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Synthesis and anticancer activity of novel 2-substituted pyranopyridine derivatives

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Abstract A novel series of 2-aminopyranopyridine derivatives (**3**–**19**) were synthesized utilizing 2-chloro-4-(thiophen-2-yl)-7,8-dihydro-5*H*-pyrano[4,3-*b*]pyridine-3-carbonitrile (**2**) as a key starting compound. The structures of the newly synthesized compounds were confirmed on the basis of elemental analyses and infrared (IR), ^{1}H, ^{13}C-nuclear magnetic resonance (NMR) and mass spectra. Anticancer evaluation was carried out for the new derivatives against Hep-G2 (human liver carcinoma), MCF-7 (human breast carcinoma), Caco-2 (human colorectal adenocarcinoma) and HCT116 (human colorectal carcinoma) cell lines using doxorubicin as a reference drug. The biological results revealed that the compounds **12** and **14** exhibited more potent anticancer activity than the reference drug.

Keywords Chloropyranopyridine · Hydrazinyls · Pyrrolinoisoindolines · Aliphatic and aromatic amines · Sulfonamides · Anticancer evaluation

Introduction

Pyridine is one of the most popular *N*-heteroaromatics incorporated into the structures of many pharmaceuticals. It constitutes the basic core in many products such as drugs, vitamins and food. Literature survey indicates that a large number of

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pyridine derivatives are associated with diverse pharmacological properties, such as antimicrobial [1–3], antiviral [4], antifungal and antimycobacterial [5], anticancer [6], sodium channel modulation [7–12] and anticonvulsant [13] activities. Also, the naturally occurring B6-vitamins pyridoxine, pyrodoxal, pyridoxamine and code-carboxylase contain a pyridine nucleus [14]. In addition, 2-amino pyridine nucleus is widely used in the production of various drugs such as piroxicam and tenoxicam (non-steroidal anti-inflammatory and analgesic drugs), sulfapyridine (antibacterial drug) and tripelennamine (antipruritic and the first generation antihistaminic), and triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) has entered phase I and II clinical trials as an antitumor chemotherapeutic agent [15]. Furthermore, the fused bicyclic ring pyranopyridine constitutes an important class of heterocyclic compounds having diverse biological activities such as anti-allergic, anti-inflammatory, estrogenic [16, 17], anti-proliferative [18], anti-bacterial (including anti-tubercular) [19], antimyopic [20], hypotensive [21], anti-rheumatic [22] and antiasthmatic [23]. On the other hand, over the past few years, some research groupshave been interested in introducing a comprehensive study program towards the synthesis of thiophenes and their fused derivatives [24–27]. The importance of such compounds is based on their uses as anti-inflammatory [28, 29], antidiabetic [30], anti-hepatocellular carcinoma agents [31], protein kinase inhibitors [32], and antibacterial [33] and antiviral agents [34].

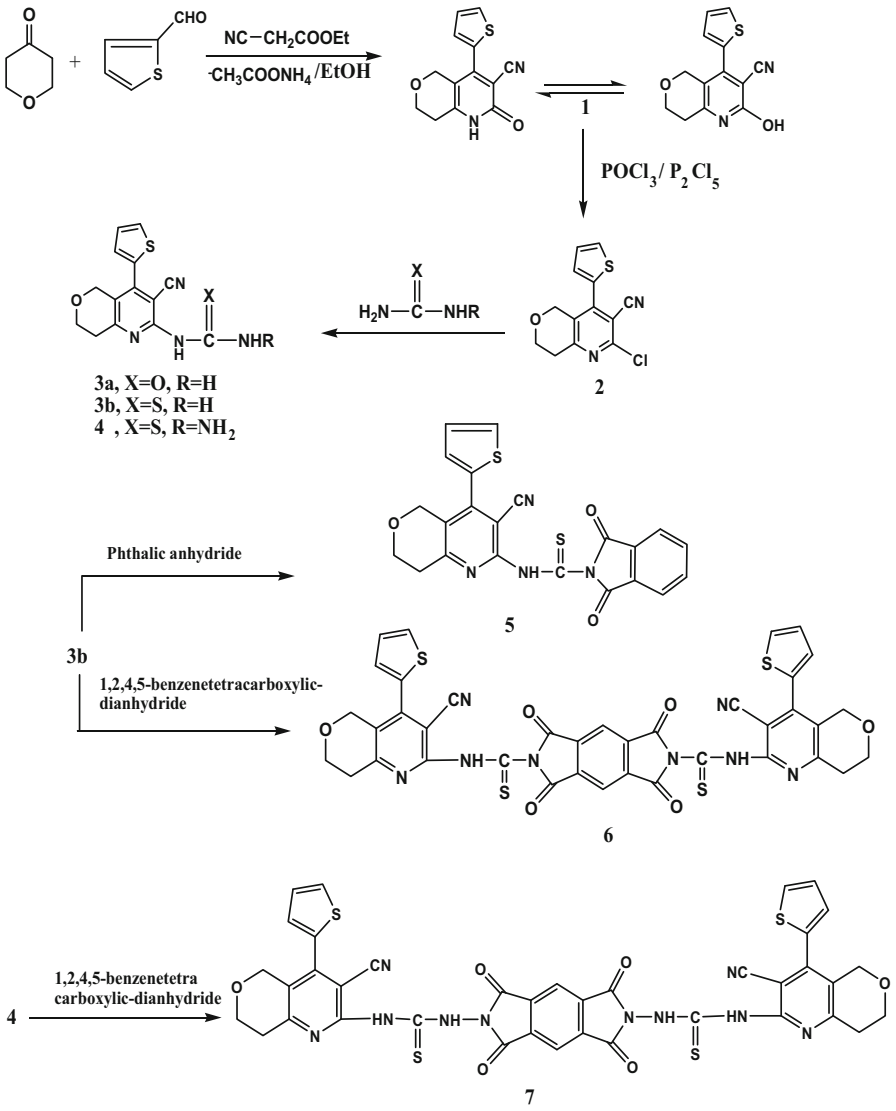
Cancer, a disease of the cell cycle, is one of the major health problems in the world. The effectiveness of many existing anticancer drugs is limited by their toxicity in normal, rapidly growing cells that may develop resistance to that drug. Another drawback is that most of the drugs currently in the market are not specific [35]. Considering this information, the development of newer chemotherapeutic scaffolds that selectively act on the target without side effects has become a primary objective of medicinal chemists. Depending on the above-mentioned criteria and with the ultimate aim of developing novel antitumor agents of significant activity and few drawbacks, it was thought worthwhile to synthesize new derivatives of pyranopyridine-thiophene scaffold clubbed with various heterocyclic/aromatic ring systems of reported anticancer activity, such as phthalic anhydride, benzenetetracarboxylic dianhydride, *p*-toluidine, 4-fluoroaniline, 9-acridine, 2-aminothiazole, morpholine and 4-methylpiperazine and different sulfa drugs [36–41]. The prepared compounds were evaluated as anticancer agents against Hep-G2 (human liver carcinoma), MCF-7 (human breast carcinoma), Caco-2 (human colorectal adenocarcinoma) and HCT116 (human colorectal carcinoma) cell lines. Compounds **12** and **14** exhibited more potency than that of the reference drug Doxorubicin against the tested carcinoma cell Lines.

