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SYNTHESIS, TUMOR INHIBITORY AND ANTIOXIDANT ACTIVITY OF NEW POLYFUNCTIONALLY 2-SUBSTITUTED 5,6,7,8-TETRAHYDRO-NAPHTHALENE DERIVATIVES CONTAINING PYRIDINE, THIOXOPYRIDINE AND PYRAZOLOPYRIDINE MOIETIES

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Abstract: 2-Acetyl-5,6,7,8-tetrahydronaphthalene (**1**) was allowed to react with different aromatic aldehydes to produce the cyanopyridones **2a** and **2b**, which were treated with phosphorous pentasulfide to afford the corresponding thioxopyridine derivatives **3a** and **3b**, respectively. The reaction of **3a** and **3b** with ethyl bromoacetate afforded the ester derivatives **4a** and **4b**, while their condensation with hydrazine hydrate gave the corresponding pyrazolopyridine derivatives **5a** and **5b**. The reaction of the precursor 2-amino-5,6,7,8-tetrahydronaphthalene (**6**) with ethoxy methylenemalonic ester led to the formation of aminomethylenemalonate derivative **7**. Cyclization of **7** in boiling diphenyl ether gave the derivative - ethyl 6,7,8,9-tetrahydro-4-hydroxybenzo[g]-quinoline-3-carboxylate (**8**) which was hydrolyzed to produce the corresponding carboxylic acid analogue **9**. Further reaction of **3a** with 3-chloropropane-1,2-diol and/or iodomethane produced the corresponding nicotinonitrile derivatives **10** and **11**. Hydrazinolysis of derivative **11** gave the hydrazinyl derivative **12**. Moreover, chlorination of compound **2a** with phosphorous oxychloride led to 2-chloro nicotinonitrile derivative **13**, which was refluxed with various amines to form the corresponding derivatives **5a**, **14** and **15**. Treatment of the pyrazolopyridine compound **5a** with formic acid and acetic anhydride afforded the corresponding formamide and acetamide analogues **16** and **17**, while its reaction with DMF-DMA yielded the corresponding formimidamide derivative **18**. The pyridopyrazolo[1,5-a]pyrimidine derivative **19** was obtained by cyclization of **5a** with acetyl acetone. The antioxidant activity evaluation of the newly synthesized compounds showed that the pyrazolopyridine derivative **5a** exhibited scavenging potency higher than that obtained by ascorbic acid. Tumor inhibitory activity screening revealed that derivatives **8** and **10** showed promising potency against the liver cancer cells (HepG-2) compared to doxorubicin as a reference drug.

Keywords: nicotinonitrile, thioxopyridine, pyrazolopyridine, antioxidant, antitumor activity

Nitrogen-containing heterocyclic compounds are one of the most fruitful and extensively developing fields of heterocyclic chemistry. These compounds exhibit various kinds of biological activities. The pyridyl heterocyclic nucleus is a widespread sub-unit in numerous biologically active compounds and natural products resembling NAD nucleotides, pyridoxol (vitamin B6) and pyridine alkaloids (1-3). Some examples of pyridine-containing agents are used as pharmaceuticals (as antimalarial, vasodilator, anesthetic, anticonvulsant, and antiepileptic), dyes, additives (as antioxidant), agrochemicals (as fungicidal, pesticidal, and herbicidal), veterinary (as anthelmintic, antibacterial, and antiparasitic), and also in qualitative and quantitative analysis (4-7). At the same time, different literature survey showed that many 3-cyanopyridone and pyrazolo[3,4-b]pyridine derivatives exhibited antimicrobial activity (8, 9) in addition to other diverse biological and pharmacological activities, such as anti-inflammatory (10-12), anticancer (13-16), anxiolytic (17), cardiotonic and Ca^{2+} channel-blocking properties in vascular smooth muscle (18) and glycogen synthesis kinase-3 (GSK-3) and phospholipase A_2 inhibition (19, 20).

Cancer is continuing to be a major health problem in developing as well as undeveloped countries

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(21). Surpassing heart diseases, it is taking the position number one killer due to various worldwide factors. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important since no currently available agents meet the criterion that would eradicate cancer cells without harming normal tissues (22).

Antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. Free radicals are constantly formed as a result of normal organ functions or excessive oxidative stress (23). High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues, resulting in mutations that can lead to malignancy. Damage to DNA by oxidative stress has been widely accepted as a major cause of cancer (24). DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been observed in various tumors. The discovery of the role of free radicals in cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders, aging and other diseases has led to new medical insight (25, 26). Minimizing oxidative damage may be an important approach to the primary prevention or treatment of these diseases, since antioxidants may stop the free-radical formation, or interrupt an oxidizing chain reaction.
Since, different studies exhibited the importance of various naphthyl and substituted chloro-nitrophenyl compounds as antitumor agents (27-31) and in continuation to our previous efforts (11, 13, 14, 16, 32), the above mentioned points had attracted a great deal of this research interest in synthesis of new derivatives bearing tetralin ring system incorporated to cyanopyridone, pyrazolo[3,4-*b*]pyridine, naphthalene and/or 2-chloro-4-nitrophenyl moieties to investigate their *in vitro* antitumor activity against liver carcinoma cell lines (HepG-2) in comparison to doxorubicin as a reference drug. Moreover, the free-radical scavenging properties were also screened using rutin and vitamin C as reference standards. In addition, the toxicity of the examined compounds against the normal cells was also evaluated.

MATERIALS AND METHODS

Chemistry

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre (NRC), Cairo, Egypt. The found values were within ±0.4% of the theoretical values. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, NRC. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury (run at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference, Faculty of Science, Cairo University, Egypt. Mass spectra were recorded at 70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan), NRC. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected glass plates and the spots were detected by exposure to UV lamp at λ 254 nm.

1-(5,6,7,8-Tetrahydronaphthalen-2-yl)ethanone (**1**)

This compound was prepared according to the previously reported procedure (33, 39).

General procedure for synthesis of 2-oxo-1,2-dihydropyridine-3-carbonitrile compounds (**2a,b**)

A mixture of 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone (**1**) (1.7 g, 10 mmol), the appropriate aromatic aldehydes, namely: 2-naphthaldehyde, 2-chloro-5-nitrobenzaldehyde, ethyl cyanoacetate (1.1 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in n-butanol (40 mL) was refluxed for 3 h. The obtained precipitate was filtered, washed successively with ethanol and recrystallized from ethanol/DMF to give compounds **2a**,**b**, respectively, as yellow crystals.

4-(Naphthalen-2-yl)-2-oxo-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1,2-dihydro pyridine-3-carbonitrile (**2a**)

Yield 86%; m.p. 304-306°C. IR (KBr, cm^{-1}): 3132 (NH), 2930 (CH, alicyclic), 2216 (CN), 1627 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.75, 2.77 (m, m, 4H, 4H, alicyclic 4CH_2 of tetrahydronaphthalene ring), 6.91 (s, 1H, Ar-H), 7.20 (d, J = 7.80 Hz, 1H, Ar-H), 7.60-7.83 (m, 5H, Ar-H), 8.01-8.11 (m, 3H, Ar-H), 8.30 (s, 1H, pyridine-H5), 12.30 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO- d_6, δ, ppm): 22.34, 22.40, 28.63, 28.68 (4CH_2), 105.50, 116.51, 124.55, 125.19, 126.81, 127.58, 128.06, 128.12, 128.53, 129.23, 129.35, 132.37, 133.33, 133.55, 137.37, 140.52 (Ar-C, CN), 161.95 (C=O). MS: m/z (%): 377 (M^+ + 1, 28), 376 (M^+, 87), 375 (M^+ - 1, 41). Analysis: for $C_{26}H_{20}N_2O$ (376.46): calcd.: C, 82.95; H, 5.35; N, 7.44%; found: C, 82.86; H, 5.44; N, 7.59%.

