Synthesis and anticancer activity of some new pyridine derivatives

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Abstract Different substituents were introduced in positions 2 and 6 of 2,6 diaminopyridine in order to obtain new heterocyclic compounds. A new series of aza pyridine, imidazopyridine, benzodiazepine, indole, pyrimidine, and benzimidazole heterocyclic derivatives were synthesized in good yields. The anticancer activities of some of the new compounds were evaluated against liver cancer cell line HEPG2. Compounds **3**, **4**, **10**, **11**, **12**, and **17** showed the highest activity when compared to 5-flurouracil (5-FU) and doxorubicin (DOX) chemotherapy.

Keywords Pyridine derivatives · Aza pyridine derivatives ·
Pyrimidine derivatives · Benzodiazepine derivatives · Anticancer activity

Introduction

The pyridine is among the most common heterocyclic compounds found in various therapeutic agents. The structure of 2,6-diaminopyridine (DAP) has the

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symmetrical feature that possesses three nitrogen lone pairs. *N*-acyl pyridine derivatives have also attracted considerable attention due to their hydrogen bonding motifs [1–3]. Poly functional pyridines are highly reactive reagents that have been used extensively in heterocyclic synthesis [4–7] and that possess biological as well as pharmacological activity [8–10]. In addition, triazolopyridines are also interesting compounds due to their pronounced biological activity, as they can be used as antidepressants [11–13]. Various pyridine derivatives are well known to possess an array of physiological activities, such as anticancer, muscle relaxant, hypnotic, anti-inflammatory, diuretic, and antihypertensive activities [14–17]. Imidazole derivatives are part of anticancer drugs like mercaptopurine. Imidazole is also a part of the theophylline molecule, found in tea leaves and coffee beans, which stimulate the central nervous system [18]. On the other hand, benzimidazole is a very important pharmacophore in drug discovery, and its derivatives are an important class of bioactive molecules in the field of drugs and pharmaceuticals [19]. Pyrimidines have been used as therapeutic agents possessing analgesics and anti-inflammatory activity [20–22]. Moreover, indoles and their derivatives are known to possess a wide variety of biological and pharmacological properties, including antibacterial and antifungal, cytotoxic, antioxidant and insecticidal activity [23, 24].

In this article, synthesis and characterization of some new heterocyclic compounds such as pyrido pyrimidine, diazepine, indole and imidazole derivatives and were performed to be evaluated against liver carcinoma cell line (HEPG2).

Experimental

Chemistry

All melting points are uncorrected and were recorded on an open glass capillary tube using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Micro analytical Unit, using Vario Elementar and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR 300 E Fourier transform infrared spectrophotometer using the KBr disc technique. The spectra of ^{1}H-NMR and C^{13}-NMR were determined using a JEOL EX-270 and/or 500NMR spectrophotometers and were run in deuterated chloroform (CDCl$_3$) or dimethyl sulphoxide (DMSO-d6). Chemical shifts were related to that of the solvent. The mass spectra were measured with a JEOL mass spectrometer and LC/MS-Finnigan at Central Services Laboratory, National Research Center, Cairo, Egypt. Follow-up of the reactions and checking purity of the compounds were made by thin-layer chromatograph technique on silica gel pre-coated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at 254 nm for a few seconds.

*Synthesis of 6-amino-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-2-one (**1**)*

A suspension of 2,6 diaminopyridine (0.1 mol), chloroacetic acid (10 g, 0.1 mol), anhydrous sodium acetate (1.5 g) in a mixture of acetic anhydride and acetic acid

(1:3) was heated under reflux with stirring at 120 °C for 10 h. The reaction mixture was then poured onto ice-water (50 mL) and set aside in refrigerator at 0 °C. The solid formed was collected by filtration and recrystallization from benzene–petroleum ether (60–80 °C).

Yield 72%, mp. 140–142 °C. ^{1}H-NMR (270 MHz, chloroform) δ: 3.60 (2H, t, CH$_2$), 3.90 (2H, t, CH$_2$), 6.50 (2H, br, $J = 11.0$ Hz, NH$_2$ D$_2$O-exchangeable), 7.20–7.60 (3H, m, Ar'H pyridine), ^{13}C-NMR (270 MHz, chloroform) δ: 39.80, 40.20 (CH$_2$), 120.50, 122.70, 131.90 (pyridine carbon), 158.90 (C=N), 160.90 (C=N), 170.90 (C=O). IR (KBr) cm^{-1}: 3350 (NH$_2$), 1720(C=O), 1640–1650 (C=N). Anal. Calcd. for C$_8$H$_9$N$_3$O: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.80; H, 5.50; N; 25.70.

Synthesis of 6-amino-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione (2) and 1-aminobenzo[e]pyrido[1,2-a][1,3]diazepine-6,11-dione (2-3): general procedure

A mixture of 2,6 diaminopyridine (0.02 mol) and (0.02 mol) malonic acid and/or phthalic acid anhydride in ethanol (25 mL) in presence of few drops of TEA was refluxed for 8–10 h. Cooling the mixture followed by filtration and recrystallization from ethanol afforded the target molecules **2** and **3**.

6-amino-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione (2)

Yield 77%, mp. 130–133 °C. ^{1}H-NMR (270 MHz, chloroform) δ: 3.95 (2H, s, $J = 7.0$ Hz, CH$_2$), 6.50 (2H, br, $J = 11.0$ Hz, NH$_2$ D$_2$O-exchangeable), 7.20–7.60 (3H, m, Ar'H pyridine). ^{13}C-NMR (270 MHz, chloroform) δ: 40.20 (CH$_2$), 120.50, 122.70, 131.90 (pyridine carbon), 158.90 (C=N), 160.90 (C=N), 168.90 (C=O), 169.90 (C=O). IR (KBr) cm^{-1}: 3350 (NH$_2$), 1720–1740 (C=O), 1640–1650 (C=N). Anal. Calcd. for C$_8$H$_8$N$_3$O$_2$: C, 53.93; H, 4.53; N, 23.58. Found: C, 53.90; H, 4.55; N, 23.68, MS: $m/z = 178$ [M$^+$].

1-aminobenzo[e]pyrido[1,2-a][1,3]diazepine-6,11-dione (3)

Yield 74%, mp. 154–156 °C. ^{1}H-NMR (270 MHz, chloroform) δ: 6.50 (2H, br, NH$_2$ D$_2$O-exchangeable), 7.20–7.50 (3H, m, $J = 9.5$ Hz, Ar'H pyridine), 7.60–7.80 (4H, m, Ar'H). ^{13}C-NMR (270 MHz, chloroform) δ: 120.50, 122.70, 131.90 (pyridine carbon), 124.90, 125.40, 129.90, 130.70, 132.90, 135.60 (aromatic carbon), 158.90 (C=N), 160.50 (C=N), 166.30 (C=O), 168.90 (C=O). IR (KBr) cm^{-1}: 3350 (NH$_2$), 1710–1720 (C=O), 1640–1650 (C=N). Anal. Calcd. for C$_{13}$H$_9$N$_3$O$_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.20; H, 3.75; N; 17.50.

General procedure for the synthesis of compounds (4–6)

A mixture of compound **1**–**3** (0.001 mol) and the appropriate aldehyde, namely, 5-methylfuran-2-carbaldehyde (0.0015 mol) in ethanol (20 mL) and glacial acetic acid (1 mL) was refluxed for 6–8 h. The reaction mixture was poured into ice-cold

water and the solid product was filtered off, washed with petroleum ether and recrystallization from methanol to form compounds **4–6**, respectively.

