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Synthesis and Antitumor Activity against HepG-2, PC-3, and HCT-116 Cells of Some Naphthyridine and Pyranopyridinecarbonitrile Derivatives[1]

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Abstract—A series of substituted and fused heterocyclic derivatives **2**–**17** were synthesized using 3,5-bis(4-methoxybenzylidene)-1-propylpiperidin-4-one (**1**) as starting material. Treatment of **1** with malononitrile or semicarbazide afforded compounds **2** and **3**, respectively. Condensation of **1** with ethyl cyanoacetate afforded naphthyridine-3-carbonitrile derivative **4**, which reacted with phosphorus pentachloride and phosphoryl chloride to give chloro derivative **5**. Treatment of **5** with thiosemicarbazide afforded compound **6**. The reaction of **1** with malononitrile gave cyano aminopyrane derivative **7** which was condensed with pyromellitic dianhydride, phthalic anhydride, succinic anhydride, or morpholine in glacial acetic acid to obtain imide derivatives **8**–**11**. Additionally, the reaction of **7** with aromatic aldehydes gave derivatives **12a**–**12c**. Acetylation of **7** with acetic anhydride in boiling acetic acid gave N-acetyl derivative **13** which was cyclized to pyridine derivative **14** by refluxing in dioxane in the presence of triethylamine. Treatment of 7 with hydrazine hydrate gave pyrazolo derivative **15**. Finally, the reaction of 7 with triethyl orthoformate in the presence of acetic anhydride gave formimidate **16** which was treated with hydrazine hydrate to form *N*-amino derivative **17**. Some of the synthesized compounds were examined *in vitro* for their antitumor activity against HepG-2, PC-3, and HCT-116 human carcinoma cell lines using MTT assay.

Keywords: propylpiperidin-4-one, thiosemicarbazide pyridine, aminocyanopyranopyridine, anticancer activity