4-(2-Chloro-5-nitrophenyl)-2-oxo-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1,2-dihydropyridine-3-carbonitrile (2b)

Yield 82%; m.p. 218-220°C. IR (KBr, cm^{-1}): 3130 (NH), 2928 (CH, alicyclic), 2222 (CN), 1640 (C=O), 1512, 1341 (NO_2). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.75, 2.75 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 6.89 (s, 1H, Ar-H), 7.20 (d, *J* = 8.10 Hz, 2H, Ar-H), 7.59-8.01 (m, 3H, Ar-H), 8.32(s, 1H, pyridine-H5), 12.92 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO- d_6, δ, ppm): 22.5, 22.6, 28.5, 28.8 ($4CH_2$), 115.2, 116.3, 120.4, 124.0, 124.6, 125.8, 128.5, 129.3, 131.1, 135.0, 135.2, 138.6, 149.8, 154.6 (Ar-H, CN), 160.9 (C=O). MS: m/z, (%): 407 (M^+ + 2, 33), 405 (M^+, 100). Analysis: for $C_{22}H_{16}ClN_3O_3$ (405.86): calcd.: C, 65.10; H, 3.97; Cl, 8.73; N, 10.35%; found: C, 64.86; H, 4.22; Cl, 8.62; N, 10.21%.

General procedure for synthesis of 2-thioxo-1,2-dihydropyridine-3-carbonitrile compounds (3a,b)

A mixture of compounds **2a,b** (10 mmol) and P_2S_5 (2 g) in pyridine (10 mL) was refluxed for 6 h. Upon reaction completion, the reaction mixture was poured into ice-cold water acidified with diluted HCl. The separated solid was filtered and recrystallized from appropriate solvent to afford the corresponding thione derivatives **3a**,**b**, respectively.

4-(Naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (3a)

Crystallized from acetic acid to give orange crystals. Yield 74%; m.p. 160–162°C. IR (KBr, cm^{-1}): 3161 (NH), 2929 (CH alicyclic), 2219 (CN), 1596 (C=N) and 1200 (C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76, 2.78 (m, m 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 7.11–8.0 (m, 10H, Ar-H), 8.23 (s, 1H, pyridine-H5) and 8.41 (s, 1H, NH, D_2O exchangeable). MS: m/z (%): 392.27 (M^+, 100). Analysis: for $C_{26}H_{20}N_2S$ (392.52): calcd.: C,79.56; H, 5.14; N, 7.14; S, 8.17%; found: C, 79.35; H, 5.49; N, 7.00; S, 7.97%.

4-(2-Chloro-5-nitrophenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (3b)

Crystallized from ethanol/DMF to give orange crystals. Yield 70%; m.p. 166–168°C. IR (KBr, cm^{-1}): 3361 (NH), 2926 (CH alicyclic), 2222 (CN), 1591 (C=N) and 1219 (C=S). ^{1}H NMR (DMSO-d_6, δ, ppm) 1.75, 2.78 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 7.11–7.23 (m, 2H, Ar-H), 7.42 (s, 1H, Ar-H), 7.47–7.88 (m, 3H Ar-H), 8.12 (s, 1H, CH-pyridine ring), 8.19 (s, 1H, NH, D_2O exchangeable). MS: m/z (%): 423.5 (M^+ + 2, 33), 421.5 (M^+, 100). Analysis: for $C_{22}H_{16}ClN_3O_2S$ (421.9): calcd.: C, 62.63; H, 3.82; Cl, 8.40; N, 9.96; S, 7.60%; found: C, 62.31; H, 3.51; Cl, 8.56; N, 10.21; S, 7.39%.

General procedure for the synthesis of pyridin-2-ylthioacetate compounds (4a,b)

To a solution of 2-thioxopyridine derivatives (10 mmol) **3a**,**b** in DMF (30 mL), ethyl bromoacetate (2 mL, 12 mmol) and anhydrous potassium carbonate (5 g) were added. The reaction mixture was refluxed on a water bath for 20 h, then poured into ice-water and allowed to stand overnight. The formed precipitate was filtered and recrystallized from the appropriate solvent to afford the corresponding ester derivatives **4a**,**b**, respectively.

Ethyl 2-(3-cyano-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridin-2-ylthio) acetate (4a)

Crystallized from ethanol/DMF to give yellow crystals. Yield 79%; m.p. 150–152°C. IR (KBr, cm^{-1}): 2936 (CH alicyclic), 2216 (CN), 1746 (C=O, ester), 1590 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3), 1.74, 2.78 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 4.23 (q, *J* = 6.8 Hz, 7.2 Hz, 2H, -CH_2CH_3), 5.81 (s, 2H, SCH_2COO), 7.16 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.62–7.69 (m, 3H, Ar-H), 7.82–7.93 (m, 3H Ar-H), 8.03–8.13 (m, 3H Ar-H), 8.16 (s, 1H, pyridine-H). MS: m/z (%): 478 (M^+, 100). Analysis: for $C_{30}H_{26}N_2O_2S$ (478.6): calcd.: C, 75.29; H, 5.48; N, 5.85, S; 6.70%; found: C, 75.05; H, 5.85; N, 5.52; S; 6.91%.

Ethyl 2-[4-(2-chloro-5-nitrophenyl)-3-cyano-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridin-2-ylthio]acetate (4b)

Crystallized from acetic acid to give pale brown crystals. Yield 73%; m.p. 148–150°C. IR (KBr, cm^{-1}): 2925 (CH alicyclic), 2223 (CN), 1737 (C=O, ester) and 1590 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.25 (t, *J* = 7.2 Hz 3H, CH_2CH_3), 1.71, 2.72 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 4.23 (q, *J* = 6.8, 7.2 Hz, 2H, CH_2CH_3,), 5.78 (s, 2H, -SCH_2COO), 7.10-7.19 (m, 2H, Ar-H), 7.51-7.90 (m, 4H, Ar-H), 8.13 (s, 1H, pyridine-H5). MS: m/z (%): 509.5 (M^+ + 2, 33), 507.5 (M^+, 100). Analysis: for $C_{26}H_{22}ClN_3O_4S$ (507.99): calcd.: C, 61.47; H, 4.37; Cl, 6.98; N, 8.27; S, 6.31%; found: C, 61.16; H, 4.21 ; Cl, 7.23 ; N, 8.11; S, 6.59%.

General procedure for synthesis of pyrazolo[3,4-*b*]pyridine compounds (5a,b)

Method A

A mixture of thioxopyridine **3a**,**b** (10 mmol) and hydrazine hydrate 80% (6 mL, 20 mmol) was refluxed in absolute ethanol (10 mL) for 24 h. After cooling, the resulting solid product was recrystallized from acetic acid to give yellow crystals **5a,b**, respectively.

Method B for synthesis of 4-naphthalen-2-yl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-*b*]pyridin-3-ylamine (5a)

To a solution of chloro compound **13** (3.9 g, 10 mmol) in absolute ethanol (30 mL), an excess of hydrazine hydrate 99% (1.2 mL, 40 mmol) was added and the reaction mixture was refluxed for 12 h. After cooling, the separated solid was filtered and recrystallized from acetic acid to give compound **5a**.

Yield 75%; m.p. 198-200°C. IR (KBr, cm^{-1}): 3418, 3333 (NH_2), 3199 (NH), 2924 (CH, alicyclic), 1600 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.77, 2.79 (m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene ring), 4.56 (s, 2H, NH_2, D_2O exchangeable), 7.18 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.56-7.63 (m, 3H, Ar-H), 7.81-8.13 (m, 6H, Ar-H), 8.26 (s, 1H, pyridine H5), 12.32 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 22.5, 28.5 ($4CH_2$), 124.3, 125.2, 127.4, 128.2, 128.7, 128.9, 129.7, 133.2, 138.4, 149.2, 150.2, 150.6, 151.1 (Ar-H), 155 (C-NH_2). MS: m/z (%): 390 (M^+, 100), 389 (M^+-1, 29). Analysis: for $C_{26}H_{22}N_4$ (390.49): calcd.: C, 79.97; H, 5.67; N, 14.35%; found: C, 80.10; H, 5.62; N, 14.12%.

4-(2-Chloro-5-nitrophenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-*b*]pyridin-3-ylamine (5b)

Yield 68%; m.p. 228-230°C. IR (KBr, cm^{-1}): 3433, 3327(NH_2), 3215 (NH), 2925 (CH, alicyclic), 1522, 1344 (NO_2). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76, 2.78 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene), 5.48 (s, 2H, NH_2, D_2O exchangeable), 6.64-6.73 (m, 2H, Ar-H), 7.15-7.34 (m, 3H, Ar-H), 7.83 (s, 1H, Ar-H), 7.85 (s, 1H, pyridine H5), 12.21 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 22.5, 28.5 ($4CH_2$), 124.0, 124.3, 125.2, 128.1, 128.6, 128.7, 129.7, 131.1, 135.0, 135.3, 138.4, 149.3, 149.9, 150.1, 150.6, 151.1 (Ar-C), 155 (C-NH_2). MS: m/z (%): 421 (M^+ + 2, 33), 419 (M^+, 100). Analysis: for $C_{22}H_{18}ClN_5O_2$ (419.86): calcd.: C, 62.93; H, 4.32; Cl, 8.44; N, 16.67%; found: C, 62.61; H, 4.89; Cl, 8.22; N, 16.84%.