Results and discussion

The synthesis of the newly pyrano[4,3-*b*]pyridinone was carried out according to the reported methods [42–44]. Heating an equimolar mixture of 4-pyranone, 2-thiophenecarboxaldehyde and ethyl cyanoacetate in the presence of ammonium acetate and ethanol in a one pot reaction afforded nicotinonitrile compound **1**. The

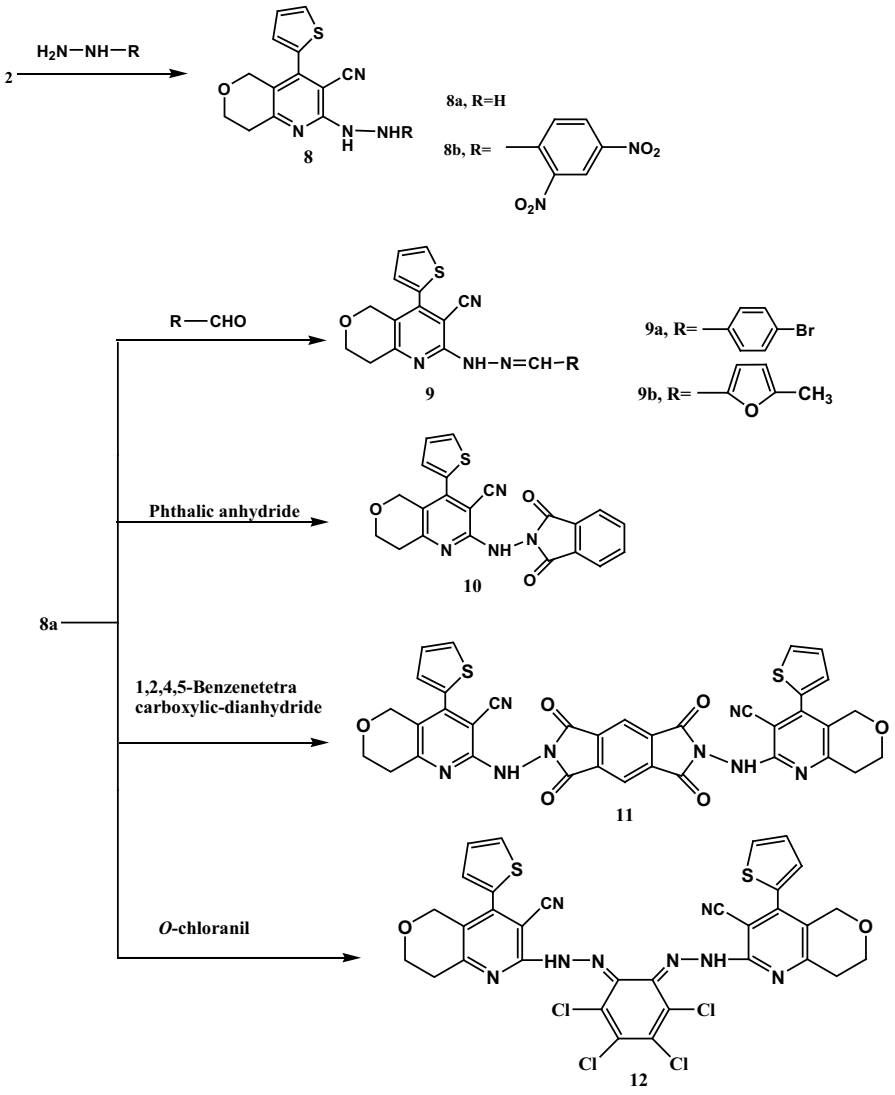
assignment of the structure was proved based on elemental analysis and spectral data. The IR spectrum showed characteristic absorption bands at 1655, 2224 and 3433 cm^{-1} due to C=O, CN and NH (keto-enol). The ^{1}H-NMR spectrum showed signals at δ 2.65, 3.85, 4.28 ppm (pyran-H), 7.20–7.80 ppm (thiophene-H), and an exchangeable signal with D_2O at δ 12.70 ppm assigned to NH. Its mass spectrum showed the molecular ion peak at *m/z* 258 (70 %). Refluxing **1** with a mixture of phosphorus oxychloride and phosphorus pentachloride gave the corresponding product 2-Chloro-4-(thiophen-2-yl)-7,8-dihydro-5*H*-pyrano[4,3-b]-pyridine-3-carbonitrile (**2**). IR spectrum of **2** showed the characteristic absorption band of CN at 2224 cm^{-1} and the disappearance of the C=O absorption band. As the chloro derivative **2** is a good precursor for the synthesis of different new heterocyclic compounds, due to its reactivity towards many types of nucleophiles, especially the nitrogen and oxygen nucleophiles [45, 46]. Thus, nucleophilic replacement of the chlorine atom of compound **2** was performed via reaction with urea, thiourea and thiosemicarbazide in boiling ethanol containing a catalytic amount of triethylamine to give the substituted derivatives **3a, b** and **4**, respectively (Scheme 1). IR spectra of the obtained compounds exhibited absorption bands at 3167–3367 cm^{-1} due to NH and NH_2 groups, 1679 cm^{-1} for C=O in case of compound **3a** and at 1432, 1162 cm^{-1} for C=S groups in case of compounds **3b** and **4**. The mass spectra showed the molecular ion peaks of compounds **3a**, **b** and **4** at *m/z* 300, 316 and 331. The ^{13}C-NMR spectrum of compound **4** showed the characteristic bands for CS and CN groups at δ 182.00 and 116.60 ppm (cf. Exp.). On the other hand, condensation of compound **3b** with different acid anhydrides, namely phthalic anhydride and benzenetetracarboxylic dianhydride, was performed in glacial acetic acid to give the corresponding thioamide derivatives **5** and **6** (Scheme 1). The structures of **5** and **6** were proven by elemental analysis and spectral date (cf. Exp.). Also, compound **4** was condensed with benzenetetracarboxylic dianhydride in glacial acetic acid to afford compound **7**. The IR spectrum of **7** showed absorption bands at 3194–3280 cm^{-1} (4NH), at 2220 cm^{-1} for CN, at 1725, 1665 cm^{-1} (anhydride C=O) and at 1375 cm^{-1} for C=S. The ^{1}H-NMR spectrum showed two characteristic singlets at δ 2.20 and 8.40 ppm for four NH groups and the disappearance of the NH_2 signlet. The mass spectrum showed the molecular ion peak at *m/z* 844 (75 %).

Reaction of **2** with hydrazine hydrate and/or 2,4-dinitrophenylhydrazine in ethanol afforded the hydrazide derivatives **8a, b**. The products showed absorption bands due to NH, NH_2 and 2NH in their IR spectra at 3195, 3350, 3182 and 3340 cm^{-1}, respectively. The ^{1}H-NMR spectrum of **8a** showed signals at δ 4.15 and 7.94 ppm for NH_2 and NH groups. The mass spectrum of **8a** showed the molecular ion peak at *m/z* 272 (100 %). Condensation of **8a** with the appropriate aldehydes, namely 4-bromobenzaldehyde and 5-methyltetrahydrofuran-2-carbaldehyde, gave the hydrazones **9a, b**. Compounds **9** showed the expected absorption bands due to NH and CN groups at the correct regions in their IR spectra. The ^{1}H-NMR showed the presence of azomethine proton (CH=N) and NH in the expected region (cf. Exp.). Mass spectra of **9** showed the expected molecular ion peaks, which were in agreement with their molecular formulae. Further condensation of **8a** with different anhydrides, namely phthalic anhydride and benzenetetracarboxylic dianhydride, in glacial acetic acid afforded compounds **10** and **11**. IR spectra of both compounds