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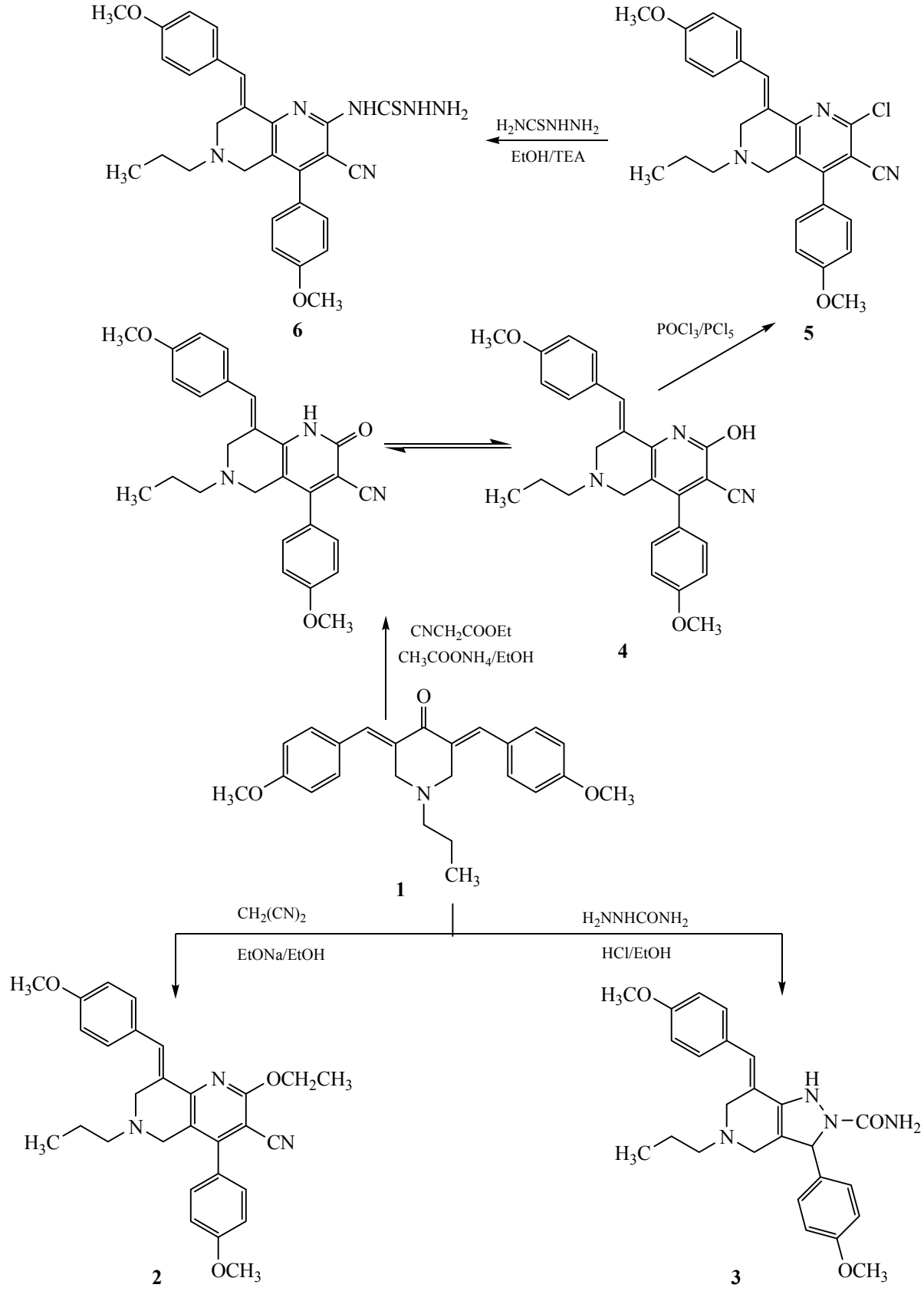
Pyridinones and their derivatives play an essential role in several biological processes and are of considerable chemical and pharmacological importance [1–3]. Moreover, pyridinones represent a unique class of pharmacophores which are encountered in various therapeutic agents [4], antibiotics [5], and antibacterial [6], antifungal [7], and cardiotonic agents [8]. On the other hand, pyran and pyridine derivatives are widely used as anticoagulant, anticancer [9, 10], antiviral [11], antitumor [12], and anti-inflammatory agents [13]. Additionally, pyran derivatives are well known for their antihistaminic [14] and antimicrobial activity [15] and inhibition of influenza virus sialidases [16]. Apart from biological importance, some 2-amino-4*H*-pyrans are widely used as photoactive materials [17]. We previously synthesized some new pyridine and pyran derivatives which were used as analgesic, anticonvulsant, anti-anxiety, and antiparkinsonian agents [18–20]. We have recently [21–26] prepared some new heterocyclic compounds and evaluated them as anti-inflammatory [21, 22], anti-HSV-1 [23], and anti-melanoma [24] activities; they can also be used as activators of tumor suppressor protein p53 [25] and SARS-CoV 3C-like protease inhibitors [26]. In continuation of our previous work in heterocyclic chemistry, we synthesized some new heterocyclic compounds and tested them for anticancer activity.