Diethyl 2-((1,2,3,4-tetrahydronaphthalen-6-ylamino)methylene)malonate (7)

A mixture of equimolar quantities of aminotetralin derivative **6** (4.41 g, 30 mmol) and diethyl ethoxymethylenemalonate (7.8 mL, 30 mmol) was heated at 120°C for 1 h. The white crystalline solid obtained upon cooling the mixture to room temperature was filtered and recrystallized from ethanol to give the corresponding malonate ester derivative **7**.

Yield 80%; m.p. 120°C. IR (KBr, cm^{-1}): 3420 (NH), 3012 (CH, aromatic), 2934 (CH, alicyclic), 1720-1710 (broad band, 2CO). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.12 (t, *J* = 7.1 Hz, 6H, $2CH_2CH_3$), 1.80, 2.79 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene), 4.21 (q, *J* = 7.1 Hz, 4H, $2CH_2CH_3$), 6.25 (s, 1H, -HN-C*H*), 7.33 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.50 (s, IH, Ar-H), 8.23 (d, *J* = 8.1 Hz,1H, Ar-H), 11.71 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%): 317 (M^+, 10). Analysis: for $C_{18}H_{23}NO_4$ (317.38): calcd.: C, 68.12; H, 7.30; N, 4.41%; found: C, 68.42; H, 7.71; N, 4.60%.

Ethyl 6,7,8,9-tetrahydro-4-hydroxybenzo[g]quinoline-3-carboxylate (8)

A suspension of diethyl malonate ester **7** (3.1 g, 10 mmol) in diphenyl ether (10 mL) was refluxed for 1 h. After dilution with petroleum ether at room temperature, the light brown precipitate was collected by filtration, washed with petroleum ether and recrstallized from DMF/water to give the carboxylate compound **8**.

Yield 80%; m.p. 260°C. IR (KBr, cm^{-1}): 3530 (OH), 3002 (CH, aromatic), 2914 (CH, alicyclic), and 1710 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.22 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 1.80, 2.79 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene), 4.01 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 7.21, 7.51, 7.92 (3s, 3H, Ar-H), 12.52 (s, 1H, O*H*, exchangeable with D_2O). MS: m/z (%): 271 (M^+, 60). Analysis: for $C_{16}H_{17}NO_3$ (271.31): calcd. C, 70.83; H, 6.32; N, 5.16%; found: C, 70.53; H, 6.62; N, 5.56%.

4-Hydroxy-6,7,8,9-tetrahydrobenzo[g]quinoline-3-carboxylic acid (9)

A suspension of ethyl carboxylate derivative **8** (5.4 g, 20 mmol) in ethanolic solution of NaOH 10% was refluxed for 40 min. The solution was allowed to cool to room temperature and neutralized with conc. HCl. The solid precipitated was filtered, washed with water several times and recrystallized from DMF/water to give the corresponding carboxylic acid derivative **9**.

Yield 90%; m.p. > 300°C. IR (KBr, cm^{-1}): 3545, 3530 (2OH), 3012 (CH, aromatic), 2944 (CH,

alicyclic), and 1720 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.80, 2.79 (m, m, 4H, 4H, 4C*H*$_2$ of tetrahydronaphthalene), 7.35, 7.61, 8.00 (3s, 3H, Ar-H), 11.21, 12.91 (2s, 2H, 2O*H*, exchangeable with D_2O). MS: m/z (%): 243 (M^+, 20). Analysis: for $C_{14}H_{13}NO_3$ (243.26): calcd.: C, 69.12; H, 5.39; N, 5.76%; found: C, 69.45; H, 5.56; N, 5.36%.

Preparation of 2-((2,3-dihydroxypropyl)thio)-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)nicotinonitrile (10)

A solution of thioxopyridine derivative **3a** (3.9 g, 10 mmol) in 10% ethanolic potassium hydroxide solution (30 mL) was refluxed for 2 h, and the reaction mixture was cooled to room temperature. Then, a solution of 3-chloropropane-1,2-diol (1.10 g, 10 mmol) in dry acetone (15 mL) was added portionwise to the previously prepared potassium salt over a period of 1 h with stirring. The stirring was continued for 10 h, whereby the formed precipitate was collected by filtration, dried and crystallized from methanol to give compound **10** as pale yellow crystals.

Yield 69%; m.p. 150–152°C. IR (KBr, cm^{-1}): 3402 (br, 2OH), 2925 (CH alicyclic), 2215 (CN), 1570 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76, 2.79 (m, m, 4H, 4H, 4CH$_2$ of tetrahydronaphthalene), 4.09 (d, *J* = 7.8 Hz., 2H, C*H*$_2$), 4.33 (m, 1H, C*H*), 4.90 (m, 2H, C*H*$_2$), 6.81–7.83 (m, 10H, Ar-H), 7.85 (s, 1H, pyridine H5), 11.60 (s, 2OH, exchangeable with D_2O). MS: m/z (%): 466 (M^+, 27). Analysis: for $C_{29}H_{26}N_2O_2S$ (466.59): calcd.: C, 74.65; H, 5.62; N, 6.00; S, 6.87%; found: C, 75.49; H, 6.12; N, 5.23; S, 6.11%.

2-(Methylthio)-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-nicotinonitrile (11)

A solution mixture of thioxopyridine **3a** (1.9 g, 50 mmol) in ethanolic potassium hydroxide 10% ethanol (30 mL) was refluxed for 2 h. Then, the reaction mixture was cooled to room temperature. Iodomethane (0.6 mL, 6 mmol) was added to the previously prepared potassium salt. The reaction mixture was allowed to reflux for further 4 h. Upon completion of reaction, the mixture was cooled to room temperature and poured in cold water. The formed precipitate was collected by filtration, washed well with cold water, dried and crystallized from dioxane to afford the titled compound **11**.

Yield 65%; m.p. 150–152°C. IR (KBr, cm^{-1}): 2928 (CH, alicyclic), 2216 (CN), 1600 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.77, 2.77 (m, m, 4H, 4H, 4CH$_2$ of tetrahydronaphthalene), 2.62 (s, 3H, C*H*$_3$), 7.21 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.65 (m, 2H, Ar-H), 7.83–8.30 (m, 7H, Ar-H), 8.36 (s, 1H, pyridine H5). MS: m/z (%): 406 (M^+, 100). Analysis: for $C_{27}H_{22}N_2S$ (406.54): calcd.: C, 79.77; H, 5.45; N, 6.89; S, 7.89%; found: C, 79.45; H, 5.12; N, 7.00; S, 7.53%.

Method A for synthesis of 2-hydrazinyl-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydro naphthalen-2-yl)-3-nicotinonitrile (12)

To the methylthio derivative **11** (4 g, 10 mmol), an excess of hydrazine hydrate (99%) (1.2 mL, 40 mmol) was added. Then, the reaction mixture was allowed to reflux for 2 h. Then, the mixture was cooled and poured in water whereby a white precipitate was produced. The newly obtained precipitate was collected upon filtration. The crystallization was achieved from ethanol/dioxane (3 : 1) mixture to produce the hydrazinyl compound **12**.

Method B for synthesis of compound 12

The chloro compound **13** (3.9 g, 10 mmol) was refluxed with hydrazine hydrate (1.2 mL, 40 mmol) in ethanol (10 mL) for 3 h. Then, the reaction mixture was poured into ice-cold water acidified with diluted hydrochloric acid and the separated solid was filtered and recrystallized from ethanol/dioxane (3 : 1) mixture to afford the corresponding hydrazinyl derivative **12**.

Yield 70%; m.p. 150–152°C. IR (KBr, cm^{-1}): 3432, 3271 (NH_2), 3186 (NH), 2926 (CH alicyclic), 2216 (CN), 1627 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76, 2.77 (m, m, 4H, 4H, 4CH$_2$ of tetrahydronaphthalene), 4.56 (s br, 2H, NH_2, D_2O exchangeable), 7.19–8.05 (m, 10H, Ar-H), 8.25 (s, 1H, pyridine H5), 12.3 (s, 1H, NH, D_2O exchangeable). MS: m/z (%): 390 (M^+, 100). Analysis: for $C_{26}H_{22}N_4$ (390.48): calcd.: C, 79.97; H, 5.67; N, 14.34%; found: C, 80.12; H, 5.42; N, 14.00%.