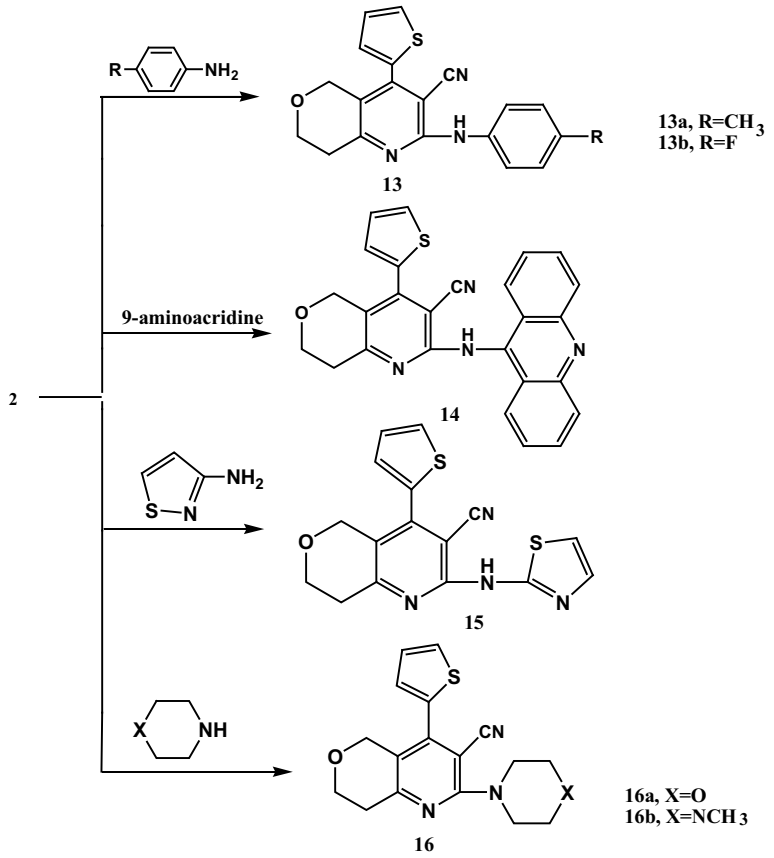
Scheme 1 Synthesis of compounds **1**–**7**

showed the carbonyl stretching bands at 1701 (2 × C=O) and 1775, 1699 (4 × C=O), in addition to NH and CN stretching bands at 3250, 3200 and 2217, 2220 cm^{-1}. The molecular ion peaks of **10** and **11** appeared at *m/z* 402 (25 %) and 726 (25 %) in their mass spectra. Also, compound **8a** reacted with *O*-chloranil in glacial acetic acid to afford **12** (Scheme 2). IR spectrum of **12** showed absorption bands due to NH and 2 × CN at their expected regions and the disappearance of NH_2. The mass spectrum of **12** showed the molecular ion peak at *m/z* 754 (100 %).



Scheme 2 Synthesis of compounds **8–12**

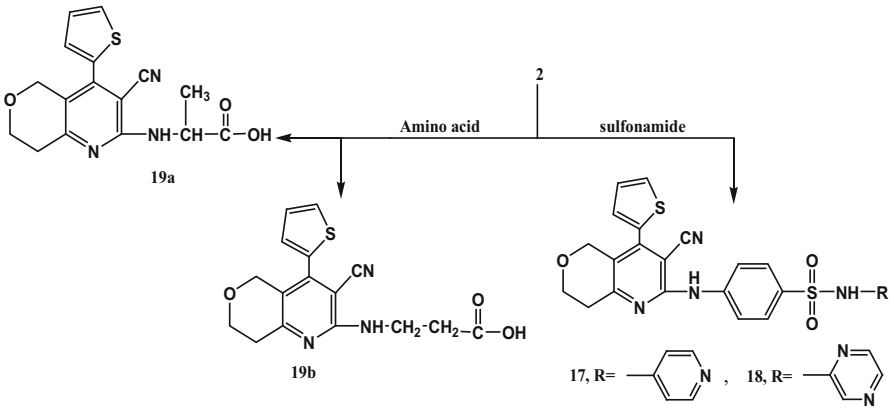
Upon nucleophile attack of compound **2** with different aromatic, heterocyclic and secondary alicyclic amines such as *p*-toluidine, 4-fluoroaniline, 9-acridine, 2-aminothiazole, morpholine, and 4-methylpiperazine, compounds **13–16** (Scheme 3) were obtained. IR spectra of compounds **13–15** revealed the appearance of new absorption bands in the range of 3250–3177 and 2220–2229 cm^{-1} due to NH and CN groups. The ^{1}H-NMR spectrum of compound **16a** displayed the methylene protons of N-$(CH_2)_2$ and O-$(CH_2)_2$ of the morpholine ring as two singlets at δ 3.77 and 2.86 ppm, while the ^{1}H-NMR spectrum of methylpiperazine **16b** showed two singlets at δ 2.65 and 3.35 ppm



Scheme 3 Synthesis of compounds **13–16**

for eight piperazine protons, while the methyl group appeared as a singlet at δ 2.40 ppm, in addition to the expected signals of the parent compound. The ^{13}C-NMR spectrum of compound **16b** showed signals at δ 49.20 and 54.50 ppm, assigned to the piperazine methylene carbons. The methyl group appeared at δ 43.50 ppm. The CN and aromatic carbons appeared at 116.50, 124.50–162.50 ppm, while the aliphatic carbons appeared at δ 36.50–73.50 ppm (cf. Exp.). The mass spectra of compounds **13–16** showed molecular ion peaks corresponding to their expected molecular formulae.

Condensation of compound **2** with sulfonamides, namely sulfapyridine and sulfadiazine, in absolute ethanol containing a catalytic amount of triethylamine as a basic medium afforded the corresponding sulfonamide derivatives **17** and **18**. The IR spectra of the latter compounds exhibited characteristic vibrational bands at 3356–3243, 2222–2223 and 1371–1157 cm^{-1} assigned to NH, CN and SO_2 groups. The mass spectra of **17** and **18** showed the molecular ion peaks at *m/z* 489 (83 %) and 490 (56 %). Compound **2** also reacted with different amino acids, δ-alanine and δ-alanine, in the presence of sodium carbonate as a catalytic base at pH 9–9.5



Scheme 4 Synthesis of compounds **17–19**

[44, 47] and afforded pyranopyridine amino acid derivatives **19a, b** (Scheme 4). IR spectrum of compound **9b** revealed absorption bands for NH, OH and C=O. Its ^{1}H-NMR spectrum showed the presence of OH, NH and 2 × CH_2 (cf. Exp.).

Biological activity

In vitro antitumor activity

Figure 1 shows the antitumor activities of the synthesized compounds against HepG2 and HCT 116 cancer cells in comparison with doxorubicin. These results revealed that compounds **1**, **2**, **7**, **9a**, **9b**, **10**, **11**, **12**, **14** and **19a** showed comparable or better activities with respect to the positive control. However, the rest of the compounds showed weak antitumor activities. In addition, Fig. 2 shows the antitumor activity of

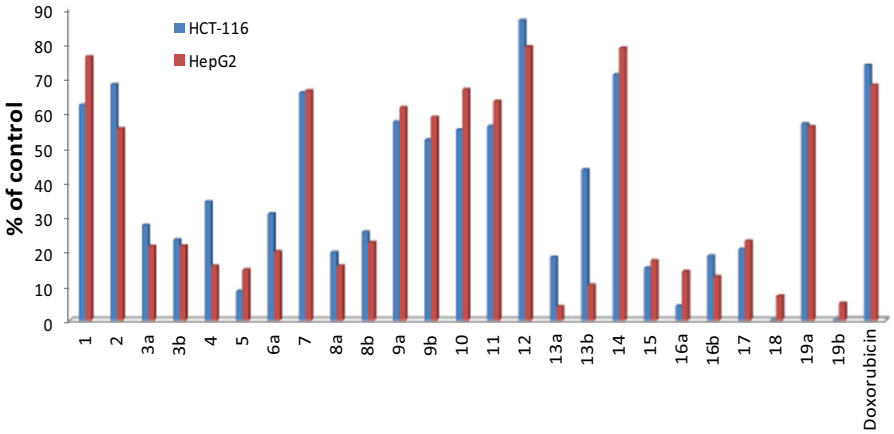


Fig. 1 The antitumor activity of the synthesized compounds against HepG2 and HCT 116 compared to doxorubicin