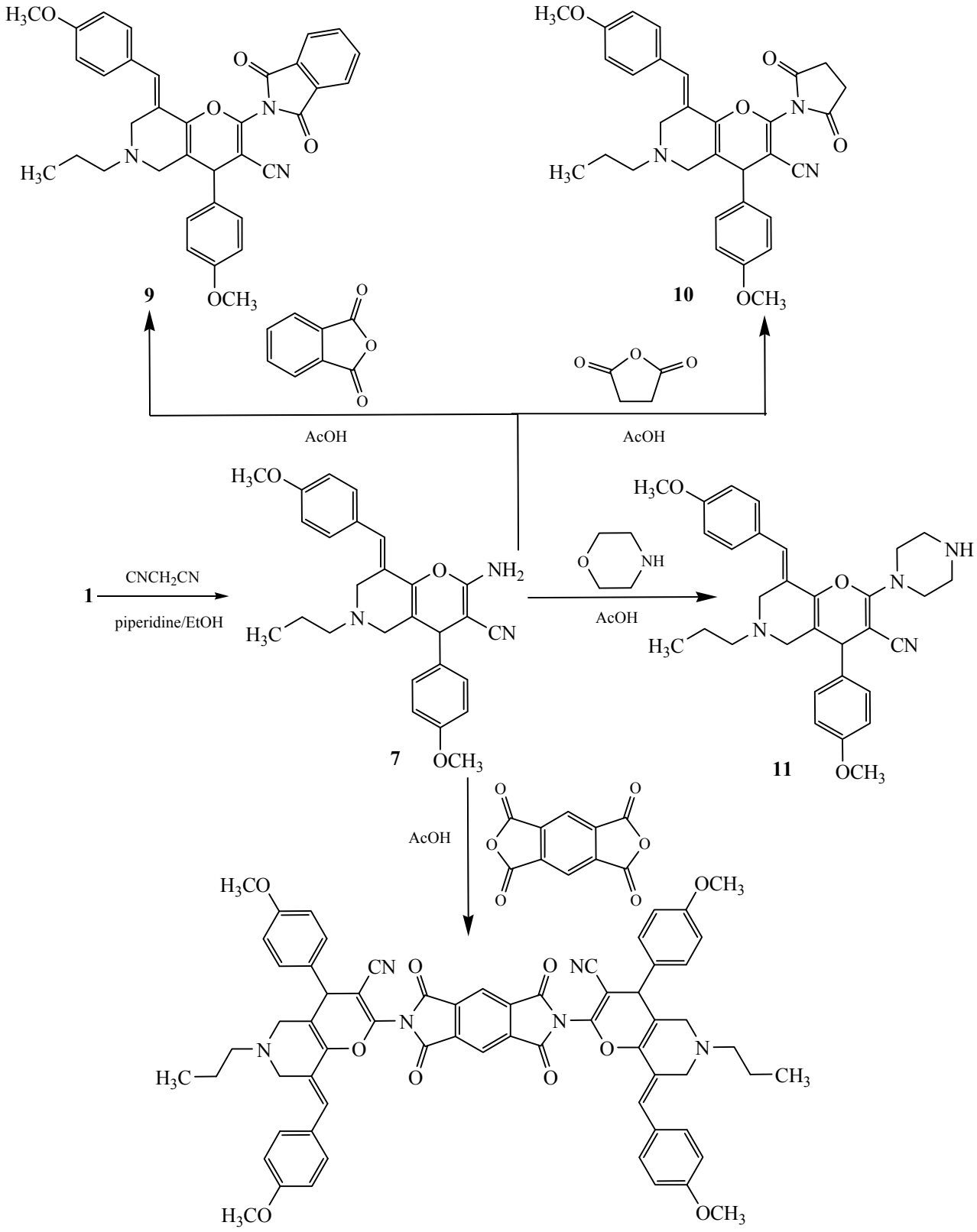
A series of substituted and fused nitrogen heterocycles were synthesized using 3,5-bis(4-methoxybenzylidene)-1-propylpiperidin-4-one (**1**) as starting material, which was prepared from 1-propylpiperidin-4-one according to known procedure [27]. Treatment of **1** with malononitrile in the presence of alcoholic

[1] The text was submitted by the authors in English.