2-Chloro-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-nicotinonitrile (13)

A suspension of pyridone compound **2a** (3.7 g, 10 mmol), PCl_5 (0.5 g) and $POCl_3$ (5 mL) was heated on a water bath for 3 h. The reaction mixture was poured gradually into ice-cold water and neutralized by dilute ammonia solution. The separated solid was filtered and recrystallized from ethanol to afford the corresponding chloro derivative **13** as yellow crystals.

Yield 64%; m.p. 138–140°C. IR (KBr, cm^{-1}): 2925 (CH alicyclic), 2223 (CN), 1575 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76, 2.78 (m, m, 4H, 4H, 4CH$_2$ of tetrahydronaphthalene), 7.19 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.65–8.38 (m, 9H, Ar-H), 8.55 (s,

1H, pyridine-H5). MS: m/z (%): 396.3 (M^+ + 2, ^{37}Cl, 32.79), 394.3 (M^+, ^{35}Cl, 100). Analysis: for $C_{26}H_{19}ClN_2$ (394.9): calcd.: C, 79.08; H, 4.85; Cl, 8.98; N, 7.09%; found: C, 78.81.12; H, 4.47; Cl, 8.73; N, 7.30%.

2-(Benzylamino)-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-nicotinonitrile (14)

The chloro compound **13** (3.9 g, 10 mmol) was refluxed with benzylamine (1.2 mL, 10 mmol) in ethanol (10 mL) for 3 h. Then, the reaction mixture was poured into ice-cold water, acidified with diluted hydrochloric acid and the separated solid was filtered and recrystallized from ethanol to produce the corresponding derivative **14**.

Yield 70%; m.p. 196–198°C. IR (KBr, cm^{-1}): 3367 (NH), 2921 (CH alicyclic), 2209 (CN), 1589 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.75 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene), 4.26 (d, *J* = 5.7 Hz, 2H, C*H*$_2$), 4.72 (d, *J* = 6.00 Hz, 1H, NH D_2O exchangeable), 7.11–8.32 (m, 16H, Ar-H). MS: m/z (%): 464 (M^+ - 1, 100), 465 (M^+, 84). Analysis: for $C_{33}H_{27}N_3$ (465.59): calcd.: C, 85.13; H, 5.85; N, 9.03%; found: C, 85.19; H, 5.42; N, 9.30%.

4-(Naphthalen-2-yl)-2-(piperidin-1-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-nicotinonitrile (15)

Chloro compound **13** (3.9 g, 10 mmol) was dissolved in ethanol (20 mL) containing piperidine (1 mL, 10 mmol) and the solution mixture was refluxed for 24 h. Upon cooling, the separated crystalline precipitate was collected by filtration and recrystallized from acetic acid to give the piperidinyl derivative **15** as creamy crystals.

Yield 78%; m.p. 150–152°C. IR (KBr, cm^{-1}): 2925 (CH alicyclic), 2206 (CN), 1598 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69 (m, 10H, $2CH_2$ of tetrahydronaphthalene and piperidine β- + γ-CH_2's), 2.78 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 3.72 (m, 4H, of piperidine α-CH_2's), 7.20-8.08 (m, 10H, Ar-H), 8.29 (s, 1H, pyridine H5). MS: m/z (%): 443 (M^+, 38), 442 (M^+ - 1, 100). Analysis: for $C_{31}H_{29}N_3$ (443.58.): calcd.: C, 83.94; H, 6.59; N, 9.47%; found: C, 84.24; H, 6.32; N, 9.60%.

N-(4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl)formamide (16)

A solution of pyrazolopyridine compound **5a** (3.9 g, 10 mmol) in formic acid (20 mL) was heated under reflux for 1 h. Upon cooling to room temperature, the solid precipitate so formed was filtered and recrystallized from ethanol to give formamide compound **16** as yellow crystals.

Yield 80%; m.p. 262–264°C. IR (KBr, cm^{-1}): 3327, 3146 (2NH), 2925 (CH, alicyclic), 1655 (C=O, amide), 1592 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.79, 2.82 (m, m, 4H, 4H, $4CH_2$ of tetrahydronaphthalene), 7.21 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.61 (m, 2H, Ar-H), 7.81 (m, 2H, Ar-H), 7.97–8.03 (m, 5H, Ar-H), 8.19 (s, 1H, pyridine-H5), 8.22 (s, 1H, CHO), 9.72, 10.32 (2s, 2H, 2NH, exchangeable with D_2O). MS: m/z (%): 418 (M^+, 67). Analysis: for $C_{27}H_{22}N_4O$ (418.49): calcd.: C, 77.49; H, 5.30; N, 13.39%; found: C, 77.86; H, 5.17; N, 13.54%.

N-(4-(Naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl)acetamide (17)

Pyrazolopyridine derivative **5a** (3.9 g, 10 mmol) was heated under reflux in acetic anhydride (20 mL) for 1 h, then, the mixture was allowed to attain room temperature. The deposited solid was filtered, washed with petroleum ether (60–80°C) and recrystallized from ethanol to give compound **17** as yellow crystals.

Yield 80%; m.p. 230–232°C. IR (KBr, cm^{-1}): 3330, 3146 (2NH), 2924 (CH, alicyclic), 1714 (C=O acetyl), 1580 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.79, 2.82 (m, m, 4H, 4H, 4C*H*$_2$ of tetrahydronaphthalene), 1.92 (s, 3H, C*H*$_3$) 7.24 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.61–7.87 (m, 4H, Ar-H), 7.97–8.07 (m, 6H, Ar-H + NH, exchangeable with D_2O), 8.13 (s, 1H, pyridine-H5), 10.32 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%): 432 (M^+, 100). Analysis: for $C_{28}H_{24}N_4O$ (432.52): calcd.: C, 77.75; H, 5.59; N, 12.95%; found: C, 77.42; H, 5.36; N, 13.12%.

(E)-N,N-Dimethyl-N'-(4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl)formimidamide (18)

A solution mixture of compound **5a** (3.9 g, 10 mmol) and dimethylformamide dimethylacetal (DMF-DMA) (1.19 g, 10 mmol) was heated under reflux for 12 h in xylene (10 mL). The solvent volume was reduced *in vacuo* and left to cool at room temperature to deposit a solid which was collected by filtration and crystallized from ethanol to give compound **18** as yellow crystals.

Yield 82%; m.p. 240–242°C. IR (KBr, cm^{-1}): 3148 (NH), 2921 (CH, alicyclic), 1618, 1584 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.77, 2.79 (m, m, 4H, 4H, 4C*H*$_2$ of tetrahydronaphthalene), 2.66 (s, 6H, -N(C*H*$_3$)$_2$), 7.19 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.57 (m, 2H, Ar-H), 7.69 (s,1H, Ar-H), 7.92–7.99 (m, 6H, Ar-H), 8.03 (s, 1H, pyridine H5), 8.44 (s, 1H, -CH=N), 12.77 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%): 445 (M^+, 8). Analysis: for

$C_{29}H_{27}N_5$ (445.56): calcd.: C, 78.17; H, 6.11; N, 15.72%; found: C, 78.36; H,5.81; N, 15.99%.

2,4-Dimethyl-10-(naphthalen-2-yl)-8-(5,6,7,8-tetrahydronaphthalen-2-yl)pyrido[2',3':3,4]pyrazolo[1,5-a]pyrimidine (19)

To a solution of compound **5a** (3.9 g, 10 mmol) in ethanol (20 mL), acetyl acetone (1 mL, 10 mmol) was added and the reaction mixture was refluxed for 10 h. The solvent was then removed under reduced pressure and the obtained residue was recrystallized from ethanol to give compound **19**.

Yield 78%; m.p. 303–305°C. IR (KBr, cm^{-1}): 2921 (CH, alicyclic), 1617, 1571 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.61 (s, 3H, C*H*$_3$), 1.80, 2.84 (m, m, 4H, 4H, 2CH_2 of tetrahydronaphthalene), 2.53 (s, 3H, CH_3), 7.23 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 7.61–7.63 (m, 2H, Ar-H), 7.92–8.10 (m, 6H, Ar-H), 8.16 (s, 1H, pyridine-H5). MS: m/z (%): 454 (M^+, 22). Analysis: for $C_{31}H_{26}N_4$ (454.56): calcd.: C, 81.91; H, 5.77; N, 12.33%; found: C, 82.31; H, 5.42; N, 12.01%.

