6$ (176.18): C, 47.72; H, 4.58; N, 47.70%. 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 ºC. IR (KBr): υ/cm^{-1} = 3314-3129 (2NH$_2$, OH), 1653 (C=N), 1645 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.33, 4.67 (2s, 4H, D$_2$O-exchangeable, 2NH$_2$), 6.85-6.96 (m, 2H, pyridine ring), 9.76 (s, 1H, D$_2$O-exchangeable, OH). MS (relative intensity) m/z: 178 [M+1]$^+$, 19.1%. Calcd for $C_7H_7N_5O$ (177.16): C, 47.46; H, 3.98; N, 39.53%. Found: C, 47.23; H, 3.66; N, 39.79%.

General procedure for the synthesis of compound: pyrido[2,3-d]pyrimidine-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 ºC. IR (KBr): υ/cm^{-1} = 3277-3134 (4NH$_2$), 1658 (C=N), 1644 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.18, 4.66, 4.92, 5.37 (4s, 8H, D$_2$O-exchangeable, 4NH$_2$), 6.73 (s, 1H, fused pyridine ring). MS (relative intensity) m/z: 191 (M$^+$, 22.4%). Calcd for $C_7H_9N_7$ (191.19): C, 43.97; H, 4.74; N, 51.28%. Found: C, 43.69; H, 4.93; N, 51.63%.

General procedure for the synthesis of compounds: 6-(phenyldiazenyl)pyrido[2,3-d]pyrimidine-2,4,5, 7-tetraamine (**12a**)*, 6-((chlorohexa-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-hydropyrimido[4,5-c]pyridazine-3-carbonitrile* (**13a**) *and 4,5-diamino-1-(chlorohexa-1,3,5-triynyl)-7-imino-1,7-dihydropyridazino[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 dimethyl-formamide (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 ºC. IR (KBr): υ/cm^{-1} = 3322-3184 (4NH$_2$), 3051 (CH aromatic), 1653 (C=N), 1646 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.25, 4.78, 5.12, 5.66 (4s, 8H, D$_2$O-exchangeable, 4NH$_2$), 7.14-7.33 (m, 5H, C$_6$H$_5$). ^{13}C 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, C$_6$H$_5$ C). MS (relative intensity) m/z: 295 (M$^+$, 17.5%). Calcd for $C_{13}H_{13}N_9$ (295.30): C, 52.87; H, 4.44; N, 42.69%. Found: 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 ºC. IR (KBr): υ/cm^{-1} = 3282-3155 (4NH$_2$), 3053 (CH aromatic), 1658 (C=N), 1649 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.17, 4.54, 4.83, 5.25 (4s, 8H, D$_2$O-exchangeable, 4NH$_2$), 7.39-7.58 (d.d, 4H, C$_6$H$_4$). ^{13}C 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, C$_6$H$_4$ C). MS (relative intensity) m/z: 329 (M$^+$, 13.4%). Calcd for $C_{13}H_{12}ClN_9$ (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 ºC. IR (KBr): υ/cm^{-1} = 3377-3134 (2NH$_2$, NH), 3047 (CH aromatic), 2221 (CN), 1656 (C=N), 1644 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.34, 4.81 (2s, 4H, D$_2$O-exchangeable, 2NH$_2$), 7.22–7.47 (m, 5H, C$_6$H$_5$), 10.23 (s, 1H, D$_2$O-exchangeable, NH) . ^{13}C 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, pyridazine C, C$_6$H$_5$ C). MS (relative intensity) m/z: 278 (M$^+$, 13.7%). Calcd for $C_{13}H_{10}N_8$ (278.27): C, 56.11; H, 3.62; N, 40.27%. Found: C, 56.37; H, 3.33; N, 40.55%.

Compound **13b**: Yellow crystals from ethanol, yield 43%, 0.672 g, m.p. 200-202 ºC. IR (KBr): υ/cm^{-1} = 3334-3121 (2NH$_2$, NH), 3055 (CH aromatic), 2223 (CN), 1654 (C=N), 1641 (C=C). ^{1}H NMR (DMSO-d$_6$) δ = 4.56, 5.34 (2s, 4H, D$_2$O-exchangeable, 2NH$_2$), 7.41-7.58 (dd, 4H, C$_6$H$_4$), 10.52 (s, 1H, D$_2$O-exchangeable, NH) . ^{13}C 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, C_6H_4 C). MS (relative intensity) m/z: 312 (M^+, 10.2%). Calcd for $C_{13}H_9ClN_8$ (312.72): C, 49.93; H, 2.90; N, 35.83%. Found: C, 49.66; H, 3.16; N, 35.58%.