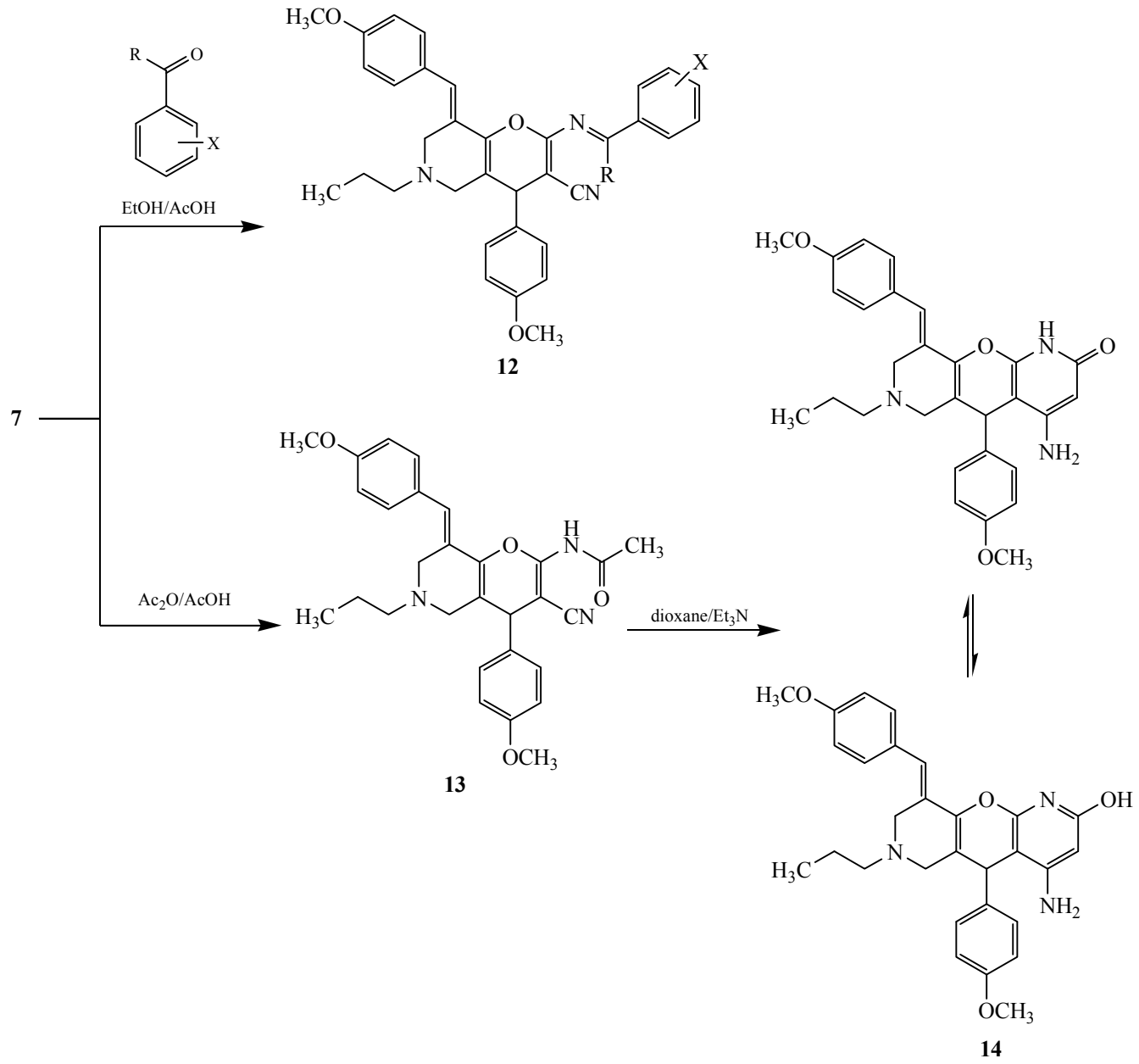
Scheme 1.


H3CO
NHCSNHNH2
H2NCSNHNH2
EtOH/TEA
Cl
CN
OCH3
6
5
POCl3/PCl5
H
N
O
OH
4
CNCH2COOEt
CH3COONH4/EtOH
1
CH2(CN)2
EtONa/EtOH
H2NNHCONH2
HCl/EtOH
OCH2CH3
CONH2
2
3

Scheme 2.


H3CO
O
N
O
H3C
N
CN
OCH3
9
10
AcOH
AcOH
1
CNCH2CN
piperidine/EtOH
NH2
7
NH
AcOH
11
AcOH
NC
CH3
8

Scheme 3.