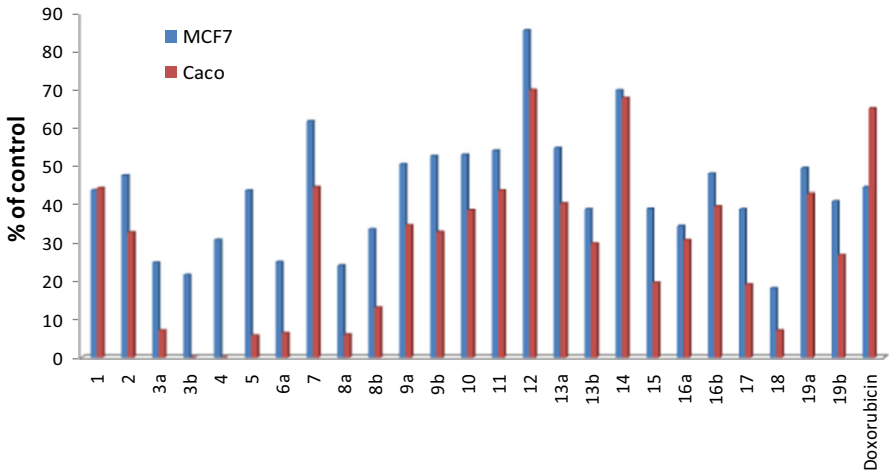


Fig. 2 The antitumor activity of the synthesized compounds against MCF-7 and Caco-2 compared to doxorubicin

the same synthesized compounds against MCF-7 and Caco-2. The results obtained showed that compounds **1**, **2**, **7**, **9a**, **9b**, **10**, **11**, **12**, **13a**, **14**, **16b** and **19a** showed comparable antitumor activities with respect to doxorubicin, and the rest of the compounds did not show significant cytotoxicity activities. Furthermore, additional experiments have been conducted in order to measure the half maximal inhibitory concentration (IC_{50}) for the most promising compounds (Table 1).

Structure activity relationship (SAR)

From the above obtained results (Figs. 1, 2; Table 1), we can conclude that the basic nucleus (thiophen-2-yl)-7,8-dihydro-5*H*-pyrano-[4,3-*b*]-3-carbonitrile conjugated

Table 1 The IC_{50} values of the selected compounds tested for their antitumor activities against HepG-2, HCt-116, MCF-7 and Caco-2 using MTT assay

Compound no.	IC_{50} (μM)			
	HepG2	HCT-116	MCF-7	Caco-2
1	68.1	56.3	87.8	100.3
2	69.4	52.6	77.5	112.1
7	60.1	56.2	71.7	88.0
9a	57.2	65.2	88.2	114.7
9b	58.7	65.9	80.8	116.4
10	50.8	68.1	73.7	136.2
11	49.7	72.4	92.2	110.0
12	60.8	58.8	30.7	86.2
14	49.1	52.5	55.3	52.8
19a	66.5	70.1	67.4	118.8
Doxorubicin	67.9	73.5	93.2	96.3

with different substituted amines at position 2 (compounds **3**–**19**) is essential for the anticancer activities against liver (HepG2), colorectal (HCT 116), breast (MCF-7) and colorectal adenocarcinoma (Caco-2) human carcinoma cell lines using doxorubicin as a reference drug. The most potent activity against all the tested cell lines that was higher than that of the reference drug (growth inhibition 75 %, IC_{50} 71.8 μM) was gained by compound **12** (growth inhibition >85 %, IC_{50} 60.8, 58.8, 30.7, 86.2 μM). This is may be due to the presence of two molecules of hydrazinylpyranopyridine conjugated with *O*-chloranil. Also, compound **14** (growth inhibition >80 %, IC_{50} 49.1, 52.2, 55.3, 52.8 μM) was found to be more potent than the reference drug due to the presence of acridine ring at position-2. The replacement of the electron releasing group C=O of compound **1** with the electron withdrawing group C–Cl (compound **2**) slightly reduced the anticancer activity. Interestingly, the conjugation of three molecules via thioamide linkages (compound **7**) improved its activity. An improvement in activity was observed upon replacement of the chlorine atom of compound **2** with δ-alanine to give compound **19a** (IC_{50} 66.5, 70.1, 67.4, 118.8 μM). While compounds **9a**, **9b** and **10** showed anticancer activity against two cell lines only (HepG2, HTC-116), this may be due to the presence of Schiff bases and phthalic ring.

Experimental

All melting points are uncorrected and were recorded on an open glass capillary tube using an Electrothermal IA 9100 digital melting point apparatus (Shimadzu, Japan). Elemental micro-analyses were carried out at Micro Analytical Unit, Central Service Lab (CSL), National Research Centre (NRS), using Vario Elementar (Germany), and were found within 0.4 % of the theoretical values. IR spectra were recorded on Jasco FT/IR-6100, Fourier Transform Infrared spectrometer (Japan), while ^{1}H- and ^{13}C-NMR spectra were obtained using JEOL EX-270 & 500 (UK), using available solvent and TMS as internal standard (δ) and the mass spectra were recorded on a Finnigan Mat SSQ-7000 mass spectrometer (USA) at CSL, NRS. TLC on silica gel precoated aluminum sheets type 60-F254 (Mercek, Darmstadt, Germany) was also used with chloroform/methanol (5:1) as solvent.

2-Oxo-4-(thiophen-2-yl)-1,5,7,8-tetrahydro-1*H*-pyrano[4,3-b]pyridine-3-carbonitrile (1)

A mixture of 4-pyranone (0.01 mol), thiophene 2-carboxaldehyde (0.01 mol), ethyl cyanoacetate (0.01 mol) and ammonium acetate (0.08 mol) in ethanol (30 mL) was refluxed for 6 h. The obtained precipitate was filtered off, washed successively with water and recrystallized from dioxane.

Yield: 84 %; mp 254–256 °C. IR (KBr): 1655 (C=O), 2224 (CN), 2926 (CH, Aliph), 3060 (CH, Ar) 3433 (NH) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.65 (2H, m, pyran-H), 3.85 (2H, m, pyran-H), 4.28 (2H, s, pyran-H), 7.20 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.35 (1H, m, thiophen-H), 7.80 (1H, d, $J = 5.4$ Hz, thiophene-H), 12.70 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50,

95.00, 116.00, 116.90, 125.50, 127.50, 127.75, 137.50, 147.60, 155.50, 162.50. MS *m/z* (%): 258 (100). Anal. Calcd for $C_{13}H_{10}N_2O_2S$ (258.3): C, 60.45, H, 3.90, N, 10.85, O, 12.39, S, 12.41. Found: C, 60.40; H, 3.92, N, 10.88; S, 12.50.

2-Chloro-4-(thiophen-2-yl)-7,8-dihydro-5*H*-pyrano[4,3-b]pyridine-3-carbonitrile (2)

A mixture of compound **1** (0.01 mol), phosphorus oxychloride (10 mL) and phosphorus pentachloride (0.5 gm) was heated on boiling water bath for 8 h. After the reaction was completed, the solution mixture was cooled and poured gradually onto crushed ice. The obtained precipitate was filtered off and dried to obtain the chloro-analogue **2**.