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 **1** (0.33 g, 0.005 mol) in 50 mL of ethanol was treated with an equimolar amount of either compounds **14a** (0.88 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. 127-128 ºC. IR (KBr): υ/cm^{-1}= 3378-3226 (NH_2), 3049 (CH aromatic), 1659 (C=N), 1649 (C=C). ^{1}HNMR (DMSO) δ = 4.86 (s, 2H, D_2O-exchangeable, NH_2), 6.67-6.77 (s, 2H, pyrimidine ring), 7.16-7.38 (m, 5H, C_6H_5). MS (relative intensity) m/z: 199 (M^+, 14.8%). Calcd for $C_{10}H_9N_5$ (199.21): C, 60.29; H, 4.55; N, 35.16%. Found: C, 60.55; H, 4.28; N, 35.44%.

Compound **15b**: Yellowish brown crystals from ethanol, yield 47%, 0.534 g, m.p. 155-157 ºC. IR (KBr): υ/cm^{-1}= 3355-3248 (NH_2), 3055 (CH aromatic), 2987 (CH_3), 1662 (C=N), 1653 (C=C). ^{1}HNMR (DMSO) δ = 1.69, 1.97 (2s, 6H, $2CH_3$), 4.53 (s, 2H, D_2O-exchangeable, NH_2), 7.21-7.42 (m, 5H, C_6H_5). MS (relative intensity) m/z: 228 $[M+1]^+$, 21.4%. Calcd for $C_{12}H_{13}N_5$ (227.27): C, 63.42; H, 5.77; N, 30.82%. Found: C, 63.70; H, 5.52; N, 30.55%.

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) acetamide* (**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 glacial acetic acid (40 mL), acetic anhydride (0.208 g, 0.002 mol) was added The reaction mixture was then refluxed for 4 h, cooled, then poured on 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 (CH_3), 1681 (C=O), 1655 (C=N), 1647 (C=C). ^{1}HNMR (DMSO) δ = 1.77 (s, 3H, CH_3), 6.44-6.53 (s, 2H, pyrimidine ring), 7.22-7.43 (m, 5H, C_6H_5), 10.12 (s, 1H, D_2O-exchangeable, NH), MS (relative intensity) m/z: 241 (M^+, 23.4%). Calcd for $C_{12}H_{11}N_5O$ (241.25): C, 59.74; H, 4.60; N, 29.03%. Found: C, 60.02; H, 4.88; N, 29.31%.

Compound **16b**: Creamy white crystals from ethanol, yield 55%, 0.296 g, m.p. 227-229 ºC. IR (KBr): υ/cm^{-1}= 3223 (NH), 3046 (CH aromatic), 2984 (CH_3), 1676 (C=O), 1652 (C=N), 1643 (C=C). ^{1}HNMR (DMSO) δ = 1.63, 1.77, 1.85 (3s, 9H, $3CH_3$), 7.33-7.54 (m, 5H, C_6H_5), 10.43 (s, 1H, D_2O-exchangeable, NH),. MS (relative intensity) m/z: 269 (M^+, 21.1%). Calcd for $C_{14}H_{15}N_5O$ (269.30): 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: 2-cyano-N-(5-(phenyldiazenyl) pyrimidin-2-yl)acetamide (**17a**) *and 2-cyano-N-(4,6-dimethyl-5-(phenyldiazenyl) pyrimidin-2-yl)acetamide* (**17b**)

To either solution of compound **15a** (0.398 g, 0.002 mol) or **15b** (0.454 g, 0.002 mol) in dimethyl formamide (10 mL) ethyl cyanoacetate (0.226 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 4 h and the reaction was monitored under TLC control then, poured onto ice/water and the formed solid product in each case was collected by filtration.

Compound **17a**: Pale brown crystals from ethanol, yield 53%, 0.282 g, m.p. 162-164 ºC. IR (KBr): υ/cm^{-1}= 3223 (NH), 3045 (CH aromatic), 2965 (CH_2), 2223 (CN), 1669 (C=O), 1654 (C=N), 1649 (C=C). ^{1}HNMR (DMSO) δ = 3.12 (s, 2H, CH_2), 6.17-6.34 (s, 2H, pyrimidine ring), 7.29-7.41 (m, 5H, C_6H_5), 9.54 (s, 1H, D_2O-exchangeable, NH),. MS (relative intensity) m/z: 266 (M^+, 15.8%). Calcd for $C_{13}H_{10}N_6O$ (266.26): C, 58.64; H, 3.79; N, 31.56%. Found: C, 58.90; H, 4.05; N, 31.81%.

Compound **17b**: Pale brown crystals from ethanol, yield 57%, 0.335 g, m.p. 149-151 ºC. IR (KBr): υ/cm^{-1}= 3318 (NH), 3052(CH aromatic), 2985 (CH_3), 2965 (CH_2), 2221 (CN), 1672 (C=O), 1657 (C=N), 1646 (C=C). ^{1}HNMR (DMSO) δ = 1.95, 2.14 (2s, 6H, $2CH_3$), 3.28 (s, 2H, CH_2), 7.18-7.33 (m, 5H, C_6H_5), 9.39 (s, 1H, D_2O-exchangeable, NH),. MS (relative intensity) m/z: 294 (M^+, 18.2%). Calcd for $C_{15}H_{14}N_6O$ (294.31): C, 61.21; H, 4.79; N, 28.55%. Found: C, 61.50; H, 4.53; N, 28.29%.