-6-((4-methylfuran-2-yl)methyleneamino)-3,4-dihydro-2H-pyrido [1,2-a]pyrimidin-2-one (4)

Yield 75%, mp. 162–164 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 1.90 (3H, s, CH$_3$), 3.60 (2H, t, CH$_2$), 3.90 (2H, t, CH$_2$), 6.65 (1H, d, furan), 6.75 (1H, d, furan), 7.20–7.50 (3H, m, $J = 9.5$ Hz, Ar'H pyridine), 7.65 (1H, s, $J = 12.0$ Hz, N=CH). IR (KBr) cm^{-1}: 1710 (C=O), 1640–1650 (C=N). Anal. Calcd. for C$_{14}$H$_{13}$N$_3$O$_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.80; H, 5.10; N, 16.40, MS: $m/z = 253[M^+]$.

6-((4-methylfuran-2-yl)methyleneamino)-2H-pyrido [1,2-a]pyrimidine-2,4(3H)-dione (5)

Yield 72%, mp. 170–172 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 1.90 (3H, s, CH$_3$), 3.95 (2H, s, CH$_2$), 6.65 (1H, d, furan), 6.75 (1H, d, furan), 7.20–7.50 (3H, m, Ar'H pyridine), 7.75 (1H, s, $J = 12.0$ Hz, N=CH), IR (KBr) cm^{-1}: 1710–1720 (C=O), 1640–1650 (C=N). Anal. Calcd. for C$_{14}$H$_{11}$N$_3$O$_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.50; H, 4.18; N, 15.66.

(4a)-1-((4-methylfuran-2-yl)methyleneamino)benzo[e]pyrido[1,2-a] [1,3]diazepine-6,11-dione (6)

Yield 75%, mp. 180–183 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 1.90(3H, s, $J = 5.0$ Hz, CH$_3$), 6.65 (1H, d, furan), 6.75 (1H, d, furan), 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.80 (4H, m, Ar'H), 7.90 (1H, s, $J = 12.0$ Hz, N=CH). IR (KBr) cm^{-1}: 1710–1720 (C=O), 1640–1650 (C=N). Anal. Calcd. for C$_{19}$H$_{13}$N$_3$O$_3$: C, 68.88; H, 3.95; N, 12.68. Found: C, 68.85; H, 3.89; N, 12.60, MS: $m/z = 331[M^+]$.

Synthesis of N2,N6-bis ((1H-indol-3-yl)methylene)pyridine-2,6-diamine (7)

A mixture of starting compound 2,6 diaminopyridine (0.001 mol) and indole 3-carboxaldehyde (0.001 mol) in ethanol and few drops of glacial acetic acid was refluxed for 8 h. The reaction mixture was poured into ice-cold water and the solid product was filtered off, washed with petroleum ether, and recrystallization from ethanol to give compound **7**.

Yield 68%, mp. 182–184 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.80(5H, m, $J = 13.00$ Hz, Ar'H), 7.90 (1H, s, $J = 11.0$ Hz, N=CH), 10.75 (1H, br, NH benzimidazole). IR (KBr) cm^{-1}: 3290 (NH), 1640 (C=N). Anal. Calcd. for C$_{23}$H$_{17}$N$_5$: C, 67.01; H, 4.71; N, 19.27. Found: C, 67.08; H, 4.76; N, 19.20. MS: $m/z = 253$ [M$^+$].

Reaction of compound (7) with oxalyl chloride: preparation of -2-(3-((6-((1-(2-chloro-2-oxoacetyl)-1H-indol-3-yl)methyleneamino)pyridin-2-yl)methyleneamino)-1H-indol-1-yl)-2-oxoacetyl chloride (8)

A mixture of compound **7** (0.01 m mol) and oxalyl chloride (0.01 mmol) in diethyl ether (30 mL) with stirring for 12 h. The reaction mixture was poured into ice water and the solid product was filtered off, washed with petroleum ether, and recrystallization from ethanol to obtain compound **8**.

Yield 65%, mp. 192–195 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.80 (5H, m, J = 13.00 Hz, Ar'H), 7.90 (1H, s, J = 11.0 Hz, N=CH). IR (KBr) cm^{-1}: 1640–1660 (C=N), 1680–1695 (C=O), 1250–1260 (C–Cl). Anal. Calcd. for $C_{29}H_{21}N_5O_4$: C, 60.64; H, 3.68; Cl, 12.34; N, 12.19. Found: C, 60.68; H, 3.62; Cl, 12.39; N, 12.15, MS: m/z = 545 [M$^+$ +1].

Synthesis of 2,2′-(3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(1H-indole-3,1-diyl))bis(1-(piperazin-1-yl)ethane-1,2-dione) (9)

A mixture of the appropriate compound **8** (0.005 mol) and piperazine (0.005 mol) was dissolved in hot absolute ethanol (20 mL) and anhydrous potassium carbonate (0.5 g) was added with stirring. The mixture was heated under reflux with stirring for 8 h. Then, an excess of solvent was evaporated under vacuum, poured onto ice and then neutralized with diluted HCl. The residue formed was treated with petroleum ether, collected and recrystallization from ethanol to form compound **9**.

Yield 62%, mp. 206–208 °C. ^{1}H-NMR (270 MHz, DMSO) δ: 2.80–3.30 (8H, m, 4CH$_2$ piprazine), 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.80 (5H, m, Ar'H), 7.90 (1H, s, J = 11.0 Hz, N=CH), 9.95 (1H, s, NH piprazine). IR (KBr) cm^{-1}: 3225 (NH), 1640–1660 (C=N), 1680–1695 (C=O). Anal. Calcd. for $C_{36}H_{35}N_9O_4$: C, 65.74; H, 5.36; N, 19.17. Found: C, 65.70; H, 5.31; N, 19.10.

Synthesis of 3,3′-(pyridine-2,6-diyl)bis (aza-1-yl-1-ylidene)) diindolin-2-one (10)

2,6 diaminopyridine (0.02 mol) was added to 2,3 indolinedione (0.02 mol, 2.94 g) in (50 mL) absolute ethanol containing few drops of glacial acetic acid. The reaction mixture was heated under reflux for 6 h and then the reaction mixture was filtered off. Recrystallization of the product from ethanol then drying afforded product **10**.

Yield 78%, mp. 225–227 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.90 (4H, m, J = 13.0 Hz, Ar'H), 10.19 (1H, s, NH). ^{13}C-NMR (500 MHz, DMSO) δ: 122.3, 127.98, 132.70 (pyridine carbon), 111.80, 118.90, 124.80, 125.70, 133.60, 135.20 (aromatic carbon), 165.77 (C=N), 167.70 (C=N), 170.90 (C=O). IR (KBr) cm^{-1}: 3340 (NH), 1645–1660 (C=N), 1690 (C=O) Anal. Calcd. for $C_{21}H_{13}N_5O_2$: C, 68.66; H, 3.57; N, 19.06. Found: C, 68.62; H, 3.52; N, 19.10. MS: m/z = 367 [M$^+$].