Yield: 72 %; mp 185–187 °C. IR (KBr): 2224 (CN), 2920 (CH-Aliph), 3060 (CH-Ar) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.70 (2H, m, pyran-H), 3.85 (2H, m, pyran-H), 4.65 (2H, s, pyran-H), 7.20 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.35 (1H, m, thiophene-H), 7.80 (1H, d, $J = 5.4$ Hz, thiophene-H). ^{13}C NMR (DMSO-d_6): δ 35.60, 64.90, 63.50, 105.50, 116.60, 125.50, 127.20, 127.30, 128.50, 138.50, 149.50, 152.20, 164.50. MS *m/z* (%): 276 (100), 278 (30). Anal. Calcd for $C_{13}H_9ClN_2OS$ (276.74): C, 56.42, H, 3.28, Cl, 12.81, N, 10.12, O, 5.78, S, 11.59. Found: C, 56.30, H, 3.35, N, 10.00; S, 11.50.

General procedure for synthesis of compounds 3a, b and 4

A mixture of compound **2** (0.01 mol) and urea, thiourea and/or thiosemicarbazide (0.01 mol) in absolute ethanol (30 mL) containing few drops of triethylamine was heated under reflux for 12 h. The mixture was concentrated and the obtained precipitate was filtered off and recrystallized from ethanol to obtain the desired derivatives.

*1-[(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)]urea (**3a**)*

Yield: 78 %; mp 212–214 °C. IR (KBr): 3436 (NH_2), 3192 (NH), 2217 (CN), 1679 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.90 (2H, m, pyran-H), 3.25 (2H, m, pyran-H), 3.95 (2H, s, pyran-H), 5.49 (2H, bs, NH_2, D_2O exchangeable), 7.20 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.38 (1H, m, thiophene-H), 7.80 (1H, d, $J = 5.4$ Hz, thiophene-H), 9.21 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.50, 73.50, 85.00, 116.90, 120.00, 125.50, 127.40, 127.70, 138.10, 151.00, 162.00, 162.50, 176.50. MS *m/z* (%): 300 (100). Anal. Calcd for $C_{14}H_{12}N_4O_2S$ (300.34): C, 55.99, H, 4.03, N, 18.65, O, 10.65, S, 10.68. Found: C, 55.50, H, 4.25, N, 18.65, 10.45.

*1-[(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)]thiourea (**3b**)*

Yield: 85 %; mp 235–237 °C. IR (KBr): 3367–3167 (NH, NH2), 2213 (CN), 1432 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.90 (2H, m, pyran-H), 3.50 (2H, m, pyran-H), 4.25 (2H, s, pyran-H), 5.49 (2H, bs, NH_2, D_2O exchangeable), 7.22 (1H, d,

$J = 5.4$ Hz, thiophene-H), 7.38 (1H, m, thiophene-H), 7.80 (1H, d, $J = 5.4$ Hz, thiophene-H), 9.26 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.45, 73.55, 85.20, 116.50, 120.00, 125.50, 127.45, 127.75, 138.20, 151.20, 162.00, 162.50, 182.00. MS *m/z* (%): 316 (42). Anal. Calcd for $C_{14}H_{12}N_5OS_2$ (316.4): C, 53.14, H, 3.82, N, 17.71, O, 5.06, S, 20.27. Found: C, 53.25, H, 3.82, N, 17.75, S, 20.50.

*N-(3-cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)hydrazinecarbo-thioamide (**4**)*

Yield: 75 %; mp 240–242 °C. IR (KBr): 3372–3179 (2NH, NH_2), 2220 (CN), 1162 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.68 (2H, m, pyran-H), 3.70 (2H,m, pyran-H), 4.35 (2H, s, pyran-H), 4.45 (2H, s, NH_2, D_2O exchangeable), 7.00 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.22 (1H, m, thiophene-H), 7.62 (1H, d, $J = 5.4$ Hz, thiophene-H), 8.50, 9.20 (2H, 2s, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.40, 73.50, 83.50, 116.60, 124.20, 125.50, 127.40, 127.70, 138.00, 151.20, 154.40, 161.50, 182.00. MS *m/z* (%): 331 (30). Anal. Calcd for $C_{14}H_{13}N_5OS_2$ (331.42): C, 50.74, H, 3.95, N, 21.13, O, 4.83, S, 19.35. Found: C, 50.55, H, 4.00, N, 21.15. S, 19.00.

*N-(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-1,3-dioxoiso-indoline-2-carbothioamide (**5**)*

To a solution of compound **3b** (0.01 mol) in glacial acetic acid (30 mL), phthalic anhydride (0.01 mol) was added. The mixture was refluxed for 8 h, then poured onto ice water. The formed precipitate was filtered off, washed with water and crystallized from dioxane to give the titled compound.

Yield: 72 %; mp >300 °C. IR (KBr): 3410 (NH), 2220 (CN), 1752, 1690 (2 × C=O), 1140 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.95 (2H, m, pyran-H), 3.20 (2H, m, pyran-H), 4.25 (2H, m, pyran-H), 7.20–8.00 (7H, m, Ar–H), 9.11 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.50, 73.50, 85.50, 116.20, 120.00, 125.50, 127.50, 127.75, 127.90, 132.20, 132.50, 138.20, 151.00, 162.20, 162.60, 174.20, 182.50. MS *m/z* (%): 446 (100). Anal. Calcd for $C_{22}H_{14}N_4O_3S_2$ (446.5): C, 59.18 H, 3.16, N, 12.55, O, 10.75, S, 14.36. Found: C, 59.20, H, 3.18, N, 12.5, S, 14.00.

General procedure for synthesis of compounds 6, 7

A mixture of either compound **3b** and/or compound **4** (0.02 mol) and 1,2,4,5-benzene-tetracarboxylic dianhydride (0.01 mol) in glacial acetic acid (50 mL) was refluxed for 5 h. The formed precipitate was filtered on hot, dried and crystallized from dioxane.

*[N-(3-cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-2-carbothioamide-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrolo[3,4-f]isoindol-2-yl]-N-(3-cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-2-carbothioamide (**6**)*

Yield: 68 %; mp 285–287 °C. IR (KBr): 3199 (2 × NH), 2221 (2 × CN), 1772, 1693 (4 × C=O, anhydride), 1377 (2 × C=S) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.85 (4*H*, m, pyran-H), 3.84 (4*H*, m, pyran-H), 4.65 (4*H*, s, pyran-H), 7.25–7.85 (6*H*, m, thiophene-Hs), 8.99 (2H, s, Ar–H), 11.85 (2H, s, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.50, 73.65, 85.00, 116.55, 120.00, 125.20, 125.50, 127.45, 127.75, 135.40, 138.00, 151.50, 154.50, 161.50, 172.50, 182.00. MS *m/z* (%): 814 (42). Anal. Calcd for $C_{38}H_{22}N_8O6S_4$ (814.89): C, 56.01, H, 2.72, N, 13.75, O, 11.78, S, 15.74. Found: C, 56.20, H, 2.65, N, 13.78, S,15.70.

*[N-(3-cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-2-carbothiourea-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrolo[3,4-f]isoindol-2-yl]-N-(3-cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-2-carbothiourea (**7**)*

Yield: 55 %; mp 262–264 °C. IR (KBr): 3194, 3280 (4 × NH), 2222 (2 × CN), 1725, 1665 (4 × C=O, anhydride), 1375 (2 × C=S) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.20 (2H, s, 2 × NH, D_2O exchangeable), 2.85 (4H, m, pyran-Hs), 3.80 (4H, m, pyran-H), 4.62 (4H, s, pyran-H), 7.27–7.88 (2 × 3H, m, thiophene-H), 8.25 (2H, s, Ar–H), 8.50 (s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 83.80, 116.60, 124.20, 125.30, 125.50, 127.40, 127.70, 135.40, 138.00, 151.20, 155.50, 161.20, 174.50, 182.00. MS *m/z* (%): 844 (75). Anal. Calcd for $C_{38}H_{24}N_{10}O6S_4$ (844.92): C, 54.02, H, 2.86, N, 16.58, O, 11.36, S, 15.18. Found: C, 54.20, H, 2.85, N, 16.70, S, 15.25.