General procedure for the synthesis of compounds: 4,6-diamino-2-oxo-1-(5-(phenyldiazenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (**18a**)*, 4,6-diamino-1-(4,6-dimethyl-5-(phenyldiazenyl) pyrimidin-2-yl)-2-oxo-1,2-dihydro- pyridine -3-carbonitrile* (**18b**)*, 4-amino-6-hydroxy-2-oxo-1-(5-(phenyldiazenyl) pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile* (**18c**) *and 4-amino-1-(4,6-dimethyl-5-(phenyldiazenyl)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 in each case was collected by filtration.

Compound **18a**: Brown crystals from ethanol, yield 61%, 0.202 g, m.p. 207-209 ºC. IR (KBr): υ/cm^{-1}= 3344-3276 ($2NH_2$), 3049 (CH aromatic), 2227 (CN), 1667 (C=O), 1652 (C=N), 1645 (C=C). ^{1}HNMR (DMSO) δ = 4.22-4.56 (2s, 4H, D_2O-exchangeable, $2NH_2$), 6.11-6.75 (3s, 3H, pyrimidine ring, pyridine ring), 7.22-7.51 (m, 5H, C_6H_5). ^{13}C NMR: δ = 114.2 (CN), 115.8, 117.4, 120.3, 122.8, 124.1, 127.2, 129.5, 131.4, 133.2, 134.8, 136.7, 137.3, 138.6 (pyrimidine C, pyridine C, C_6H_5 C), 163.4 (C=O). MS (relative intensity) m/z: 332 (M^+, 28.3%). Calcd for $C_{16}H_{12}N_8O$ (332.32): C, 57.83; H, 3.64; N, 33.72%. Found: C, 57.61; H, 3.91; N, 33.44%.

Compound **18b**: Brown crystals from ethanol, yield 57%, 0.205 g, m.p. 214-216 ºC. IR (KBr): υ/cm^{-1}= 3376-3233 ($2NH_2$), 3056 (CH aromatic), 2988, 2983 ($2CH_3$), 2229 (CN), 1669 (C=O), 1655 (C=N), 1646 (C=C). ^{1}HNMR (DMSO) δ = 1.74, 1.93 (2s, 6H, $2CH_3$), 4.47-4.69 (2s, 4H, D_2O-exchangeable, $2NH_2$), 6.23 (s, 1H, pyridine ring), 7.38-7.64 (m, 5H, C_6H_5). ^{13}C NMR: δ = 22.3, 24.7 ($2CH_3$), 112.7 (CN), 116.2, 117.9, 120.8, 121.7, 123.4, 125.5, 128.2, 130.7, 132.6, 134.3, 136.9, 137.6, 138.3 (pyrimidine C, pyridine C, C_6H_5 C), 167.1 (C=O). MS (relative intensity) m/z: 360 (M^+, 31.4%). Calcd for $C_{18}H_{16}N_8O$ (360.37): C, 59.99; H, 4.48; N, 31.09%. Found: C, 59.72; H, 4.77; N, 31.35%.

Compound **18c**: Brown crystals from ethanol, yield 52%, 0.173 g, m.p. 240-242 ºC. IR (KBr): υ/cm^{-1}= 3412-3354 (OH, NH_2), 3058 (CH aromatic), 2224 (CN), 1663 (C=O), 1650 (C=N), 1642 (C=C). ^{1}HNMR (DMSO) δ = 4.28-4.53 (s, 2H, D_2O-exchangeable, NH_2), 6.32-6.56 (3s, 3H, pyrimidine ring, pyridine ring), 7.17-7.39 (m, 5H, C_6H_5), 9.84 (s, 1H, D_2O-exchangeable, OH) . ^{13}C NMR: δ = 117.2 (CN), 118.6, 119.4, 120.8, 122.3, 123.8, 125.7, 128.1, 130.7, 132.5, 134.3, 136.3, 137.8, 139.2, 140.4 (pyrimidine C, pyridine C, C_6H_5 C), 164.9 (C=O). MS (relative intensity) m/z: 333 (M^+, 24.5%). Calcd for $C_{16}H_{11}N_7O_2$ (333.30): C, 57.66; H, 3.33; N, 29.42%. Found: C, 57.93; H, 3.60; N, 29.64%.