Biological evaluation

***In-vitro* antioxidant activity**

1,1-Diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and methanol were of HPLC grade and all other reagents and chemicals were of analytical reagent grade.

Antioxidant activity of each compound and standards (ascorbic acid and rutin) was assessed based on the radical scavenging effect of stable DPPH free radical (34). Ten µL of each tested compound or standard (from 0.0 to 500 µM/mL) was added to 90 µL of a 100 µM methanolic solution of DPPH in a 96-well microtitre plate (Sigma-Aldrich Co., St. Louis, MO, USA). After incubation in dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using an ELISA microplate reader (Model 550, Bio-Rad Laboratories Inc., California, USA). Absorbance of blank sample containing the same amount of DMSO and DPPH solution was also prepared and measured. All experiments were carried out in triplicate. The scavenging potential was compared with a solvent control (0% radical scavenging) and the standard compounds. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Reduction of absorbance} = [(AB - AA) / AB] \times 100$$

where: AB – absorbance of blank sample and AA – absorbance of tested compound (t = 30 min). The concentration of each compound required to scavenge 50% of DPPH (IC_{50}) was determined as well (35, 36).

***In-vitro* anticancer activity**

Cell culture of Hep-G2 (liver carcinoma cell line) was purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI-1640 medium. Wish cell line was maintained in DMEM medium. Both media were 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 cytotoxicity assay

The cytotoxicity activity against Hep-G2 human cell line and the normal human cell line (wish) was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells (37). Cells were dispensed in a 96 well sterile microplate (5 × 104 cells/well), and incubated at 37°C with 100 µM/mL of each tested compound or doxorubicin (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (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 acidified isopropanol. The absorbance was measured at 570 nm using a microplate ELISA reader (Biorad, USA). The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

Lactate dehydrogenase (LDH) assay

To determine the effect of each compound on membrane permeability in both Hep-G2 and wish cell lines, a lactate dehydrogenase (LDH) release assay was used (38). The cells were seeded in 96-well culture plates at a density of 2×10^4 cells/well in 100 µL volume and allowed to grow for 18 h before treatment. After treatment with 100 µM/mL of each tested compound, the plates were incubated for 48 h. Then, the supernatant (40 µL) was transferred to a new 96 well to determine LDH release and 6% triton X-100 (40 µL) was added to the original plate for determination of total LDH. An aliquot of 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 4.6 mM pyruvic acid was mixed

with the supernatant using repeated pipetting. Then, 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to the wells. The kinetic changes were read for 1 min using ELISA microplate reader in absorbance at wavelength 340 nm. This procedure was repeated with 40 µL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well.

Statistical analysis

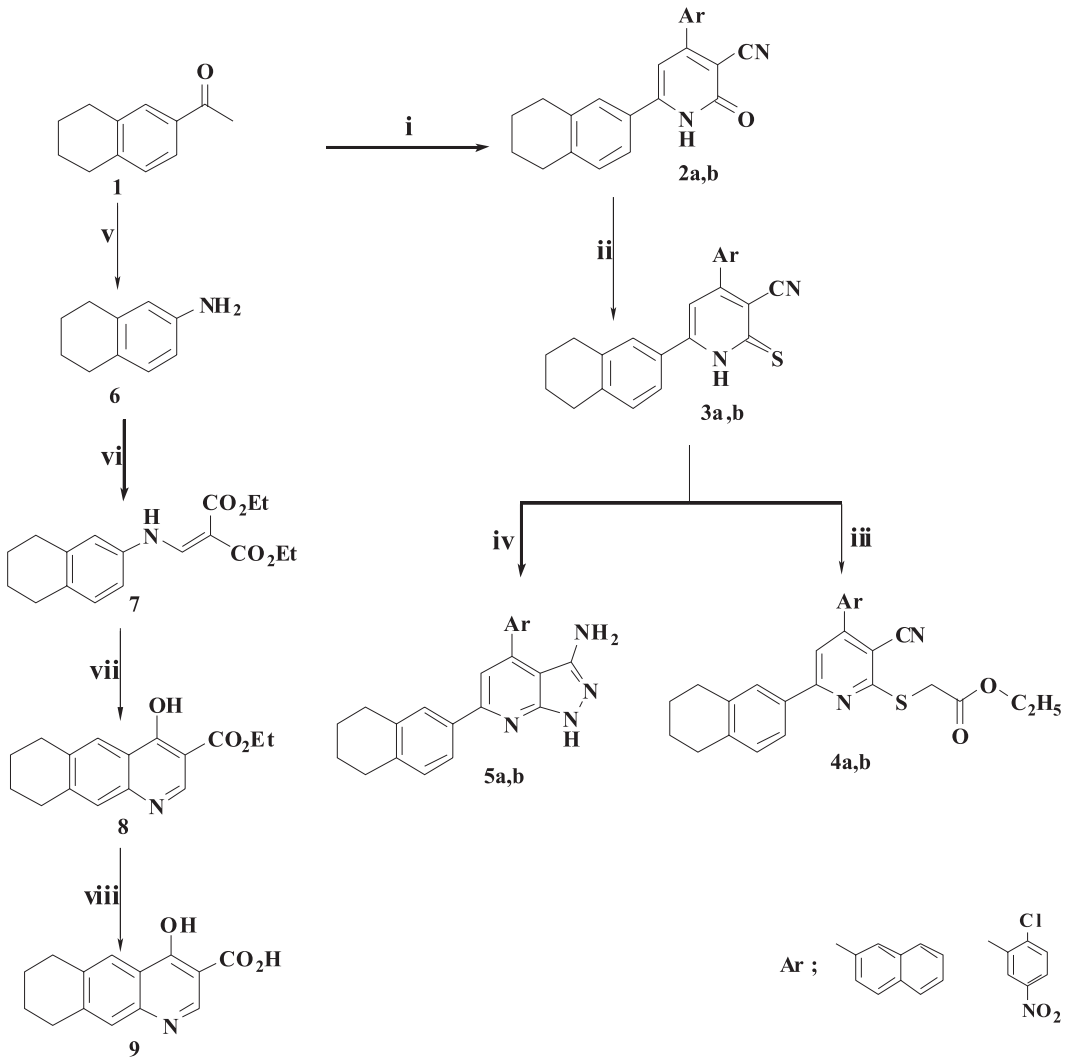
All experiments were conducted in triplicate (n = 3). All the values were represented as the mean ± SD. Significant differences between the means of parameters as well as IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Chemistry

The starting material - 2-acetyl-5,6,7,8-tetrahydronaphthalene (**1**) prepared following the literature method (33) was subjected to cyclocondensation by one pot reaction method (39) using a mixture of the appropriate aromatic aldehydes, namely: naphthalene aldehyde and/or 2-chloro-5-nitrobenzaldehyde, ethylcyanoacetate and excess ammonium acetate in refluxing n-butanol to give the corresponding 2-oxopyridine-3-carbonitriles **2a**,**b**.