General procedure for synthesis of compounds 8a,b

A mixture of compound **2** (0.01 mol) and hydrazine hydrate 99 % (5 mol) or 2,4-dinitrophenyl-hydrazine (0.01 mol) in ethanol (30 mL) was stirred under reflux for 8 h. The formed precipitate was filtered off, dried and recrystallized from dioxane.

*2-Hydrazino-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**8a**)*

Yield: 84 %; mp 235–237 °C. IR (KBr): 3350 (NH_2), 3195 (NH), 3050 (Ar–CH), 2925 (Aliph-CH), 2214 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.22 (2H, m, pyran-H), 3.32 (2H, m, pyran-H), 4.15 (2H, br, NH_2, D_2O exchangeable), 4.42 (2H, s, pyran-H), 7.32 (1H, d, J = 5.4 Hz, thiophene-H), 7.33 (1H, m, thiophene-H), 7.85 (1H, d, J = 5.4 Hz, thiophene-H), 7.94 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.20, 64.50, 73.50, 83.50, 116.50, 124.50, 125.50, 127.50, 127.80, 138.50, 151.00, 155.50, 161.00. MS *m/z* (%): 272 (100). Anal. Calcd for $C_{13}H_{12}N_4OS$ (272.33): C, 57.34, H, 4.44, N, 20.57, O, 5.88, S, 11.77. Found: C, 57.35, H, 4.40, N, 20.57, S, 11.82.

*2-(2-(2,4-Dinitrophenyl)hydrazino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]-pyridine-3-carbonitrile (**8b**)*

Yield: 73 %; mp 285–287 °C. IR (KBr): 3340, 3187 (2NH), 2990 (Ar–CH), 2928 (Aliph-CH), 2240 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.25 (2H, m, pyran-H), 3.35 (2H, m, pyran-H), 4.20 (1H, s, NH, D_2O exchangeable), 4.62 (2H, s, pyran-H), 7.20–8.95 (7H, 6Ar-H, 1NH, D_2O, m, exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.45, 64.60, 73.50, 83.60, 116.20, 116.60, 120.50, 124.50, 125.50, 127.50, 127.80, 130.50, 134.50, 138.10, 139.20, 151.20, 155.00, 162.20, 162.50. MS *m/z* (%): 438 (70). Anal. Calcd for $C_{19}H_{14}N_6O_5S$ (438.43): C, 52.00, H, 3.19, N, 19.16, O, 18.25, S, 7.30. Found: C, 52.15, H, 3.19, N, 19.00, S, 7.35.

General procedure for synthesis of compounds 9a, b

A solution of the hydrazinyl derivative **8a** (0.01 mol) and the appropriate aldehydes, namely 4-bromoenzaldehyde and 5-methyl-2-furaldehyde (0.01 mol), in glacial acetic acid (30 mL) containing a few drops of piperidine was refluxed for 3 h. The reaction mixture was kept at room temperature overnight, and the separated solid was filtered off, dried and recrystallized from acetic acid.

*2-(2-(4-Bromobenzylidene)hydrazinyl)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano [4,3-b]-pyridine-3-carbonitrile (**9a**)*

Yield: 55 %; mp 263–265 °C. IR (KBr): 3190 (NH), 3083 (Ar–CH), 2917 (Aliph-CH), 2220 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.99 (2H, m, pyran-H), 3.75 (2H, m, pyran-H), 4.65 (2H, s, pyran-H), 7.25–7.75 (7H, m, Ar–H), 8.20 (1H, s, N=CH), 10.50 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 116.20, 124.20, 125.20, 125.50, 127.30, 127.80, 131.40, 131.80, 132.80, 138.20,151. 162.20, 162.70, 143.50. MS *m/z* (%): 439 (40). Anal. Calcd for $C_{20}H_{15}BrN_4OS$ (439.33): C, 54.68, H, 3.44, Br, 18.19, N, 12.75, O, 3.64, S, 7.30. Found: C, 54.65, H, 3.55, N, 12.70, S, 7.15.

*2-(2-((5-Methylfuran-2-yl)methylene)hydrazinyl)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**9b**)*

Yield: 48 %; mp 251–253 °C. IR (KBr): 3114 (NH), 3083 (Ar–CH), 2917 (Aliph-CH), 2220 (CN) cm^{-1}. ^1H NMR (DMSO-d_6):δ 2.29 (3H, s, -CH3), 2.99 (2H, m, pyran-H), 3.70 (2H, 2H, pyran-H), 4.62 (2H, s, pyran-H), 6.33–7.81 (6H, m, 6Ar–H, 1H, N=CH), 8.22 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 13.60, 36.50, 64.60, 73.50, 85.20, 108.00, 112.50, 116.60, 124.00, 125.50, 127.50, 127.75, 136.60, 138.10, 148.00, 151.00, 152.50, 162.20, 162.50. MS *m/z* (%): 364 (55). Anal. Calcd for $C_{19}H_{16}N_4O_2S$ (364.42): C, 62.62, H, 4.43, N, 15.37, O, 8.78, S, 8.80. Found: C, 62.65, H, 4.40, N, 15.35, S, 8.70.

General procedure for synthesis of compounds 10, 11

To a solution of compound **8a** (0.01 mol) and/or (0.02 mol) in glacial acetic acid (30 mL), phthalic anhydride and/or 1,2,3,4-benzenetetracarboxylic dianhydride (0.01 mol), respectively, was added. The mixture was refluxed for 8 h, then poured onto ice/water. The formed precipitate was filtered off, washed with water and recrystallized from dioxane.

*2-(1,3-Dioxoisoindolin-2-ylamino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**10**)*

Yield: 65 %; mp >300 °C. IR (KBr): 3250 (NH), 2217 (CN), 1701 (2 × C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.98 (2H, 2H, pyran-H), 3.78 (2H, m, pyran-H), 3.99 (1H, s, NH, D_2O exchangeable), 4.60 (2H, s, pyran-H), 6.85–7.85 (7H, m, Ar–H).^{13}C NMR (DMSO-d_6): δ 36.50, 64.80, 73.50, 83.50, 116.20, 124.20, 125.50, 127.50, 127.60, 127,90, 132.00, 132.30, 138.20, 151.50, 155.70, 161.00, 167.20 (2 × C=O). MS *m/z* (%): 402 (25). Anal. Calcd for $C_{21}H_{14}N_4O_3S$ (402.43): C, 62.68, H, 3.51, N, 13.92, O, 11.93, S, 7.97. Found: C, 62.52, H, 3.55, N, 13.95, S, 8.02.

*[N-(2-amino-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-3-carbonitrile-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrolo[4,3-f]isoindol-2-yl]-N-(2-amino-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)-3-carbonitrile (**11**)*

Yield: 76 %; mp 262–264 °C. IR (KBr): 3200 (2 × NH), 1775, 1698 (4 × C=O), 2220 (2 × CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.99 (4H, m, pyran-H), 3.75 (4H, m, pyran-H), 4.20 (2H, s, 2NH, D_2O exchangeable), 4.65 (4H, s, pyran-H), 6.85–7.20 (6H, m, thiophen-H), 7.98 (2H, s, Ar–H). H).^{13}C NMR (DMSO-d_6): δ 36.50, 64.50, 73.50, 83.50, 116.20, 124.20, 125.25, 125.50, 127.50, 127.75, 135.50, 138.00, 151.20, 155.50, 161.20, 167.50. MS *m/z* (%): 726 (25). Anal. Calcd for $C_{36}H_{22}N_8O_6S_2$ (726.74): C, 59.50, H, 3.05, N, 15.42, O, 13.21, S, 8.82. Found: C, 59.52, H, 3.15, N, 15.40, S, 8.80.