*Synthesis of -1-(2-hydroperoxyethyl)-2-oxoindolin-3-ylideneamino) pyridin-2-ylimino)-2-oxoindolin-1-yl) acetic acid derivative (**11**)*

A mixture of potassium hydroxide (0.01 mol) and compound **10** (0.01 mol) was dissolved in (25 mL) absolute ethanol. Then chloroacetic acid (0.01 mol, 1.22 g) was added drop wisely with stirring and then the reaction mixture was refluxed for 8 h. After cooling, the solvent was evaporated under vacuum and the formed solid was recrystallization from ethanol.

Yield 74%, mp. 285–287 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 4.20 (2H, s, $J = 7.5$ Hz, CH$_2$), 7.20–7.50 (3H, m, Ar'H pyridine), 7.60–7.95 (4H, m, $J = 9.0$ Hz, Ar'H), 11.20 (1H, s, OH). ^{13}C-NMR (500 MHz, DMSO) δ: 40.50 (CH$_2$), 122.3, 127.98, 132.70 (pyridine carbon), 115.90, 117.30, 124.50, 125.98, 129.47, 131.77 (aromatic carbon), 155.30 (C=N), 163.22 (C=N), 170.90 (C=O), 173.90 (COOH). IR (KBr) cm^{-1}: 3370 (OH), 1645–1660 (C=N), 1690 (C=O), 1750 (COOH). Anal. Calcd. for C$_{25}$H$_{17}$N$_5$O$_6$: C, 62.11; H, 3.54; N, 14.49. Found: C, 62.18; H, 3.54; N, 14.55. MS: $m/z = 483$ [M$^+$].

*Synthesis of 3,3'-(pyridine-2,6-diyl)bis(aza-1-yl-1-ylidene))bis(2-oxoindoline-1-yl-3-ylidene)bis(methylene)bis(1H-benzo[d]imidazole-6-carboxylic acid) (**12**) and 3,3'-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))bis(1-((3H-imidazo[4,5-b]pyridin-2-yl)methyl)indolin-2-one) (**13**) derivatives: general procedure*

Compound **11** (0.01 mol) was dissolved in ethanol (10 mL) and was added to 2,3 diaminopyridine and/or *o*-phenylendiamine-5-carboxylic acid (0.01 mol) in ethanol (10 mL) in presence of few drops of TEA. The reaction mixture was heated under reflux with stirring for 10–12 h. The solvent was evaporated under vacuum and then crystallization from methanol to afford the products **12–13**.

*3,3'-(pyridine-2,6-diyl)bis(aza-1-yl-1-ylidene))bis(2-oxoindoline-1-yl-3-ylidene)bis(methylene)bis(1H-benzo[d]imidazole-6-carboxylic acid) derivative (**12**)*

Yield 75%, mp. 196–198 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 4.20 (2H, s, $J = 7.0$ Hz, CH$_2$), 6.90–7.10 (4H, m, $J = 12.0$ Hz, Ar'H), 7.20–7.50 (4H, m, Ar'H pyridine), 7.60–7.90 (4H, m, Ar'H), 10.90 (1H, s, NH), 11.20 (1H, s, OH). ^{13}C-NMR (500 MHz, DMSO) δ: 39.96 (CH$_2$), 122.3, 127.98, 132.70 (pyridine carbon), 114.20, 115.90, 116.50, 117.80, 118.50, 119.20, 121.90, 124.50, 125.50, 126.80, 128.60, 131.70 (aromatic carbon), 163.20 (C=N), 165.90 (C=N), 167.22 (C=N), 170.90 (C=O), 174.90 (COOH), IR (KBr) cm^{-1}: 3340 (OH), 1640–1665 (C=N), 1690 (C=O), 1750 (COOH). Anal. Calcd. for C$_{39}$H$_{25}$N$_9$O$_6$: C, 65.45; H, 3.52; N, 17.61. Found: C, 65.52; H, 3.46; N, 17.55.

*3,3'-(pyridine-2,6-diyl)bis(aza-1-yl-1-ylidene))bis(1-((3H-imidazo[4,5-b]pyridin-2-yl)methyl)indolin-2-one) derivative (**13**)*

Yield 78%, mp. 175–177 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 4.23 (2H, s, CH$_2$), 6.90–7.10 (3H, m, Ar'H pyridine), 7.20–7.50 (3H, m,, Ar'H pyridine), 7.60–7.90

(3H, m, $J = 11.0$ Hz, Ar'H), 10.50 (1H, s, NH). ^{13}C-NMR (500 MHz, DMSO) δ: 40.90 (CH$_2$), 122.89, 127.80, 132.50 (pyridine carbon), 115.70, 117.90, 123.80, 126.90, 130.90 (pyridine carbon), 115.90, 117.80, 119.90, 124.50, 126.98, 131.91 (aromatic carbon), 158.40 (C=N), 161.90 (C=N), 163.22 (C=N), 165.24 (C=N), 170.90 (C=O). IR (KBr) cm^{-1}: 3350 (NH),1640–1660 (C=N), 1695 (C=O) Anal. Calcd. for C$_{35}$H$_{23}$N$_{11}$O$_2$: C, 66.77; H, 3.68; N, 24.47. Found: C, 66.70; H, 3.61; N, 24.40.

Synthesis of 1,1'-(pyridine-2,6-diyl) bis (3-isopropylthiourea) (14), 1,1'-(pyridine-2,6-diyl) bis (3-butylurea) (15) and N,N'-(pyridine-2,6-diyl)bis (aza-diyl)) bis (thioxomethylene) dibenzamid (16) derivatives: general procedure

To a solution of 2,6 diaminopyridine (0.01 mol) in ethanol (20 mL) containing isocyanate or isothiocyanate derivatives; namely, isopropyl isothiocyanate, butylis-ocyanate, and benzoyl isothiocyanate (0.02 mol) added dropwisely with stirring. The reaction mixture was heated under reflux for 6–8 h. After cooling, the reaction mixture washed with water. The formed solids were collected by filtration, dried, and crystallization from methanol to produce compounds **14–16**.

1,1'-(pyridine-2,6-diyl) bis (3-isopropylthiourea) derivative (14)

Yield 70%, mp. 103–105 °C. ^{1}H-NMR (500 MHz, DMSO) δ:1.20–1.34 (6H, m, 2CH$_3$), 4.55–4.70 (1H, m, CH), 7.60–7.90 (3H, m, Ar'H pyridine), 8.90 (1H, s, NH),11.50 (1H, s, NH). IR (KBr) cm^{-1}: 3280–3290 (NH), 1640 (C=N), 1260 (C=S). Anal. Calcd. for C$_{13}$H$_{21}$N$_5$S$_2$: C, 50.13; H, 6.80; N, 22.48; S, 20.59 Found: C, 50.20; H, 6.70; N, 20.60; S, 20.55. MS: $m/z = 311$ [M$^+$].

1,1'-(pyridine-2,6-diyl) bis (3-butylurea) derivative (15)

Yield 67%, mp. 130–132 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 1.18–1.30 (3H, m, CH$_3$), 4.25–4.50 (6H, m, 3CH$_2$), 7.20–7.50 (3H, m, Ar'H pyridine), 8.90 (1H, s, NH), 9.60 (1H, s, NH). IR (KBr) cm^{-1}: 3250–3270 (NH), 1690 (C=O), 1640 (C=N). Anal. Calcd. for C$_{15}$H$_{25}$N$_5$O$_2$: C, 58.61; H, 8.20; N, 22.78. Found: C, 58.70; H, 4.28; N, 22.86. MS: $m/z = 307$ [M$^+$].