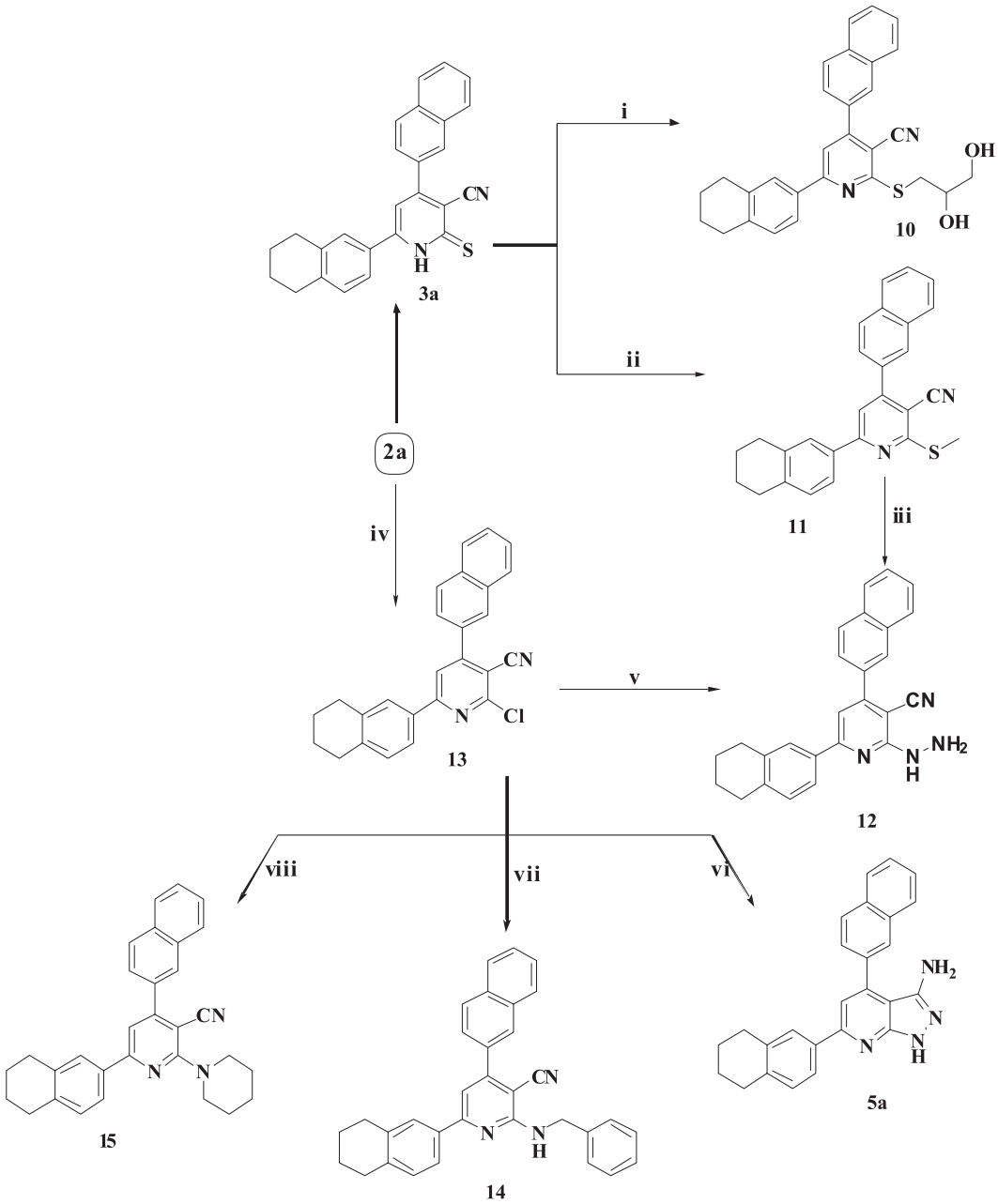
Heating a mixture of the obtained cyanopyridone derivatives **2a**,**b** with phosphorous pentasulfide in dry pyridine (39) afforded the corresponding thioxopyridine derivatives **3a**,**b**. The structures of all of the new compounds were confirmed on the basis of their elemental analyses and spectral data. For example, IR spectra of the compounds **3a**,**b** revealed the disappearance of C=O absorption bands and the presence of absorption bands at 1200 and 1219 cm^{-1}, respectively, due to C=S groups. Also, their mass spectra showed their molecular ions peaks as base peaks at m/z 392 for compound **3a** and two isotopic peaks at 421 and 423 (3 : 1) for compound **3b** (Scheme 1). Compounds **3a**,**b** underwent a series of chemical reactions, such as their treatment with ethyl bromoacetate in dimethylformamide to afford the corresponding 3-cyanopyridinyl-2-thioacetate derivatives **4a**,**b**. IR spectra of the two ester compounds showed the appearance of the respective absorption bands at 1746 and 1737 cm^{-1} due to the ester C=O groups. ^{1}H NMR spectra showed the characteristic triplet-quartet pattern of the ethyl ester groups at δ 1.25 and 4.23 ppm and a singlet signal referring to the methylene protons of -SCH_2 appeared at ≈ δ 5.81 ppm. Mass spectra of the same derivatives showed the molecular ion peak of **4a** at m/z 478 and the two isotopic peaks of **4b** at m/z 507, 509 (3 : 1) (Scheme 1). Furthermore, the reaction of thioxopyridines **3a**,**b** with excess hydrazine hydrate 80% in refluxing ethanol for 24 h gave the corresponding 3-aminopyrazolo[3,4-b]pyridine derivatives **5a**,**b**. Hamdy et al. have previously synthesized the latter compounds **5a**,**b** using an alternative method (39). The structures of **5a**,**b** were established by examining the spectral data and the elemental analysis. IR spectra indicated the absence of CN and C=S absorption bands, and the presence of two characteristic absorption bands at the range 3418-3327 cm^{-1} due to NH_2 groups and at 3215-3199 for the NH groups. With respect to ^{1}H NMR spectra, **5a**,**b** showed broad singlet signals at δ 4.56 and 4.58 ppm, respectively, assigned to the NH_2 protons and other singlets at δ 12.32, 12.21 ppm, respectively, assigned to the N*H* groups, in addition to multiplet signals at the range δ 7.11-7.89 ppm referring to the aromatic protons. The data of IR and ^{1}H NMR spectra of compounds **5a**,**b** were in accordance with those obtained previously (39). On the other hand, 2-acetyl-5,6,7,8-tetrahydronaphthalene (**1**) was converted to 2-amino-5,6,7,8-tetrahydronaphthalene **6**, according to the reported method (40), to be used as a good precursor for the preparation of novel tricyclic tetrahydronaphthalene derivatives. Thus, condensation of 2-aminotetralin derivative **6** with ethoxy methylenemalonic ester yielded the corresponding crystalline tetrahydronaphthyl aminomethylenemalonate derivative **7**. IR spectrum of the malonate analogue exhibited absorption bands at 3420 cm^{-1} and 1720-1710 cm^{-1} due to NH and 2 C=O groups, respectively. At the same time, ^{1}H NMR spectrum showed the triplet-quartet pattern of the two ethyl groups at δ 1.12 and 4.21 ppm, whereas the methine proton of –N=C*H* group appeared as a singlet signal at δ 6.25 ppm. Further cyclization of the ester derivative **7** was carried out in boiling diphenyl ether to afford the corresponding ethyl 6,7,8,9-tetrahydro-4-hydroxybenzo[g]quinoline-3-carboxylate (**8**) in 80% yield. Alkaline hydrolysis of compound **8** afforded the corresponding carboxylic acid analogue **9**. IR spectrum of the ester derivative **8** exhibited absorption bands at 3545 cm^{-1} and 1710 cm^{-1} referring to OH and C=O groups, respectively. ^{1}H NMR spectra elucidated the chemical structures of the obtained derivatives **8** and **9** by exhibiting triplet-quartet signals at δ 1.22 and 4.01 ppm and a singlet signal at δ 12.52 ppm (exchangeable with



Scheme 1. Reaction conditions: i – aromatic aldehydes, ethyl cyanoacetate, ammonium acetate, butanol, reflux, 3 h; ii – P_2S_5, pyridine, reflux, 6 h; iii – ethyl bromoacetate, DMF, anhyd. potassium carbonate, reflux, 20 h; iv, v – hydrazine hydrate, absolute ethanol, reflux, 24 h, vi – DEEM, reflux at 120°C for 1 h; vii – diphenyl ether reflux for 1 h; viii – ethanolic solution of NaOH 10% , reflux for 40 min

D_2O) assigned for the ethyl and OH groups, respectively, in case of the ester derivative **8**, while that of the acid derivative **9** revealed the disappearance of the characteristic ethyl group signals and represented two singlets at δ 11.21 and 12.91 ppm (exchangeable with D_2O) due to 2 O*H* groups (Scheme 1).

On the other hand, treatment of the thioxopyridine derivative **3a** with 3-chloropropane-1,2-diol afforded the dihydroxypropyl derivative **10,** while its treatment with methyl iodide provided the methylthio derivative **11**, which was treated with hydrazine hydrate to produce the hydrazinyl cyanopyridine derivative **12** (Scheme 2). Chlorination of the cyanopyridone compound **2a** was carried out by its reaction with phosphorous oxychloride/phosphorous pentachloride mixture leading to the formation of the chloro cyanopyridine derivative **13**. The hydrazinyl cyanopyridine analogue **12** was further obtained by refluxing the chloro compound **13** with hydrazine hydrate in ethanol for 3 h, but when reflux was continued for 24 h, led to regain the pyrazolopyridine analogue **5a** (Scheme 2). IR spectrum of the pyrazolopyridine compound **5a** showed the disappearance of the absorption band due to CN group and the presence of absorption bands at 3418, 3333 and 3199 cm^{-1} due to NH_2 and NH, respective-



Scheme 2. Reaction conditions: i – 3-chloropropane-1,2-diol, anhyd. KOH, ethanol, reflux for 2 h; ii – iodomethane, anhyd. KOH, ethanol, reflux for 6 h; iii – hydrazine hydrate, ethanol, reflux for 3 h; iv – PCl_5, $POCl_3$, heat, 3 h; v, vi – excess hydrazine hydrate, ethanol, reflux for 12 h; vii – benzylamine, ethanol, reflux for 3 h; viii – piperidine, ethanol reflux for 24 h

ly, while that of the hydrazinyl derivative **12** exhibited the absorption band of CN group at 2216 cm^{-1}, in addition to the absorption bands of NH_2, NH groups at 3432, 3271 and 3186 cm^{-1}, respectively.