*2,2′-(2Z,2′Z)-2,2′-(Perchlorocyclohexa-3,5-diene-1,2-diylidene)bis(hydrazin-1-yl-2-ylidene)-bis(4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**12**)*

A mixture of the hydrazinyl derivate **8a** (0.02 mol) and *O*-chloranil (0.01 mol) in glacial acetic acid (30 mL) was refluxed for 8 h, then poured onto ice/water. The formed precipitate was filtered off, washed with water and recrystallized from methanol/dioxane.

Yield: 73 %; mp >300 °C. IR (KBr): 3200 (2NH), 2220 (2CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.01 (4H, m, pyran-H), 3.76 (4H, m, pyran-H), 4.65 (4H, s, pyran-H), 6.98–7.65 (8H, m, 6H, thiophene-H, 2H, 2 × NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 116.20, 120.20, 123.60, 125.50, 127.50, 127.75, 132.00, 138.00, 151.20, 155.00, 162.20, 162.60. MS *m/z* (%): 754 (100),

756 (72). Anal. Calcd for $C_{32}H_{20}Cl_4N_8O_2S_2$ (754.50): C, 50.94, H, 2.67, Cl, 18.80, N, 14.85, O, 4.24, S, 8.50. Found: C, 50.95, H, 2.69, N, 14.80, S, 8.22.

General procedure for synthesis of compounds 13–15

A mixture of the chloropyridine derivative **2** (0.01 mol), aromatic and hetero amines, namely *p*-toluidine, 4-fluoroaniline, 9-aminoacridine or 2-aminothiazole (0.01 mol), in ethanol/acetic acid (30 mL) was heated under reflux for 6 h. The reaction mixture was concentrated and the obtained precipitate was filtered off and recrystallized from acetic acid or dioxane.

*2-(p-Toluidino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**13a**)*

Yield: 65 %; mp 220–222 °C. IR (KBr): 3200 (NH), 2220 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.16 (3H, s, CH_3), 2.92 (2H, m, pyran-H), 3.52 (2H, m, pyran-H), 4.60 (2H, s, pyran-H), 6.95–7.90 (7H, m, Ar–H), 9.80 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 34.50, 36.50, 64.60, 73.50, 85.50, 116.20, 116.40, 120.20, 125.50, 127.50, 127.75, 128.50, 129.50, 138.20, 142.50, 149.50, 151.50, 162.50. MS *m/z* (%): 347 (85). Anal. Calcd for $C_{20}H_{17}N_3OS$ (347.43): C, 69.14, H, 4.93, N, 12.09, O, 4.61, S, 9.23. Found: C, 69.00, H, 5.02, N, 12.23, S, 9.25.

*2-(4-Fluorophenylamino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b] pyridine-3-carbo-nitrile (**13b**)*

Yield: 66 %; mp 238–240 °C. IR (KBr): 3250 (NH), 2220 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.95 (2H, m, pyran-H), 3.55 (2H, m, pyran-H), 4.66 (2H, s, pyran-H), 6.66–7.77 (7H, m, Ar–H), 9.68 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 116.50, 117.00, 118.00, 124.30, 125.50, 127.50, 127.80, 138.20, 138.50, 149.50, 151.00, 152.90, 162.70. MS *m/z* (%): 351 (100). Anal. Calcd for $C_{19}H_{14}FN_3OS$ (351.40): C, 64.94, H, 4.02, F, 5.41, N, 11.96, O, 4.55, S, 9.12. Found: C, 64.95, H, 4.22, F, 5.40, N, 12.10, S, 9.00.

*2-(Acridin-9-ylamino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbo-nitrile (**14**)*

Yield: 66 %; mp 198–200 °C. IR (KBr): 3177 (NH), 2220 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.95 (2H, m, pyran-H), 3.55 (2H, m, pyran-H), 4.67 (2H, s, pyran-H), 7.20–8.25 (11H, m, Ar–H), 9.75 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 115.00, 116.20, 120.00, 124.80, 125.50, 126.25, 127.50, 127.75, 129.20, 129.40, 138.00, 148.20, 149.30, 149.75, 151.50, 162.50. MS *m/z* (%): 434 (35). Anal. Calcd for $C_{26}H_{18}N_4OS$ (434.51): C, 71.87, H, 4.18, N, 12.89, O, 3.68, S, 7.38. Found: C, 71.85, H, 4.20, N, 12.15, S, 7.40.

*2-(Thiazol-2-ylamino)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbo-nitrile (**15**)*

Yield: 70 %; mp 191–193 °C. IR (KBr): 3199 (NH), 2229 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.02 (2H, m, pyran-H), 3.80 (2H, m, pyran-H), 4.66 (2H, s, pyran-H), 7.23–7.95 (5H, m, Ar-H), 12.75 (1H, s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 116.90, 118.00, 124.20, 125.50, 127.50, 127.60, 138.20, 138.50, 149.20, 151.00, 158.90, 162.60. MS *m/z* (%): 340 (70). Anal. Calcd for $C_{16}H_{12}N_4OS_2$ (340.42): C, 56.45, H, 3.55, N, 16.46, O, 4.70, S, 18.84. Found: C, 56.45, H, 3.52, N, 16.49, S, 18.80.

General procedure for synthesis of compounds 16a, b

A mixture of compound **2** (0.01 mol) and the appropriate 2ry amine morpholine and/or 4-methyl piperazine (0.01 mol) in absolute ethanol (30 mL) was heated under reflux for 12 h. The reaction mixture was concentrated and the obtained precipitate was filtered off and crystalized from ethanol.

*2-Morpholino-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridine-3-carbonitrile (**16a**)*

Yield: 75 %; mp 266–268 °C. IR (KBr): 2216 (CN), 2966 (Aliph-CH), 3060 (Ar–CH) cm^{-1}. ^1H-NMR (DMSO-d_6): δ 2.86 (4H, m, O-$(CH_2)_2$), 3.09 (2H, m, pyran-H), 3.73 (2H, m, pyran-H), 3.77 (4H, m, N-$(CH_2)_2$), 4.43 (2H, s, pyran-H), 7.23 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.28 (1H, m, thiophene-H), 7.85 (1H, d, $J = 5.4$ Hz, thiophene-H). ^{13}C NMR (DMSO-d_6): δ 36.50, 45.25, 64.60, 66.25, 73.50, 85.50, 116.50, 120.00, 125.50, 127.50, 127.75, 138.00, 151.00, 162.20, 162.50. MS *m/z* (%): 327 (42). Anal. Calcd for $C_{17}H_{17}N_3O_2S$ (327.40): C, 62.36, H, 5.23, N, 12.83, O, 9.77, S, 9.79. Found: C, 62.40, H, 5.20, N, 12.82, S, 9.75.

*2-(4-Methylpiperazin-1-yl)-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b] pyridine-3-carbonitrile (**16b**)*

Yield: 68 %; mp 272–274 °C. IR (KBr): 2219 (CN), 2963 (Aliph-CH) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.40 (3H, s, CH_3), 2.65 (4H, m, piperazine-H), 3.10 (2H, m, 2H, pyran-H), 3.35 (4H, m, piperazine-H), 3.75 (2H, m, pyran-H), 4.56 (2H, s, pyran-H),7.23 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.28 (1H, m, thiophene-H), 7.85 (1H, d, $J = 5.4$ Hz, 1H, thiophene-H).). ^{13}C NMR (DMSO-d_6): δ 36.50, 43.50, 49.20, 54.50, 64.60, 73.50, 85.50, 116.50, 124.50, 125.50, 127.50, 127.75, 138.20, 151.00, 162.20, 162.60. MS *m/z* (%): 340 (30). Anal. Calcd for $C_{18}H_{20}N_4OS$ (340.44): C, 63.50, H, 5.92, N, 16.46, O, 4.70, S, 9.42. Found: C, 63.48, H, 5.95, N, 16.45, S, 9.50.