N,N'-(pyridine-2,6-diyl)bis(aza-diyl))bis(thioxomethylene)dibenzamide derivative (16)

Yield 70%, mp. 120–122 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 6.90–7.50 (5H, m, Ar'H), 7.60–7.90 (3H, m, Ar'H pyridine), 10.50 (1H, s, NH), 11.40 (1H, s, NH). IR (KBr) cm^{-1}: 3260–3280 (NH), 1700 (C=O), 1640 (C=N), 1265 (C=S). Anal. Calcd. for C$_{21}$H$_{17}$N$_5$O$_2$S$_2$: C, 57.91; H, 3.93; N, 16.08; S, 14.72. Found: C, 57.82; H, 4.10; N, 16.02; S, 14.67.

Synthesis of 3,3′-(pyridine-2,6-diyl)bis(dihydropyrimidine-4,6(1H,5H)-dione)
*derivatives (**17–19**): general procedure*

A mixture of compounds **14–16** (0.001 mol) and (0.002 mol) of malonic acid in
presence of sodium methoxide (20 mL) added dropwisely and heated under reflux
for 5–6 h. The mixture was cooled, followed by filtration and recrystallization from
methanol afforded the target molecules **17–19**.

3,3′-(pyridine-2,6-diyl)bis(1-isopropyl-2-thioxodihydropyrimidine-4,6(1H,5H)-
*dione) derivative (**17**)*

Yield 70%, mp. 156–158 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 1.20–1.40 (6H, m,
2CH$_3$), 4.10 (2H, s, CH$_2$), 4.50–4.70 (1H, m, CH), 7.60–7.90 (3H, m, Ar'H
pyridine). ^{13}C-NMR (500 MHz, DMSO) δ: 19.90 (CH$_3$), 29.70 (CH$_3$), 39.20 (CH$_3$),
50.90 (CH), 124.77, 129.66, 135.32 (pyridine carbon), 166.33 (C=N), 168.92
(C=O), 170.40 (C=O), 179.50 (C=S). IR (KBr) cm^{-1}: 1680–1695 (C=O), 1645
(C=N), 1265 (C=S). Anal. Calcd. for C$_{19}$H$_{21}$N$_5$O$_4$S$_2$: C, 51.06; H, 4.69; N, 15.65, S,
14.33 Found: C, 51.02; H, 4.74; N, 15.70; S, 14.30. MS: $m/z = 447$ [M$^+$].

3,3′-(pyridine-2,6-diyl)bis(1-butyl-2-dihydropyrimidine-4,6(1H,5H)-dione)
*derivative (**18**)*

Yield 67%, mp. 178–180 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 1.20–1.30 (3H, m,
CH$_3$), 4.10 (2H, s, CH$_2$), 4.25–4.50 (6H, m, 3CH$_2$), 7.60–7.90 (3H, m, Ar'H
pyridine). ^{13}C-NMR (500 MHz, DMSO) δ: 20.90(CH$_3$), 27.90 (CH$_2$), 29.20 (CH$_2$),
30.99 (CH$_2$), 39.70 (CH$_2$), 124.50, 135.74, 137.70 (pyridine carbon), 149.80 (C=O),
165.70 (C=O), 169.70 (C=O). IR (KBr) cm^{-1}: 1680–1698 (C=O), 1645 (C=N).
Anal. Calcd. for C$_{21}$H$_{25}$N$_5$O$_6$: C, 56.88; H, 5.64; N, 15.80. Found: C, 56.80; H, 5.60;
N, 15.86. MS: $m/z = 443$ [M$^+$].

3,3′-(pyridine-2,6-diyl)bis(1-benzoyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione)
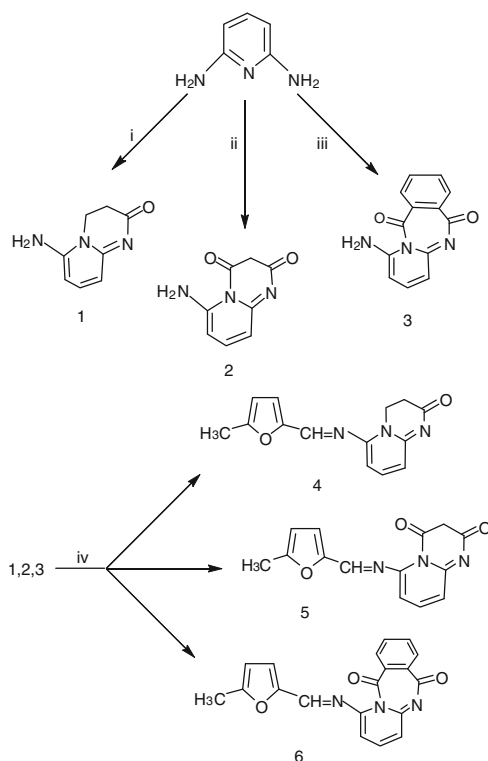*derivative (**19**)*

Yield 72%, mp. 146–148 °C. ^{1}H-NMR (500 MHz, DMSO) δ: 4.20 (2H, s, $J = 5.0$
CH$_2$), 6.90–7.50 (5H, m, $J = 10.2$, Ar'H), 7.60–7.90 (3H, m, Ar'H pyridine). IR (KBr)
cm^{-1}: 1680–1690 (C=O), 1645 (C=N), 1265 (C=S). Anal. Calcd. for C$_{27}$H$_{17}$N$_5$O$_6$S$_2$:
C, 56.74; H, 3.00; N, 12.25; S, 11.22. Found: C, 56.70; H, 3.10; N, 12.28; S, 11.26.

Results and discussion

In the present work, we report on the synthesis and preliminary biological activity
screening of several heterocycles compounds based on 2,6 diaminopyridine (DAP)
could be considered as a starting material was reacted with chloroacetic acid as well
as malonic acid and phthalic acid in refluxing ethanol containing TEA afforded
a single product identified as 6-amino-3,4-dihydro-2H-pyrido[1,2-a]pyrimidin-2-

one (1), 6-amino-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione (2) and 1-aminobenzo [e]pyrido[1,2-a][1,3]diazepine-6,11-dione (3), respectively (Scheme 1). The structures of compounds 1–3 were identified by elemental analyses and spectroscopic data. The structure of compounds 1, 2, and 3 were confirmed by their IR, ^{1}H-NMR and ^{13}C-NMR spectra. The IR spectra of compounds 1–3 appearance of bands at 3,350 cm^{-1} attributed to NH$_2$ stretching frequency are good evidence for the structure given to those compounds, 1710–1740 cm^{-1} due to the presence of two C=O groups for compound 2 and 3 in addition to C=N groups at 1640–1650 cm^{-1}. All the synthesized compounds 1–3 were confirmed by thin-layer chromatograph.