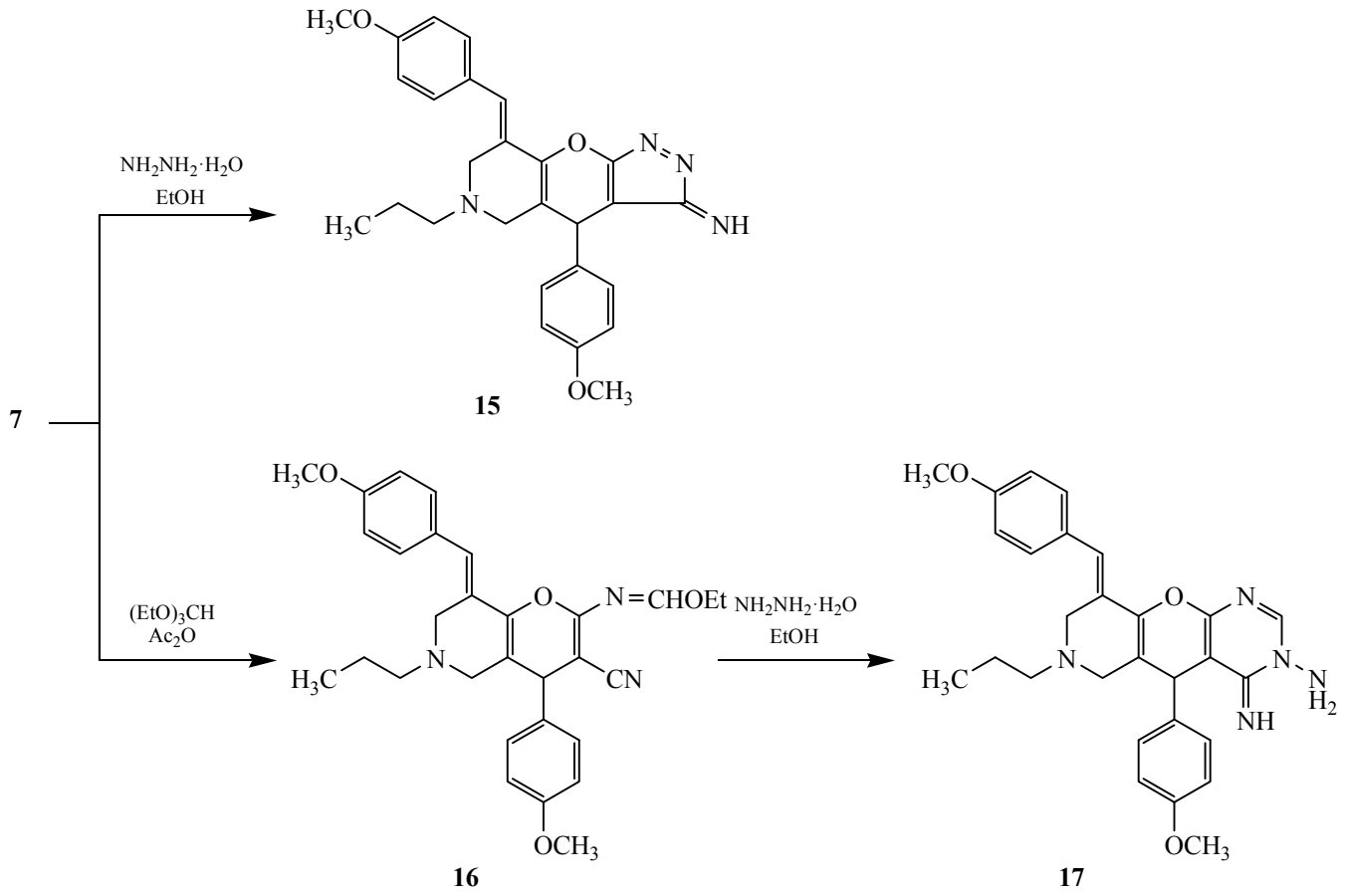


12, R = H, X = 4-Cl (**a**), 3-MeO (**b**); R = Me, X = H (**c**).

sodium ethoxide afforded the corresponding propyl-1,6-naphthyridine-3-carbonitrile **2**. Pyrazolo[4,3-*c*]pyridine-2-carboxamide **3** was synthesized by condensation of **1** with semicarbazide in the presence of hydrochloric acid. The condensation of **1** with ethyl cyanoacetate in the presence of anhydrous ammonium acetate in anhydrous ethanol gave the corresponding naphthyridine-3-carbonitrile derivative **4**. Treatment of the latter with a mixture of phosphorus pentachloride and phosphoryl chloride furnished chloro derivative **5**. The reaction of **5** with thiosemicarbazide in the presence of few drops of triethylamine furnished thiosemicarbazide **6** (Scheme 1).