Compound **18d**: Brown crystals from ethanol, yield 63%, 0.227 g, m.p. 197-199 ºC. IR (KBr): υ/cm^{-1}= 3433-3322 (OH, NH_2), 3056 (CH aromatic), 2990, 2982 ($2CH_3$), 2227 (CN), 1672 (C=O), 1655 (C=N), 1643 (C=C). ^{1}HNMR (DMSO) δ = 2.06, 2.16 (2s, 6H, $2CH_3$), 4.42-4.55 (s, 2H, D_2O-exchangeable, NH_2), 6.47 (s, 1H, pyridine ring), 7.33-7.58 (m, 5H, C_6H_5), 9.48 (s, 1H, D_2O-exchangeable, OH) . ^{13}C NMR: δ = 18.3, 21.6 ($2CH_3$), 115.6 (CN), 117.3, 119.2, 120.6, 122.9, 123.7, 124.5, 127.1, 129.6, 131.4, 133.2, 135.4, 137.2, 139.5, 140.7 (pyrimidine C, pyridine C, C_6H_5 C), 169.2 (C=O). MS (relative intensity) m/z: 361 (M^+, 22.6%). Calcd for $C_{18}H_{15}N_7O_2$ (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-(phenyldiazenyl)pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile (**20a**)*, 1-(4,6-dimethyl-5-(phenyldiazenyl)pyrimidin-2-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile* (**20b**)*, 6-hydroxy-4-methyl-2-oxo-1-(5-(phenyldiazenyl) pyrimidin-2-yl)-1,2-dihydropyridine-3-carbonitrile* (**20c**) *and 1-(4,6-dimethyl-5-(phenyldiazenyl) 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 acetoacetate (**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 ºC. IR (KBr): υ/cm^{-1}= 3056 (CH aromatic), 2988 (CH_3), 2229 (CN), 1671 (C=O), 1655 (C=N), 1649 (C=C).

^{1}HNMR (DMSO) δ = 1.65-1.77 (2s, 6H, $2CH_3$), 6.33-6.53 (3s, 3H, pyrimidine ring, pyridine ring), 7.11-7.28 (m, 5H, C_6H_5). ^{13}C NMR: δ = 18.9, 24.0 ($2CH_3$), 119.1 (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 (pyrimidine C, pyridine C, C_6H_5 C), 161.3 (C=O). MS (relative intensity) m/z: 330 (M^+, 21.5%). Calcd for $C_{18}H_{14}N_6O$ (330.34): C, 65.44; H, 4.27; N, 25.44%. Found: C, 65.18; H, 3.99; N, 25.72%.

Compound **20b**: Off white crystals from ethanol, yield 63%, 0.226 g, m.p. 102-104 ºC. IR (KBr): υ/cm^{-1}= 3051 (CH aromatic), 2992 (CH_3), 2222 (CN), 1663 (C=O), 1651 (C=N), 1644 (C=C). ^{1}HNMR (DMSO) δ = 1.65-2.17 (4s, 12H, $4CH_3$), 6.48 (s, 1H, pyridine ring), 7.18-7.43 (m, 5H, C_6H_5). ^{13}C NMR: δ = 18.2, 19.6, 20.3, 21.5 ($4CH_3$), 117.2 (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 (pyrimidine C, pyridine C, C_6H_5 C), 160.9 (C=O). MS (relative intensity) m/z: 358 (M^+, 27.6%). Calcd for $C_{20}H_{18}N_6O$ (358.40): C, 67.02; H, 5.06; N, 23.45%. Found: C, 67.22; H, 5.33; N, 23.70%.

Compound **20c**: Faint brown crystals from ethanol, yield 55%, 0.182 g, m.p. 108-110 ºC. IR (KBr): υ/cm^{-1}= 3387-3224 (OH), 2985 (CH_3), 3052 (CH aromatic), 2223 (CN), 1661 (C=O), 1647 (C=N), 1642 (C=C). ^{1}HNMR (DMSO) δ = 1.86 (s, 3H, CH_3), 6.44-6.65 (3s, 3H, pyrimidine ring, pyridine ring), 7.13-7.48 (m, 5H, C_6H_5), 9.22 (s, 1H, D_2O-exchangeable, OH) . ^{13}C NMR: δ = 19.5 (CH_3), 115.8 (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 (pyrimidine C, pyridine C, C_6H_5 C), 161.4 (C=O). MS (relative intensity) m/z: 332 (M^+,15.5%). Calcd for $C_{17}H_{12}N_6O_2$ (332.32): C, 61.44; H, 3.64; N, 25.29%. Found: C, 61.19; H, 3.91; N, 25.54%.

Compound **20d**: Brown crystals from ethanol, yield 62%, 0.223 g, m.p. 155-157 ºC. IR (KBr): υ/cm^{-1}= 3366-3231 (OH), 2986 (CH_3), 3056 (CH aromatic), 2226 (CN), 1663 (C=O), 1649 (C=N), 1643 (C=C). ^{1}HNMR (DMSO) δ = 1.63-2.12 (3s, 9H, $3CH_3$), 6.49 (s, 1H, pyridine ring), 7.17-7.36 (m, 5H, C_6H_5), 8.85 (s, 1H, D_2O-exchangeable, OH) . ^{13}C NMR: δ = 16.8, 18.3, 19.5 ($3CH_3$), 112.9 (CN), 118.2, 119.5, 120.6, 121.7, 122.8, 124.3, 126.4, 128.7, 130.9, 131.6, 133.5, 135.4, 137.2, 140.1 (pyrimidine C, pyridine C, C_6H_5 C), 162.7 (C=O). MS (relative intensity) m/z: 360 (M^+,19.2%). Calcd for $C_{19}H_{16}N_6O_2$ (360.37): C, 63.32; H, 4.48; N, 23.32%. Found: C, 63.60; H, 4.21; N, 23.51%.