On the other hand, compounds 1–3 were reacted similarly with 5-methylfuran-2-carbaldehyde in refluxing ethanol containing a few drops of acetic acid to yield the corresponding 6-((4-methylfuran-2-yl)methyleneamino)-3,4-dihydro-2H-pyrido [1,2-a]pyrimidin-2-one (4), 6-((4-methylfuran-2-yl)methyleneamino)-2H-pyrido [1,2-a]pyrimidine-2,4(3H)-dione (5) and -1-((4-methylfuran-2-yl)methyleneamino)benzo[e]pyrido[1,2-a][1,3]diazepine-6,11-dione (6), respectively (Scheme 1). The identities of the isolated products were assigned by their elemental analysis and spectral data. The ^{1}H-NMR analysis of compounds (4–6) showed characteristic


Scheme 1 Synthesis of 2H-pyrido[1,2-a]pyrimidine derivatives 4–5 and pyrido[1,2-a][1,3]diazepine derivative 6 from 2,6 diaminopyridine (DAP). Reagents and conditions: (i) chloro acetyl chloride/ anhydrous sodium acetate/reflux, (ii) malonic acid/ethanol/TEA/reflux, (iii) phthalic acid/ethanol/TEA/ reflux, (iv) 5-methylfuran-2-carboxaldehyde/ethanol/acetic acid/reflux

resonance at δ 7.20–7.50 ppm due to the presence of (Ar'H pyridine) function and 7.65–7.90 ppm due to the presence of (N=CH) group of compounds **4**–**6** and 1.90 ppm for (CH$_3$) group of compounds **4**–**6**, respectively. All synthesized compounds were confirmed by thin-layer chromatography.

In addition, condensation reaction of 2,6 diaminopyridine with indole 3-carboxaldehyde afforded the corresponding of *N*2,*N*6-bis ((*1H*-indol-3-yl)methylene) pyridine-2,6-diamine (**7**), which was reacted with oxalyl chloride to give -2-(3-((6-((1-(2-chloro-2-oxoacetyl)-*1H*-indol-3-yl)methyleneamino)pyridin-2-yl)methyleneamino)-*1H*-indol-1-yl)-2-oxoacetyl chloride (**8**). The condensation reaction of compound **8** with piperazine led to the formation of 2,2′-(3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(*1H*-indole-3,1-diyl))bis (1-(piperazin-1-yl)ethane-1,2-dione) (**9**) (Scheme 2). All the synthesized compounds **7**–**9** were characterized by their physical, chemical, and spectral data. Thus, the IR spectra of compound **7** shows the presence of characteristic absorption peaks around 3,290 cm^{-1} (NH stretching), C=N at 1,640 cm^{-1} and ^{1}H-NMR spectra of compound **7** showed broad band's at 10.75 ppm that revealed the presence of NH absorption peak in addition to MS at $m/z = 253$ [M$^+$].

In a similar manner, treatment of compound 2,6 diaminopyridine with 2,3 indolinedione upon refluxing in ethanol and few drops of acetic acid gave the corresponding Schiff's base 3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))diindolin-2-one (**10**), which reacted with chloroacetic acid yielded the corresponding
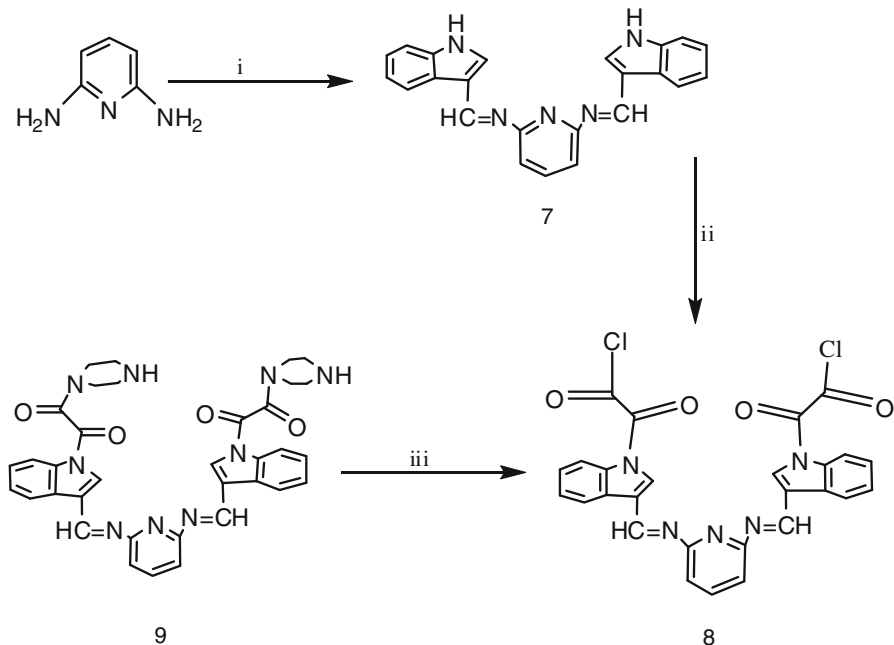
Scheme 2 Synthesis of 2,2′-(3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene) bis(*1H*-indole-3,1-diyl))bis(1-(piperazin-1-yl)ethane-1,2-dione) **9** from 2,6 DAP. Reagents and conditions: (i) indole 3-carboxaldehyde/ethanol/glacial acetic acid/reflux, (ii) oxally chloride/diethylether/rt, (iii) piprazine/ethanol/anhydrous potassium carbonate/reflux

1-(-2-hydroperoxyethyl)-2-oxoindolin-3ylideneamino)pyridin-2-ylimino)-2-oxo-indolin-1-yl) acetic acid derivative (**11**), which continue to react with diamines, namely, *o*-phenylendiamine-5-carboxylic acid and 2,3 diaminopyridine yielded the corresponding *3,3′*-(pyridine-2,6-diyl) bis (aza-1-yl-1-ylidene))bis(2-oxoindoline-1-yl-3-ylidene)bis(methylene)bis(1H-benzo[d] imidazole-6-carboxylic acid) derivative (**12**) and 3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene)) bis (1-((3H-imidazo [4,5-b] pyridin-2-yl) methyl) indolin-2-one) derivative (**13**), respectively (Scheme 3). The ^{1}H -NMR spectra of compounds **10** showed broad band at 10.19 ppm revealed the presence of the NH peak. The structure of compound **10** was further supported by its MS spectra, indicating the molecular ion peak was at *m/z* 367. The structures of compounds **10**, **11**, **12**, and **13** were confirmed on the basis of elemental analyses as well as spectral data. The synthesized compounds were confirmed by thin-layer chromatography.