On the other hand, the chloro compound **13** was refluxed with benzylamine in ethanol to afford the corresponding benzylamino derivative **14** (Scheme 2). IR spectrum of the compound **14** exhibited the appearance of two absorption bands at 3367 cm^{-1} and 2209 cm^{-1} representing NH and CN functionalities, whereas its ^{1}H NMR spectrum revealed the appearance of a doublet signal at δ 4.26 ppm due

to the methylene protons of -HN–CH_2 group and a doublet signal at δ 4.72 ppm due to N*H*. Nucleophilic substitution of the chlorine atom of the derivative **13** was also performed by its reflux with a secondary nitrogen nucleophile such as piperidine for 24 h to give compound **15** in a high yield. The chemical structure of the novel derivative **15** was confirmed on the basis of microanalyses and spectral data. For example, ^{1}H NMR spectrum exhibited singlet signals at δ 1.69 ppm due to β, γ CH_2 groups of piperidine, while α 2CH_2 groups appeared as a multiplet signal at δ 3.72 ppm. Also, its mass spectrum showed a peak corresponding to its molecular ion at m/z 443 (M^+ 38%) (Scheme 2).

This study also deals with the reaction of the pyrazolo[3,4-*d*]pyridine derivative **5a** with different reagents. It was refluxed with formic acid to afford the pyrazolo[3,4-b]pyridine formamide compound **16** in a good yield, while its reflux with acetic anhydride led to the formation of the corresponding acetamide derivative **17**. IR spectrum of compound **16** indicated the appearance of two characteristic absorption bands at 3326 and 3146 cm^{-1} representing the two NH groups and the amide C=O group appeared at 1655 cm^{-1}. Also, ^{1}H NMR spectrum of the same derivative showed a singlet signal at δ 8.22 ppm contributing to the aldehydic proton-CO-*H* and two D_2O exchangeable singlets at 9.72 and 10.32 ppm assigned to the 2N*H* protons. At the same time, IR spectrum of compound **17** indicated the presence of characteristic absorption bands at 3330 and 3146 cm^{-1} assigned for the two NH groups and at 1714 cm^{-1} assigned for the acetylic C=O group. Also, ^{1}H NMR spectrum showed a singlet signal at δ 1.92 ppm corresponding to the CH_3 protons and two D_2O exchangeable singlets at δ 8.07 and 10.32 due to the two N*H* protons.

Further treatment of compound **5a** with dimethylformamide dimethylacetal (DMF-DMA) in refluxing xylene afforded the corresponding formimidamide derivative **18** in an excellent yield. ^{1}H NMR spectrum of the isolated product exhibited two singlets at δ 2.66 and 8.44 ppm due to the six protons of –N(CH_3)$_2$ and the methine proton of –N=C*H*- groups, respectively, in addition to a D_2O exchangeable singlet at δ 12.77 ppm due to N*H* proton.

Moreover, cyclocondensation reaction has been carried out by refluxing compound **5a** with acetyl acetone to give the analogue pyridopyrazolo[1,5-a]pyrimidine **19** (Scheme 3). IR spectrum of **19** revealed the absence of NH_2, NH absorption bands, while its ^{1}H NMR spectrum showed two singlet signals at δ 1.61 and 2.53 ppm for six protons of 2 CH_3, in addition to the other signals of the molecule that appeared at their expected regions. Mass spectrum of the same derivative showed the molecular ion peak at m/z 454 (M^+ 22%).

Biological activity

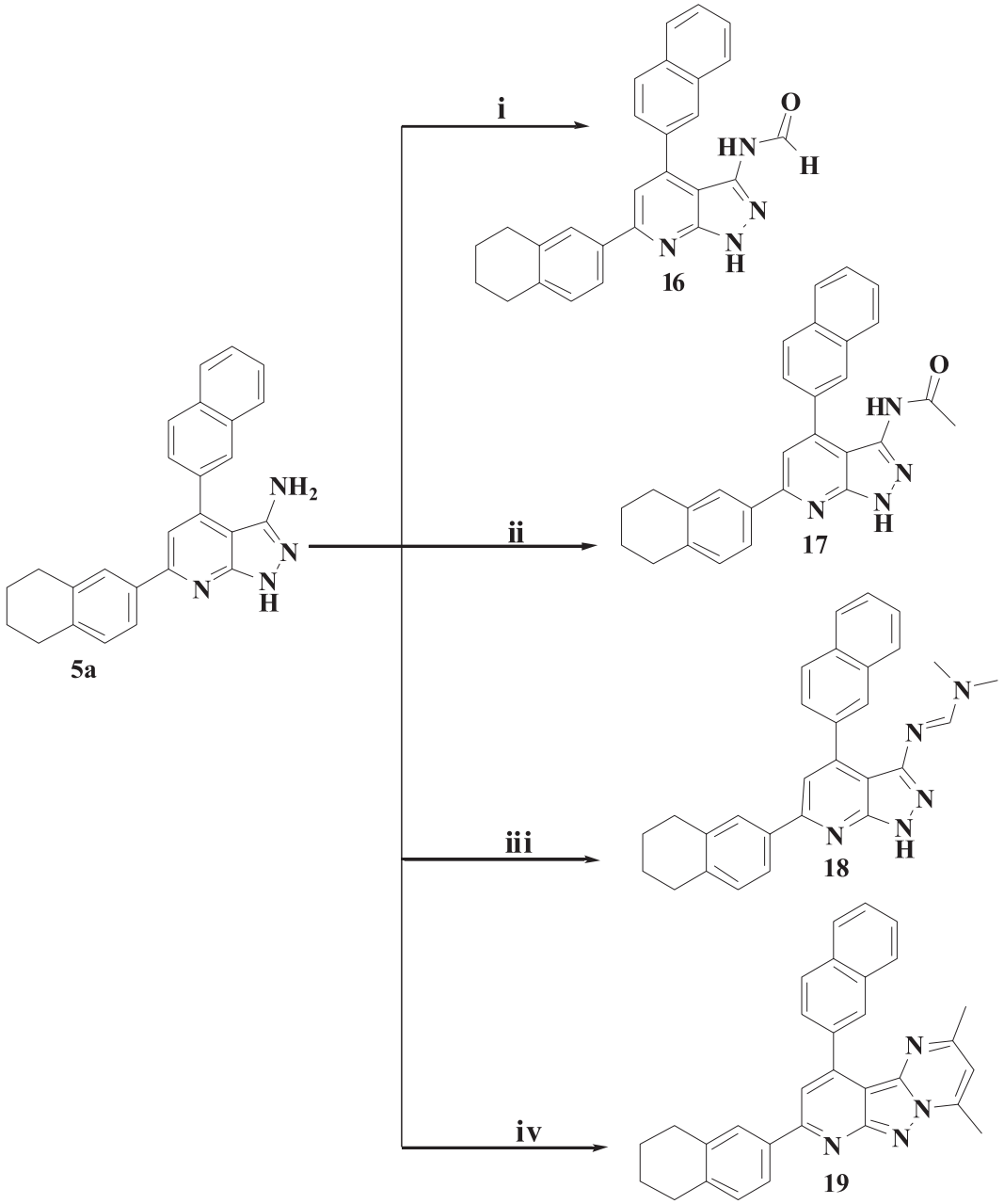
In vitro antioxidant activity

The twenty compounds obtained were examined *in vitro* for their antioxidant activities against DPPH radicals. Our results indicated that all of the tested compounds showed dose dependent DPPH inhibition activities, which were reflected by their IC_{50} values as summarized in Table 1. The activities of the compounds appeared in the following order: rutin > **5a** > Vit. C > **12** > **3a** > **15** > **5b** > **4b** > **8** > **10** > **9** > **11** > **13** > **19** > **14** > **16** > **2b** > **3b** > **18** > **2a** > **17** > **4a**.