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).

The three different human tumor cell lines that were used for such test are SF-268 (CNS cancer), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer). The MCF-7 was afforded from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460, normal fibroblast cells (WI-38), SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt).

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 μgm L^{-1}), at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 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),[16] 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 μmoL^{-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.[17] 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**[18,19] and **15a**,**b**.[20] 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 aryldiazenylpyrimidine derivatives **5a**,**b**,**c**,**d**. The of compounds **5a**,**b**,**c**,**d** were established applying analytical and spectral data, in compound **5a** ^{1}HNMR spectrum indicated the presence of three singlets, D_2O-exchangeable at δ = 4.22, 4.63, 4.91 ppm which indicate the presence of $3NH_2$ groups, multiplet at δ = 6.87-7.23 ppm corresponding to 5H of phenyl group. Furthermore, the ^{13}C NMR spectrum revealed eight signals at δ = 122.3, 125.4, 126.8, 129.2, 131.1, 134.1, 135.3, 137.2, 138.8, 140.5 ppm for the pyrimidine 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, ^{1}HNMR spectrum showed the presence of four singlets, D_2O-exchangeable at δ = 4.18, 4.66, 4.92, 5.37 ppm which indicate the presence of $4NH_2$ 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, ^{1}HNMR spectrum of compound **12a** showed the presence of four singlets, D_2O-exchangeable at δ = 4.25, 4.78, 5.12, 5.66 ppm which indicate the presence of $4NH_2$ groups, multiplet at δ = 7.14-7.33 ppm corresponding to 5H of benzene ring. The ^{13}C 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, ^{1}HNMR spectrum of compound **13a** showed the presence of two singlets, D_2O-exchangeable at δ = 4.34, 4.81 ppm which indicate the presence of $2NH_2$ groups, multiplet at δ = 7.22-7.47 ppm corresponding to 5H of benzene ring, singlet, D_2O-exchangeable at δ = 10.23 ppm which indicate the presence of NH group. Finally, the reaction of guanidine (**1**) with pheylhydrazono-dicarbonyl 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, ^{1}HNMR spectrum of compound **18a** presented the presence of two singlets, D_2O-exchangeable at δ = 4.22-4.56 ppm which indicate the presence of $2NH_2$ 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 $[C_6H_5]^+$ 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. ^{1}HNMR spectrum of compound **20a** presented the presence of two singlets at δ = 1.65-1.77 ppm which indicate the presence of $2CH_3$ 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 ^{13}C 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.