Moreover, 2,6-diaminopyridine, which upon treatment with appropriate isocyanate and/or isothiocyanate derivatives, namely, isopropyl isothiocyanate, butyl
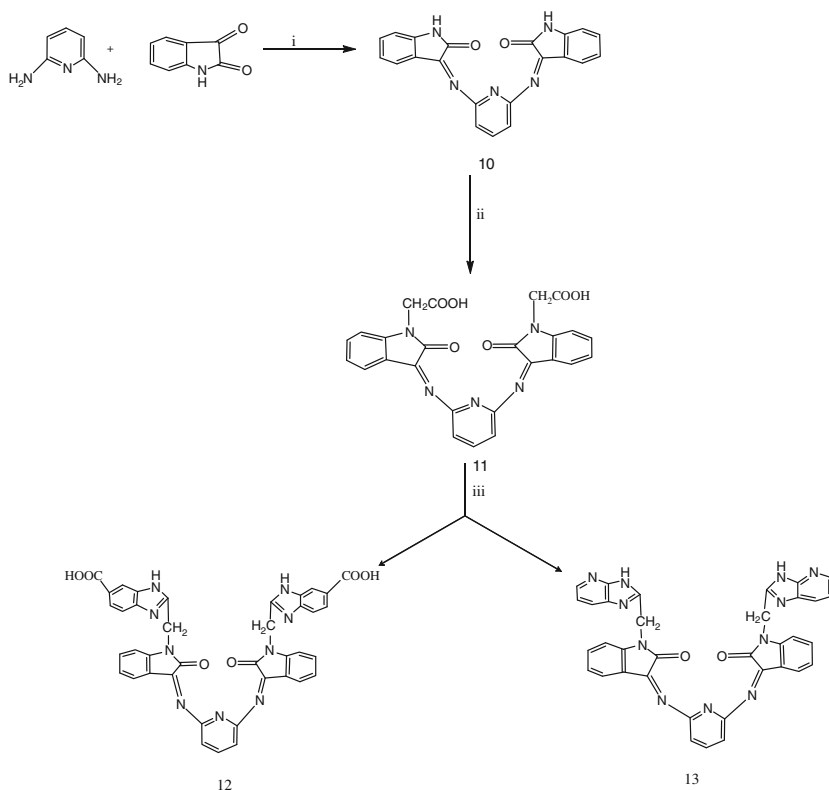
Scheme 3 Synthesis of 3,3′-(pyridine-2,6-diyl)bis(aza-1-yl-1-ylidene))bis(2-oxoindoline-1-yl-3-ylidene) bis(methylene)bis(*1H*-benzo[d]imidazole-6-carboxylic acid) **12** and 3,3′-(pyridine-2,6-diylbis(aza-1-yl-1-ylidene))bis(1-((*3H*-imidazo[4,5-b]pyridin-2-yl)methyl)indolin-2-one) **13** from 2,6 diaminopyridine (DAP). Reagents and conditions: (i) 2,3 indolinedione/ethanol/acetic acid/reflux, (ii) chloroacetic acid/ethanol/reflux, (iii) 2,3 diaminopyridine and/or *o*-phenylendiamine-5-carboxylic acid/ethanol/TEA/reflux

isocyanate and benzoyl isothiocyanate, afforded the corresponding urea and thiourea derivatives **14–16**, respectively, which was followed by cyclization when treated with malonic acid in presence of sodium methoxide to gave the corresponding pyrimidine derivatives; 3,3′-(pyridine-2,6-diyl)bis(1-isopropyl-2-thioxodihydropyrimidine-4,6(*1H,5H*)-dione) (**17**), 3,3′-(pyridine-2,6-diyl)bis(1-butyl-2-dihydropyrimidine-4,6(*1H,5H*)-dione) (**18**) and 3,3′-(pyridine-2,6-diyl)-bis(1-benzoyl-2-thioxodihydropyrimidine-4,6(*1H,5H*)-dione) (**19**) derivatives. The spectral data of **14–19** are in agreement with the assigned structures (Scheme 4). All the synthesized compounds **14–19** were confirmed by thin-layer chromatography.

$R_1 = CH(CH_3)_2$
$R_2 = (CH_2)_3 CH_3$
$R_3 = CO C_6H_5$
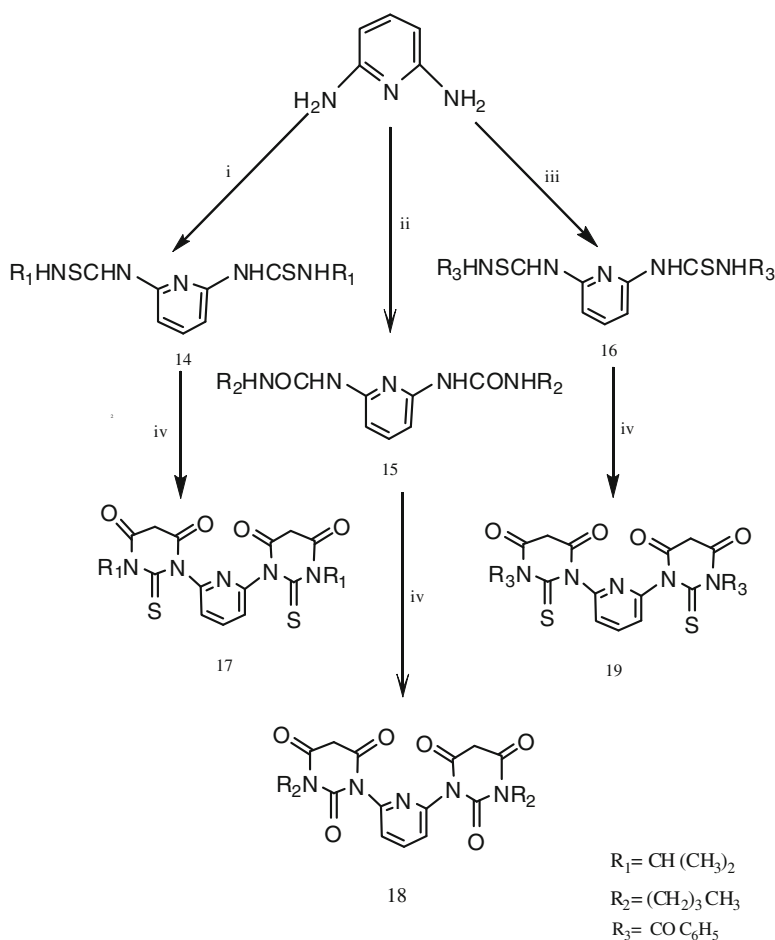
Scheme 4 Synthesis of 3,3′-(pyridine-2,6-diyl)bis(1-isopropyl-2-thioxodihydropyrimidine-4,6(*1H,5H*)-dione) derivative **17**, 3,3′-(pyridine-2,6-diyl)bis(1-butyl-2-dihydropyrimidine-4,6(*1H,5H*)-dione) derivative **18** and 3,3′-(pyridine-2,6-diyl)bis(1-benzoyl-2-thioxodihydropyrimidine-4,6(*1H,5H*)-dione) derivative **19** from 2,6 diaminopyridine. Reagents and conditions: (i) $(CH_3)_2CHNCS$/ethanol/reflux, (ii) $CH_3(CH_2)_3NCO$/ethanol/reflux, (iii) C_6H_5CONCS/ethanol/reflux, (iv) $HCOOCH_2COOH$/sodium methoxide/reflux

Pharmacology

Antitumor activity

Cytotoxic and biological effects of tested compounds against liver cancer cell line

Compounds **3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **17,** and **18** were soluble in DMSO at concentrations high enough to allow cell experiments; the in vitro biological activity of these compounds was evaluated by their growth-inhibitory potency in liver HEPG2 cancer cell lines. The cytotoxic potency of compounds **3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **17,** and **18** were studied in comparison to the known anticancer drugs 5-flurouracil (5-FU) and doxorubicin (DOX). Moreover, the biochemical effects of the tested compounds on some enzymes such as aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphates (ALP), in addition to albumin, globulins, creatinine, total lipids, cholesterol, triglycerides, and bilirubin in serum of mice were investigated.

Measurement of potential cytotoxicity by SRB assay

Compounds (**3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **17**, and **18**) were evaluated for their antitumor activity against liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and DOX. Potential cytotoxicity of the tested derivatives was tested according the method of Skehan et al. [25] as follows:

Cells were plated in 96-multiwell plates (10^4 cells/well) for 24 h before treatment with the tested compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM un-buffered Tris base [tris (hydroxymethyl) amino methane] for determination of optical density in a computer-interfaced, 96-well micro titer plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays modified by Zor and Selinger [26] at densities ranging from sparse sub-confluence to multilayered supra-confluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compounds.