Comparing the activity of these twenty compounds to the standard antioxidants and well known potent DPPH inhibitors (rutin and vitamin C) it is

Table 1. *In-vitro* antioxidant activity of the twenty newly synthesized compounds using DPPH free radical scavenging assay.

Compound No.	SC_{50} (μM) means % ± SD (n = 3)
2a	191.2 ± 3.92
2b	182.3 ± 6.12
3a	117.7 ± 2.34
3b	188.1 ± 1.09
4a	202.9 ± 2.96
4b	130.4 ± 5. 25
5a	42.2 ± 1.82
5b	129.6 ± 3.91
8	134.5 ± 4.05
9	141.6 ± 2.03
10	136.6 ± 6.23
11	151.5 ± 5.34
12	113.9 ± 3.81
13	153.2 ± 2.97
14	175.9 ± 4.59
15	128.9 ± 3.09
16	178.3 ± 3.79
17	195.8 ± 2.84
18	190.5 ± 4.91
19	160.6 ± 2.98
Ascorbic acid	54.1 ± 0.95
Rutin	27.5 ± 0.98



Scheme 3. Reaction conditions: i - formic acid, reflux for 1 h; ii - acetic anhydride, reflux for 1 h; iii - DMF-DMA, xylene, reflux for 12 h; iv- acetyl acetone, ethanol, reflux for 10 h

clear that the best scavenging properties even higher than those of vitamin C (SC_{50} = 54.1 μM) was gained by compound **5a** (SC_{50} = 42.2 μM) that bears the pyrazolopyridine nucleus incorporated to tetralin ring. The replacement of the 3-aminopyrazole moiety with hydrazinyl side chain or sulfur atom at 2-position of the cyanopyridine ring, as in case of compounds **12** and **3a**, respectively, led to a remarkable reduction in the antioxidant activity (SC_{50} = 113.9 and 117.7 μM). The rest of the tested compounds exhibited moderate to weak potency. It is noteworthy that all the tested compounds were safe and did not show any cytotoxic effects against the normal cells, thus the dose could be increased for

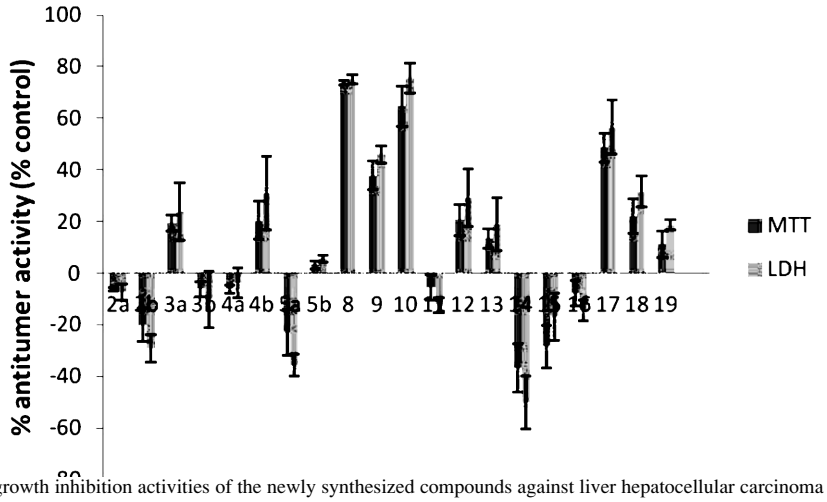


Figure 1. *In vitro* tumor growth inhibition activities of the newly synthesized compounds against liver hepatocellular carcinoma (HepG-2) at concentration of 100 µM

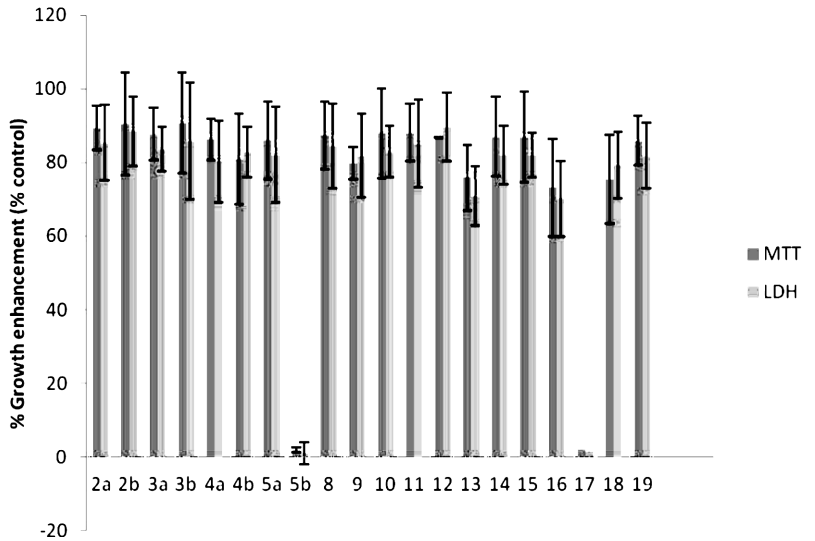


Figure 2. *In vitro* growth enhancement activities of the newly synthesized compounds against human normal cell lines (Wish) at concentration of 100 µM

possible enhancement in the scavenging potency without danger of cytotoxicity.

Anti-tumor activity

The twenty newly synthesized compounds were examined *in vitro* for their anti-tumor activities against Hep-G2 human liver cancer cell line and their cytotoxic activities against normal human cell line (wish) using MTT assay. The percentage of the intact cells was measured and compared to the control. LDH assay was used to measure the cellular membrane permeabilization (rupture) and severe

irreversible cell damage (Figs. 1 and 2). The activities of the derivatives against carcinoma cells and normal cells were compared with the cytotoxicity of doxorubicin.

The obtained results showed that only two compounds: **8** and **10** showed high cytotoxic activities against the liver cancer cells (HepG-2) with inhibition percentages of 73.6 and 64.6, respectively. Compounds **17** and **9** showed moderate cytotoxic activities with cellular growth inhibition percentages of 48.6 and 37.8, respectively. Low activities were obtained by compounds **18**, **12**, **4b**, **3a**, **13**, **19** and **5b**, while compounds **11**, **14**, **15**, **16**, **2a**, **2b**, **3b**, **4a** and **5a** did not show any cytotoxic activity against HepG-2 cell lines. All the twenty investigated compounds did not show any toxic effects against the normal cells (wish). On the contrary, they all caused growth enhancement for the normal cells ranging from 73.1 to 90.8% except for compounds **5a** and **17** which showed very low growth enhancement (Fig. 2). The resultant data can be analyzed with respect to the chemical structures of the examined compounds; it can be noticed that the derivative **8** that bears the fused tricyclic tetrahydrobenzo[g]quinoline ester ring system showed the highest potency as growth inhibiting agent against liver carcinoma cell lines, which might be due to its lipophilicity that allows its accumulation inside tumor tissues inducing growth inhibition effect (41). At the same time, the hydrolysis of ester functionality to the corresponding acid **9** led to remarkable reduction in the potency to reach the inhibition growth of 37%, which might be due to the decrease in the lipophilicity of the derivative. Also, the incorporation of dihydroxypropylthio side chain to the parent cyanopyridine nucleus as in derivative **10**, exhibited promising inhibition of the cancer cells, which might be due to hydrogen bond formation and good fitting with the binding sites of the protein receptor acting on it. Remarkable drop in the activity was observed by the pyrazolo[3,4-*b*]pyridinyl acetamide **17**.

CONCLUSION

The scope of this study was focused on the synthesis of a new series of 2-substituted-5,6,7,8-tetrahydronaphthalene derivatives containing pyridine, thioxopyridine and pyrazolopyridine heterocyclic rings. Antioxidant evaluation of the novel derivatives exhibited that the greatest potency even higher than that of vitamin C was gained by the pyrazolopyridine analogue **5a**, thus it can be considered as an antioxidant supplement after further biological studies. But a remarkable reduction in the activity was observed by the derivatives carrying hydrazinyl side chain or sulfur atom at 2-position of the cyanopyridine ring as in compounds **12** and **3a**, respectively. In addition, the derivatives were *in vitro* examined as cytotoxic agents against liver carcinoma cell lines. It can be noticed that the highest growth inhibition effect was obtained by the derivative bearing tetrahydrobenzo[g]quinoline ester functionality **8**, followed by the derivative **10** carrying dihydroxypropylthio side chain attached to the parent cyanopyridine nucleus. It is noteworthy that all of the compounds are not only safe to the normal cells but also enhance their growth.

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