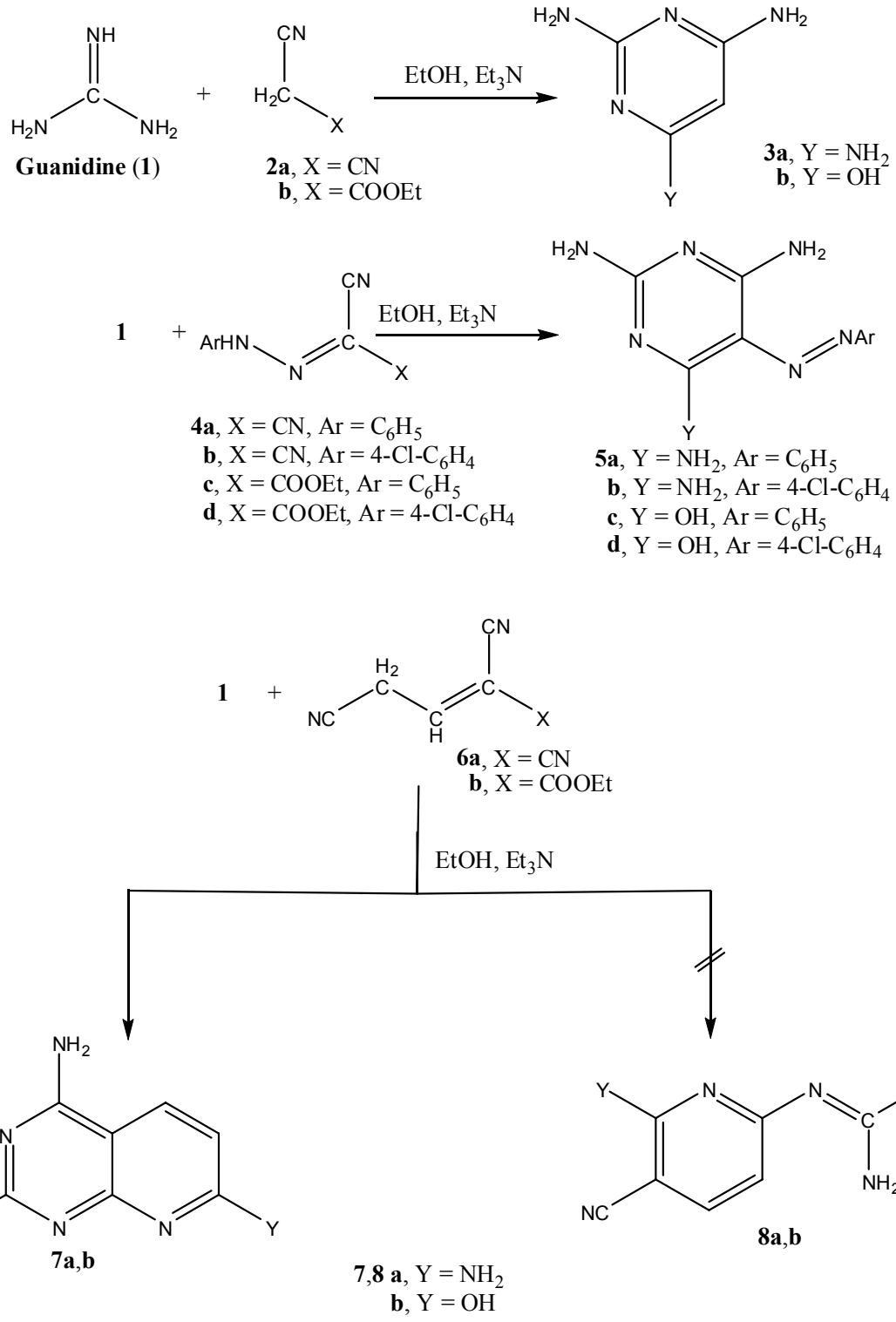
On comparing cyano-acetamidopyrimidine derivatives **17a**,**b** it was found that compound **17a** is more effective as antitumor active compound against three different cell lines, it may be due to the presence of free pyrimidine ring with no alkyl groups. On the other hand, pyridopyrimidine derivatives **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