Biochemical analysis

Animals Male albino mice weighing 18–20 g were used in the present study. Mice were divided into three main groups as follows:

1- Group (1): untreated or control group (five mice).
2- Group (2): divided into two subgroups (five mice for each subgroup) and treated with 5-FU or DOX as reference anticancer drugs.
3- Group (3): divided into eight subgroups (five mice for each subgroup) and treated with the tested compounds.
4- Treatment.

Group (1): each mouse was given a single intraperitoneal injection of 0.1 mL DMSO.

Group (2): each mouse was given a single intraperitoneal injection of 0.1 mL containing 12 mg/kg body weight 5-FU or DOX dissolved in sterile water.

Group (3): each mouse was given a single intraperitoneal injection of 0.1 mL containing 12 mg/kg body weight of the tested compounds (**3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **17** and **18**, respectively) dissolved in DMSO. Blood was collected after 7 days from all mice groups.

The biochemical effects of the tested compounds, on some liver enzymes such as aspartate and alanine, aminotransferases (AST and ALT) [27] and alkaline phosphatase (ALP) [28], were done using blood auto analyzer (Olympus AV 400, Japan). Moreover, albumin [29], globulins [30] and creatinine [31], total lipids [32], cholesterol [33], triglycerides [34] and bilirubin [35] in serum of mice were evaluated in comparison to 5-FU and DOX.

Statistical analysis of the results was performed using Chi-square values (SPSS computer program).

Preliminary screening of the tested compounds showed that all compounds exhibited a moderate to strong growth inhibition activity on the tested cell line between 1 and 10 μg/mL concentrations in comparison to the known anticancer drugs: 5-flurouracil and doxorubicin. Table 1 indicated the cytotoxic activity of the tested compounds (**3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **17**, and **18**) against liver HEPG2 cancer cell lines in comparison to the traditional anticancer drugs: 5-FU and DOX. It can be deduced from the results that compounds (**3**, **4**, **10**, **11**, **12** and **17**) were the most active and induced a reasonable growth inhibition, in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX (IC_{50} equals 2.08, 2.89, 3.02, 3.09, 3.15, and 3.36 μg/mL, while 5-FU and DOX were 5 and 3.56 μg/mL).

Effect of antitumor compounds on the biochemical parameters

Data obtained in Table 2 represented the liver enzymatic activities (ALT, AST, and ALP) in serum of control and treated groups of mice. The results showed that the values recorded for AST and ALT were significantly higher ($p < 0.001$) with 5-Fu and DOX treated groups of mice than the control. On the other hand, treatment of compounds, caused inverse effects, where some values recorded for AST and ALT were non-significant (n.s.) or slightly higher ($p < 0.01$) in comparison to control. Moreover, the recorded data showed that ALP activities were significantly increased ($p < 0.001$) with the treatment of 5-Fu and DOX, while there were no significant changes in ALP activities upon treatment with some of the new compounds.

Data listed in Table 3 demonstrated the comparison between the levels of total lipids, cholesterol, triglycerides, and bilirubin in serum of treated mice and the control group. It can be deduced from the present data that 5-FU and DOX caused a significant increase in the level of these parameters while treatment with the selected compounds showed moderate or no significant changes.

Table 4 represented a comparison between the levels of albumin, globulins, and creatinine in serum of control and treated groups of mice. It is clear from the results in the table that there was a slight increase in the level of albumin and creatinine and globulins in the 5-FU and DOX treated groups of mice while there were moderate or non-significant changes in the other treated groups.

Cytotoxic drugs are being administered with novel ways of therapy such as inhibitors of signals [36]. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents. The antitumor activities of compounds were assessed against HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-Fu and DOX. Regarding the antitumor activity study, some of the compounds showed reasonable antitumor activity in comparison to 5-FU and DOX. Moreover, study of the induced biochemical parameters of the tested compounds in mice showed insignificant differences relative to the control group, which indicates a moderate margin of safety for the tested compounds. Comparable to 5-FU and DOX, a dose augmentation of compounds **11** and **4** may be higher potency. Furthermore, the tested compounds have important potential advantages over 5-FU and DOX because of their lower toxicity and their ability to induce lower biochemical parameters. These results are in agreement with Espinosa et al. [37] and Kamalakannan and Venkappayya [38].

On the basis of monitoring the inhibition of the growth of human cancer cells, a series of synthesized compounds possessing a broader spectrum of antitumor activity and fewer toxic side-effects than traditional anticancer drugs have been

Table 1 Effect of some tested newly synthesized compounds on liver carcinoma cell line (HEPG2)

Compound	IC_{50} (μg/mL)
5-Flurouracil	5
Doxorubicin	3.56
3	3.36
4	2.89
5	4.1
6	7.11
7	3.92
8	3.62
10	3.02
11	2.08
12	3.15
13	3.56
17	3.09
18	5.23

IC_{50} dose of the compounds which reduces survival to 50%

Table 2 Biochemical effects of treatment with 5-FU, DOX, and the benzofuran derivatives on serum ALT, AST, and ALP in mice

Mice groups	Biochemical parameters		
	Alanine amino transferase mean ± SD ALT (IU/mL)	Aspartate amino transferase mean ± SD AST (IU/mL)	Alkaline phosphatase mean ± SD ALP (k.k./dL)
Control	43.5 ± 2.03	108.32 ± 4.19	18.70 ± 1.10
5-FU	51.47 ± 9.02	130.431 ± 8.92	25.485 + 6.03
p<	0.001	0.001	0.001
Doxorubicin	59.26 ± 12.03	147.226 ± 16.34	30.317 ± 5.14
p<	0.001	0.001	0.001
3	80.7 ± 19.09	162.17 ± 34.5	38.58 ± 12.61
p<	0.001	0.001	0.001
4	38.9 ± 8.9	123.9 ± 11.4	18.83 ± 6.29
p<	n.s.	0.01	n.s.
5	39.56 ± 6.7	112.54 ± 12.7	19.94 ± 4.35
p<	n.s.	0.01	n.s.
6	46.21 ± 4.17	107.81 ± 4.25	21.94 ± 3.4
p<	n.s.	n.s.	0.01
7	53.7 ± 10.08	142.3 ± 29.7	45.42 ± 10.41
p<	0.001	0.001	0.001
8	81.34 ± 27.3	151.52 ± 45.6	43.7 ± 8.36
p<	0.001	0.001	0.001
10	33.42 ± 7.05	111.2 ± 11.05	18.32 ± 3.07
p<	n.s.	n.s.	n.s.
11	60.5 ± 9.7	156.22 ± 20.1	44.26 ± 7.01
p<	0.001	0.001	0.001
12	68.34 ± 11.9	146.4 ± 28.1	36.9 ± 9.8
p<	0.001	0.001	0.001
13	50.81 ± 12.01	119 ± 9.56	22.07 ± 3.42
p<	0.01	0.01	0.01
15	73.09 ± 14.2	140.09 ± 31.01	30.41 ± 9.22
p<	0.001	0.001	0.001
17	46.09 ± 6.13	110.06 ± 8.91	18.76 ± 3.02
p<	n.s.	n.s.	n.s.