Compound **1** reacted with malononitrile in the presence of piperidine to produce aminocyanopyran derivative **7** which was condensed with pyromellitic dianhydride in acetic acid to obtain the corresponding bis-imide **8**. The condensation of **7** with phthalic anhydride, succinic anhydride, or morpholine in glacial

Scheme 4.



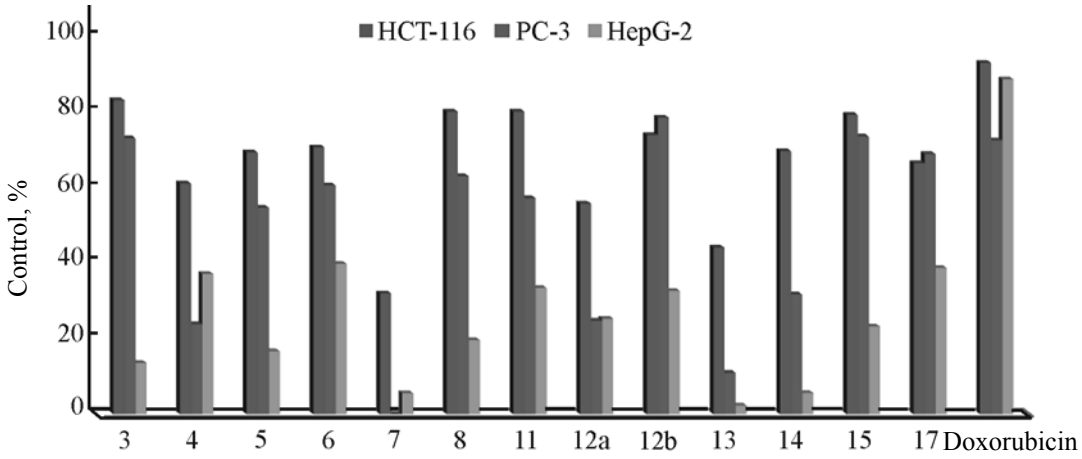
acetic acid gave imide derivatives **9**–**11**, respectively (Scheme 2).

Furthermore, the condensation of **7** with aromatic aldehydes or acetophenone in refluxing acetic acid gave Schiff bases **12a**–**12c**. Acetylation of **7** with a boiling mixture of acetic acid and acetic anhydride afforded *N*-acetyl derivative **13** which underwent cyclization in dioxane under reflux in the presence of triethylamine to give pyridine derivative **14** (Scheme 3).

Treatment of **7** with hydrazine hydrate in refluxing ethanol gave fused pyrazole **15**. Finally, the reaction of **7** with triethyl orthoformate in the presence of acetic anhydride afforded formimidate **16**, and treatment of the latter with hydrazine hydrate produced *N*-amino derivative **17** (Scheme 4).

Thirteen compounds were examined *in vitro* for their antitumor activity against HepG-2, PC-3, and HCT-116 human carcinoma cell lines using MTT assay. The percentage of the intact cells was measured and compared to Doxorubicin® used as control (see figure). The results indicated dose-dependent anticancer activity of all compounds against the three cancer cells. Compound **3**, **8**, **11**, and **15** at a concentration of 100 μg/mL showed good anticancer activities against HCT-116 carcinoma cells. Six compounds showed moderate activities (**4**–**6**, **12b**, **14**, **17**) against HCT-116 cells, and the others showed weak activity. Good anticancer activity against PC-3 cancer cells was observed for three compounds (**3**, **12b**, **15**), compounds **6**, **8**, and **17** were moderately active, and the others showed weak or no antitumor activity. All the tested compounds were weakly active or inactive against HepG-2 liver cancer. The IC_{50} values are given in table.