GI_{50} (μmol L^{-1})				
Compd.				
No.	**MCF-7**	**NCI-H460**	**SF-268**	**WI-38**
3a	28.2 ± 7.9	19.8 ± 3.6	23.5 ± 5.7	>100
3b	22.3 ± 8.3	27.5 ± 6.3	25.5 ± 4.6	>100
5a	34.6 ± 5.2	33.1 ± 5.7	40.3 ±7.5	65.5±11.7
5b	42.2 ± 8.2	44.4 ± 7.3	40.1 ± 7.3	na
5c	47.2 ± 9.2	52.3 ± 8.4	44.5 ±10.5	>100
5d	40.2± 8.1	42.1 ± 9.4	41.5 ± 8.5	>100
7a	48.0 ± 10.9	42.2 ± 9.7	44.7 ± 9.5	>100
7b	42.8 ± 8.3	43.1 ± 7.5	43.9 ± 8.9	na
10	50.1 ± 8.6	42.2 ± 6.4	46.6 ± 8.5	>100
12a	39.1 ± 8.2	35.2 ± 6.4	37.2 ± 8.3	>100
12b	35.5 ± 9.5	39.2± 8.1	30.7 ± 6.5	na
13a	39.1 ±10.2	37.1 ± 8.2	32.8 ± 9.1	27.1 ± 11.2
13b	40.6 ± 8.4	41.9 ± 7.2	33.8 ± 8.5	25.4 ± 10.2
15a	37.2 ± 8.5	28.3 ± 6.4	26.1 ± 8.9	>100
15b	29.3 ± 7.8	34.3 ± 8.2	31.9 ± 10.2	>100
16a	29.2 ± 8.8	30.2 ± 8.1	38.1 ± 8.5	>100
16b	28.5 ± 7.9	41.9 ± 5.3	33.7 ± 6.5	>100
17a	1.4 ± 0.06	2.1 ± 0.9	1.1 ±0.5	>100
17b	25.7 ± 5.5	30.4 ± 6.9	32.8 ± 8.5	na
18a	3.9 ± 1.1	2.5 ± 1.2	2.9 ±0.9	>100
18b	40.1 ± 10.2	43.5 ± 11.7	36.0 ± 6.5	na
18c	0.05 ± 0.02	0.08 ± 0.5	0.09 ±0.03	>100
18d	44.1 ± 12.2	43.4 ± 10.4	34.8 ± 7.9	na
20a	30.7 ± 7.5	42.4 ± 8.4	30.6 ± 10.8	na
20b	44.7 ± 8.5	43.4 ± 6.5	29.8 ± 12.5	na
20c	34.3 ± 6.9	32.1 ± 5.7	38.6 ± 7.8	>100
20d	39.7 ± 8.2	37.4 ± 9.9	32.6 ± 10.5	>100
Doxorubicin	0.03 ±0.008	0.07±0.008	0.09±0.007	>100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NH2
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+
CN
H2C
X
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b, X = COOEt
EtOH, Et3N
H2N
N
NH2
N
Y
3a, Y = NH2
b, Y = OH
1
+
CN
ArHN
C
N
X
EtOH, Et3N
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b, X = CN, Ar = 4-Cl-C6H4
c, X = COOEt, Ar = C6H5
d, X = COOEt, Ar = 4-Cl-C6H4
5a, Y = NH2, Ar = C6H5
b, Y = NH2, Ar = 4-Cl-C6H4
c, Y = OH, Ar = C6H5
d, Y = OH, Ar = 4-Cl-C6H4
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+
CN
H2
C
C
NC
C
H
X
6a, X = CN
b, X = COOEt
EtOH, Et3N
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N
H2N
N
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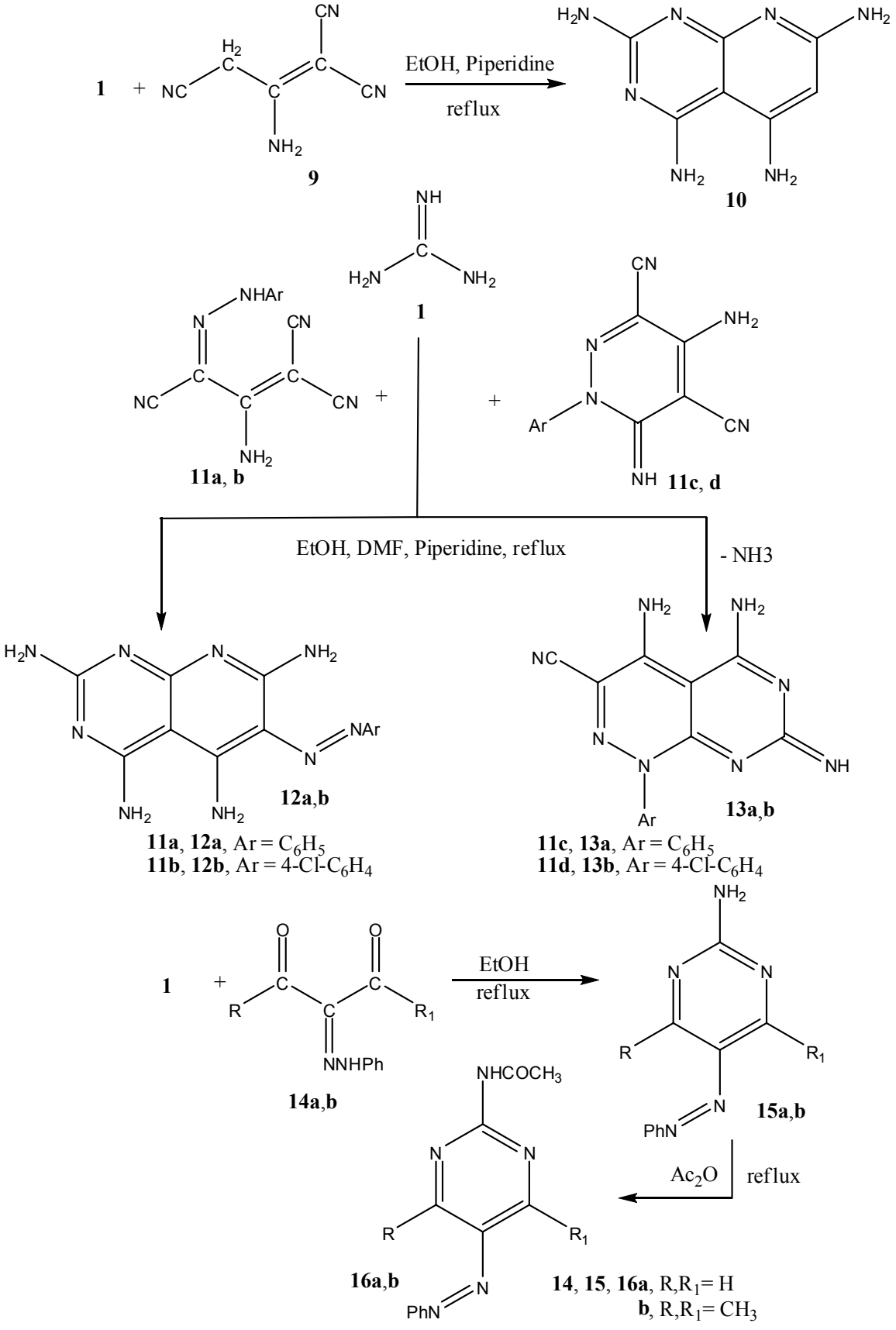
8a,b
7,8 a, Y = NH2
b, Y = OH