Data are expressed as mean + SD

n.s. non significant

$p > 0.05$ insignificant, $p < 0.01$: significant, $p < 0.001$: highly significant

studied. Twenty tested compounds (**3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 17,** and **18**) were subjected to a screening system for investigation of their antitumor potency against liver (HEPG2) cell line. Moreover, the biochemical effects of the tested compounds on some enzymes such as aspartate and alanine aminotransferases (AST and ALT)

Table 3 Biochemical effects of treatment with 5-FU, DOX and the benzofuran derivatives on serum total lipids, cholesterol, triglycerides, and bilirubin in mice

Mice groups	Biochemical parameters			
	Total lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Bilirubin (mg/dL)
Control	323.41 ± 27.1	94.32 ± 13.5	108.7 ± 16.8	0.63 ± 0.04
5-FU	378.2 ± 31.4	105.9 ± 11.7	126.5 ± 19.4	0.75 ± 0.10
$p<$	0.001	0.001	0.001	0.001
Doxorubicin	366.7 ± 6.10	109.3 ± 14.2	137.8 ± 17.10	0.81 ± 0.19
$p<$	0.001	0.001	0.001	0.001
3	363.6 ± 29.3	116.4 ± 8.3	98.4 ± 10.6	0.96 ± 0.8
$p<$	0.001	0.01	n.s.	0.001
4	329.34 ± 19.7	95.3 ± 9.4	119.7 ± 18.8	0.65 ± 0.9
$p<$	n.s.	n.s.	0.01	n.s.
5	331.63 ± 17.5	96.4 ± 10.5	118.6 ± 19.70	0.68 ± 0.11
$p<$	n.s.	n.s.	0.01	n.s.
6	317.4 ± 30.7	93.24 ± 19.53	116.23 ± 20.5	0.51 ± 0.08
$p<$	n.s.	n.s.	n.s.	0.01
7	372.8 ± 37.6	110.3 ± 17.8	93.6 ± 9.5	1.08 ± 0.7
$p<$	0.001	0.01	n.s.	0.001
8	382.09 ± 23.6	111.76 ± 34.3	172.9 ± 36.3	0.84 ± 0.4
$p<$	0.001	0.01	0.001	0.01
10	328.3 ± 13.7	96.8 ± 17.2	112.3 ± 10.6	0.67 ± 0.01
$p<$	n.s.	n.s.	n.s.	n.s.
11	374.9 ± 36.6	123.3 ± 26.09	132.8 ± 26.03	0.99 ± 0.07
$p<$	0.001	0.001	0.001	0.001
12	326.3 ± 18.7	95.6 ± 14.9	113.7 ± 8.6	0.61 ± 0.04
$p<$	n.s.	n.s.	n.s.	n.s.
13	364.19 ± 23.8	105.6 ± 17.4	119.8 ± 19.3	0.76 ± 0.15
$p<$	0.001	0.01	0.01	0.01
15	371.23 ± 26.7	110.9 ± 31.2	152.6 ± 34.5	0.77 ± 0.2
$p<$	0.001	0.01	0.001	0.01
17	329.71 ± 21.5	97.48 ± 16.7	112.54 ± 17.8	0.53 ± 0.04
$p<$	n.s.	n.s.	n.s.	0.01

Data are expressed as mean + SD

n.s. non significant

$p > 0.05$ insignificant, $p < 0.01$: significant, $p < 0.001$: highly significant

and alkaline phosphatase (ALP), in addition to albumin, globulins, creatinine, total lipids, cholesterol, triglycerides and bilirubin in serum of mice were studied in comparison to 5-flurouracil and doxorubicin.

The antitumor activity results indicated that most of the compounds showed good growth inhibition activity against the tested cell line but with varying intensities

Table 4 Biochemical effects of treatment with 5-FU, DOX, and the benzofuran derivatives on serum albumin, globulin, and creatinine in mice

Mice groups	Biochemical parameters			
	Albumin (mg/dL)	Globulin (mg/dL)	A/G ratio	Creatinine (mg/dL)
Control	5.63 ± 0.51	4.32 ± 0.9	1.3	0.69 ± 0.03
5-FU	6.49 ± 0.92	5.75 ± 0.8	1.13	0.81 ± 0.06
$p<$	0.01	0.01	0.01	0.01
Doxorubicin	6.37 ± 0.85	5.91 ± 0.63	1.078	0.78 ± 0.04
$p<$	0.01	0.01	0.01	0.01
3	7.1 ± 0.31	7.62 ± 0.76	1.003	0.78 ± 0.03
$p<$	0.01	0.01	0.001	0.01
4	7.2 ± 0.61	6.62 ± 0.86	1.006	0.8 ± 0.1
$p<$	0.01	0.01	0.001	0.01
5	5.97 ± 0.34	4.09 ± 0.63	1.46	0.65 ± 0.09
$p<$	n.s.	n.s.	n.s.	n.s.
6	5.92 ± 0.82	5.12 ± 0.9	1.15	0.73 ± 0.04
$p<$	n.s.	n.s.	n.s.	n.s.
7	6.87 ± 0.49	6.86 ± 0.8	1.02	1.7 ± 0.43
$p<$	0.01	0.01	0.001	0.001
8	11.43 ± 1.48	8.97 ± 0.9	1.13	0.76 ± 0.25
$p<$	0.001	0.001	0.01	n.s.
10	5.62 ± 0.68	4.68 ± 1.06	1.12	0.68 ± 0.08
$p<$	n.s.	n.s.	n.s.	n.s.
11	6.47 ± 0.46	6.42 ± 0.7	1.001	0.77 ± 0.05
$p<$	0.01	0.01	0.001	0.01
12	5.92 ± 0.81	4.72 ± 0.91	1.15	0.84 ± 0.6
$p<$	n.s.	n.s.	n.s.	0.01
13	7.73 ± 0.52	6.25 ± 0.82	1.23	0.84 ± 0.06
$p<$	0.01	0.01	0.01	0.01
15	10.22 ± 1.35	8.96 ± 0.91	1.14	0.72 ± 0.21
$p<$	0.001	0.001	0.01	n.s.
17	5.53 ± 0.71	4.88 ± 1.01	1.13	0.67 ± 0.1
$p<$	n.s.	n.s.	n.s.	n.s.

n.s. non significant

Data are expressed as mean + SD

$p > 0.05$ insignificant, $p < 0.01$: significant, $p < 0.001$: highly significant

extents in comparison to the known anticancer drugs: 5-flurouracil and doxorubicin. Moreover, compounds (**11**, **4**, **10**, **17**, **12** and **3**) showed cytotoxic activity (IC_{50} equals 2.08, 2.89, 3.02, 3.09, 3.15, and 3.36 µg/mL, respectively). Results of the biochemical investigations indicated that 5-flurouracil and doxorubicin caused significant changes in the level of all parameters tested while treatment with the tested compounds showed slight, moderate, or no significant changes.

Conclusions

In the present investigation, we report the design and synthesis of a new series of 2, 6 disubstituted pyridine, which include in their structures various heterocyclic moieties directly attached to the pyridine nucleus at positions 2 and 6 aiming to potentiate the biological activity as anticancer. In addition, our study was extended to the synthesis of additional derivatives containing a substituted pyridine moiety directly attached to diazepine, indole, benzimidazole, pyrimidine, and imidazole nucleus at positions 2 and 6. The anticancer activity data indicate that compounds **11**, **4**, **10**, **17**, **12**, and **3** were the most active compounds against HEPG2.

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