General procedure for synthesis of compounds 17, 18

A mixture of the chloro compound **2** (0.01 mol) and the appropriate sulfonamide, namely sulfapyridine and sulfapyrazine (0.01 mol), in ethanol (30 mL) containing

triethylamine (5 mL) was refluxed for 10 h. The reaction mixture was cooled and poured onto cold water, then acidified by dil. HCl. The solid obtained was collected by filtration, dried and crystallized from dioxane and/or acetic acid.

*4-(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-ylamino)-N-(pyridine-4-yl)benzenesulfonamide (**17**)*

Yield: 85 %; mp 220–222 °C. IR (KBr): 3313, 3243 (2NH), 2222 (CN), 1370 (S=O, asym), 1125 (S=O, sym) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.05 (2H, m, pyran-H), 3.82 (2H, m, pyran-H), 4.65 (2H, s, pyran-H), 6.75–8.00 (11H, m, Ar–H), 8.50, 8.92 (2H, 2s, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 36.50, 64.60, 73.50, 85.50, 110.50, 116.20, 116.75, 119.40, 125.50, 127.30, 127.60, 128.20, 129.50, 138.20, 146.50, 149.25, 150.30, 151.00, 155.20, 162.50. MS *m/z* (%): 489 (83). Anal. Calcd for $C_{24}H_{19}N_5O_3S_2$ (489.57): C, 58.88, H, 3.91, N, 14.31, O, 9.80, S, 13.10. Found: C, 58.90, H, 3.93, N, 14.25, S, 13.30.

*4-(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-ylamino)-N-(pyrazin-2-yl)benzenesulfonamide (**18**)*

Yield: 75 %; mp 238–240 °C. IR (KBr): 3356, 3260 (2NH), 2224 (CN), 1371 (S=O, asym), 1157(S=O, sym) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.10 (2H, m, pyran-H), 3.48 (2H, m, pyran-H), 4.65 (2H, s, pyran-H), 6.75–8.20 (10H, m, Ar–H), 8.52, 8.97 (2H, 2s, 2NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 36.40, 64.50, 73.60, 85.50, 116.50, 117.20, 119.40, 125.50, 127.20, 127.50, 128.20, 129.50, 133.40, 136.20, 138.20, 143.50, 147.00, 149.20, 151.10, 156.20, 162.50. MS *m/z* (%): 490 (56). Anal. Calcd for $C_{23}H_{18}N_6O_3S_2$ (490.56): C, 56.31, H, 3.70, N, 17.13, O, 9.78, S, 13.07. Found: C, 56.35, H, 3.67, N, 17.15, S, 13.00.

General procedure for synthesis of compounds 19a,b

The amino acids α-alanine and β-alanine (0.02 mol) and Na_2CO_3 (30 mL) were dissolved in water (15 mL) and the pH was adjusted to 9–9.5. To this mixture, compound **2** (0.01 mol) dissolved in ethanol (30 mL) was added, and the reaction mixture was stirred for 8 h at the controlled pH. The reaction mixture was left overnight at room temperature, then treated with cold formic acid. The solid formed was filtered off, washed with H_2O and crystallized from ethanol.

*2-(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-ylamino) propanoic acid (**19a**)*

Yield: 68 %; mp 164–166 °C. IR (KBr): 3400 (br OH), 3225 (NH), 2232 (CN), 1717 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.23 (3H, d, CH_3), 2.98 (2H, m, pyran-H), 3.89 (2H, m, pyran-H), 3.97 (1H, m, chiral carbon-H), 4.67 (2H, s, pyran-H), 7.23 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.42 (1H, m, thiophene-H), 7.95 (1H, d, $J = 5.4$ Hz, thiophene-H), 9.05 (1H, s, NH, D_2O exchangeable), 12.40 (1H, br, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 18.50, 36.50, 60.00, 64.60, 73.50,

85.50, 116.20, 119.40, 125.50, 127.50, 127.75, 138.20, 151.20, 162.20, 162.50, 174.20. MS *m/z* (%): 329 (85). Anal. Calcd for $C_{16}H_{15}N_3O_3S$ (329.37): C, 58.34, H, 4.59, N, 12.76, O, 14.57, S, 9.74. Found: C, 58.39, H, 4.55, N, 12.77, S, 9.69.

*3-(3-Cyano-4-(thiophen-2-yl)-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-ylamino) propanoic acid (**19b**)*

Yield: 71 %; mp 182–184 °C. IR (KBr): 3400 (br OH), 3100 (NH), 3090 (Ar–CH), 2920 (aliph-OH), 2230 (CN), 1718 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.50 (2H, m, $–CH_2$), 3.02 (2H, m, pyran-H), 3.31 (2H, m, $–CH_2$), 3.99 (2H, m, pyran-H), 4.56 (2H, s, pyran-H), 7.20 (1H, d, $J = 5.4$ Hz, thiophene-H), 7.32 (1H, m, thiophene-H), 7.78 (1H, d, $J = 5.4$ Hz, thiophene-H), 8.19 (1H, s, NH, D_2O exchangeable), 12.20 (1H, br, OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 36.50, 37.00, 37.80, 64.60, 73.50, 85.60, 116.60, 119.60, 125.50, 127.50, 127.60, 138.30, 151.20, 162.20, 162.60, 177.50. MS *m/z* (%): 329 (70). Anal. Calcd for $C_{16}H_{15}N_3O_3S$ (329.37): C, 58.34, H, 4.59, N, 12.76, O, 14.57, S, 9.74. Found: C, 58.30, H, 4.62, N, 12.75, S, 8.70.

Biological evaluation

In-vitro antitumor activity

Hep-G2 (human liver carcinoma); MCF-7 (human breast carcinoma); Caco-2 (human colorectal adenocarcinoma); and HCT116 (human colorectal carcinoma) were supplied by Applied Research Sector, VACSERA-Egypt and maintained in RPMI-1640 that was supplemented with 10 % heat-inactivated FBS, 100 U/mL penicillin and 100 U/mL streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5 % CO_2.

MTT antitumor assay

The antitumor activity against HepG2, HCT 116, MCF-7 and HCT-116 cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay [48, 49]. Cells were dispensed in a 96 well sterile microplate (2 × 104 cells/well), and incubated at 37 ° C with series of different concentrations of each tested compound or Doxorubicin (positive control) in DMSO for 48 h in a serum-free medium prior to the MTT assay. After incubation, media were carefully removed, and 40 μL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 μL of DMSO. The absorbance was measured at 590 nm using Spectra Max® Paradigm® Multi-Mode microplate reader (Molecular Devices). The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were carried out in triplicate in three different weeks. The results were represented as mean ± SD. IC_{50} were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

Conclusion

In the present study, 2-chloro-4-(thiophen-2-yl)-7,8-dihydro-5*H*-pyran[4,3-*b*]pyridine-3-carbo-nitrile (**2**) was used to synthesize novel series of 2-(substituted derivatives) aminopyranopyridine **3**–**19**. Most of the synthesized compounds were tested for their anticancer activities. The results showed that the three compounds **7**, **12** and **14** showed the highest effect against liver (HepG2), Colorectal (HCT-116), breast (McF7) and Colorectal adenocarcinoma (Caco-2) human carcinoma cell lines using doxorubicin as a reference drug. Compounds **1**, **2, 9a, 9b, 10, 11 and 19a** showed anticancer activity equal or slightly less than the reference drug.

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