Scheme 1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reflux
10
1
11a, b
11c, d
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- NH3
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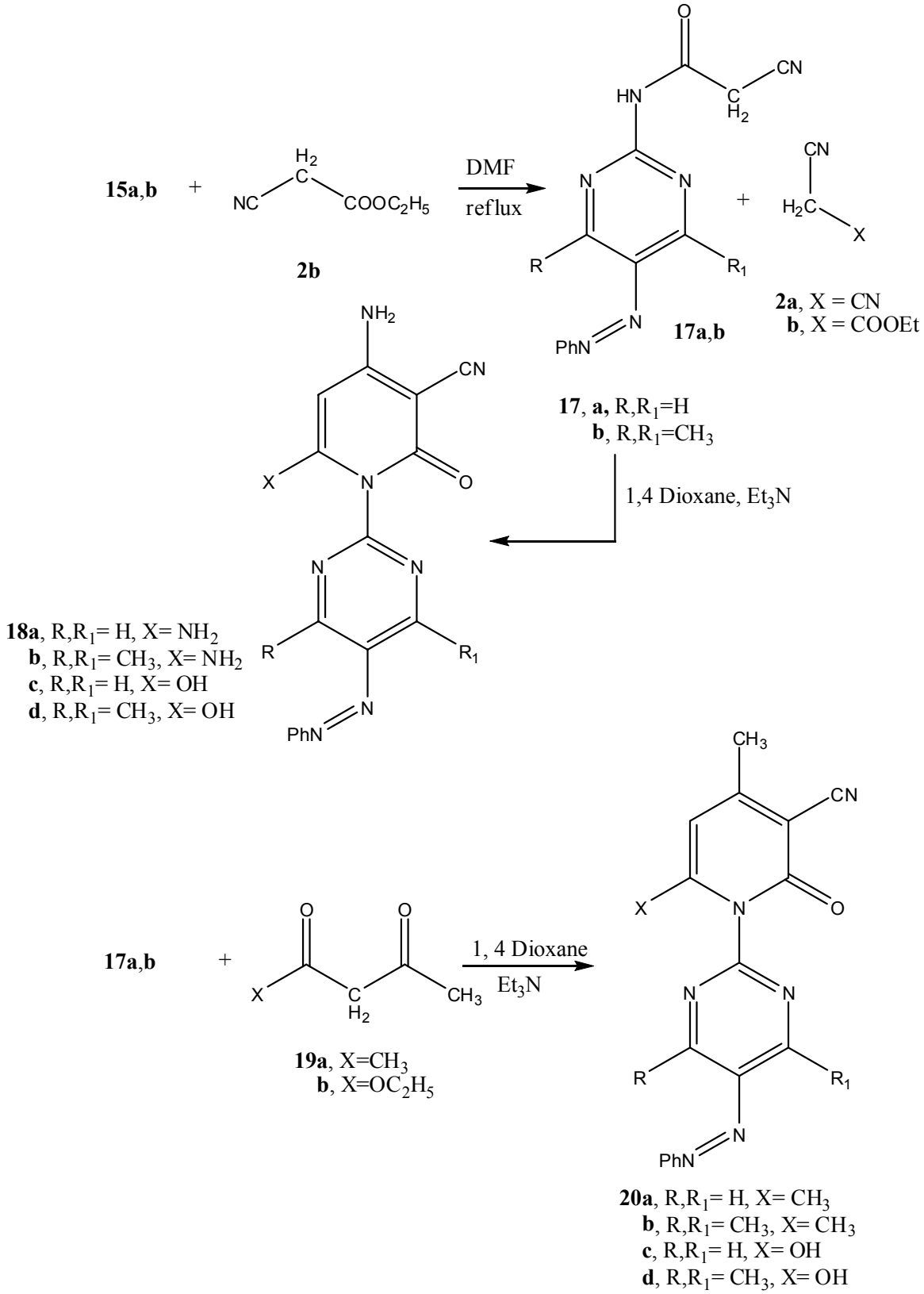
13a,b
11a, 12a, Ar = C6H5
11b, 12b, Ar = 4-Cl-C6H4
11c, 13a, Ar = C6H5
11d, 13b, Ar = 4-Cl-C6H4
EtOH
reflux
14a,b
15a,b
Ac2O
reflux
16a,b
14, 15, 16a, R,R1= H
b, R,R1= CH3

Scheme 2


15a,b
2b
DMF
reflux
17a,b
2a, X = CN
b, X = COOEt
17, a, R,R1=H
b, R,R1=CH3
1,4 Dioxane, Et3N
18a, R,R1= H, X= NH2
b, R,R1= CH3, X= NH2
c, R,R1= H, X= OH
d, R,R1= CH3, X= OH
17a,b
19a, X=CH3
b, X=OC2H5
1, 4 Dioxane
Et3N
20a, R,R1= H, X= CH3
b, R,R1= CH3, X= CH3
c, R,R1= H, X= OH
d, R,R1= CH3, X= OH

Scheme 3

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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