Our results suggests that enhanced anticancer activity of some tested compounds against HCT-116 (**3**, **8**, **11**, **15**) and PC-3 (**3**, **12b**, **15**) human carcinoma cell lines may be determined by the following factors: heteroaromaticity, large number of nitrogen atoms, naphthyridine moiety, *meta* substitution, and/or fused five-membered pyrazole or pyrrole ring.



Anticancer activity of compounds **3**–**8**, **11**–**15**, and **17** against three human cancer cell lines according to the MTT assay at 100 μg/mL.

EXPERIMENTAL

The melting points were determined in open glass capillaries with an Electro Thermal IA9100 digital melting point apparatus and are uncorrected. The elemental microanalyses for carbon, hydrogen, and nitrogen (Microanalytical Unit, NRC) were within the acceptable limits. The IR spectra (KBr) were recorded on a Nicolet Nexus 670 FTIR spectrometer. The ^{1}H and ^{13}C NMR spectra were run in DMSO-d_6 (unless otherwise stated) on a Jeol instrument (500 MHz). The mass spectra (electron impact, 70 eV) were obtained on a Finnigan MAT SSQ 7000 spectrometer. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum sheets (E. Merck). (3*E*,5*E*)-3,5-bis(4-methoxybenzylidene)-1-propylpiperidin-4-one (**1**) was synthesized from 1-propylpiperidin-4-one according to the reported procedure [27].

(8*E*)-2-Ethoxy-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (2). A mixture of 3.77 g (0.01 mol) of compound **1** and 0.66 g (0.01 mol) of malononitrile in a solution of sodium ethoxide prepared from 1 g of sodium metal and 30 mL of ethanol was refluxed for 4 h. The mixture was cooled, poured into ice water, and acidified with aqueous HCl to pH ~3.5. The precipitate was filtered off, washed with water, and recrystallized from methanol. Yield 86%, mp 236°C. IR spectrum: ν 2225 cm^{-1} (C≡N). ^{1}H NMR spectrum, δ, ppm: 6.42–7.86 m (9H, H_{arom}, 8-CH), 4.34–4.61 m (2H, OCH_2), 3.85 s and 3.81 s (3H each, OCH_3), 3.26–3.52 m and 2.67–3.15 m (4H, 5-H, 7-H), 2.23 m (2H, 6-CH_2), 1.45 m (2H, 6-$CH_2C\underline{H}_2$), 1.06 t (3H, $C\underline{H}_3CH_2O$), 0.81 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.93, 17.92, 19.01, 20.30, 53.16, 54.82, 54.98, 55.30, 55.72, 55.80, 56.50, 57.88, 111.85, 113.71, 114.39, 115.94, 126.13, 128.17, 129.96, 129.98, 130.02, 130.05, 139.21, 152.63, 158.31, 160.94, 161.20, 161.49, 163.15. Mass spectrum: *m*/*z* 469 (I_{rel} 100%). Found, %: C 74.15; H 6.66; N 8.97. $C_{29}H_{31}N_3O_3$. Calculated, %: C 74.18; H 6.65; N 8.95. *M* 469.24.

Cytotoxicity (IC_{50}, MTT assay) of compounds **3**–**8**, **11**–**15**, and **17** against human cancer cell lines

Compound no.	IC_{50},[a] μg/mL		
	HCT-116	PC-3	HepG-2
3	60.5	68.6	372.1
4	82.1	211.7	135.9
5	72.5	91.7	303.4
6	71.0	82.8	126.8
7	157.6	NA	NA
8	62.7	79.6	257.2
11	62.8	87.7	151.1
12a	90.1	203.8	199.6
12b	67.9	64.1	154.6
13	63.4	68.2	217.5
14	75.4	72.9	130.3
15	114.5	460.4	NA
17	72.1	159.3	NA

[a] NA stands for no activity.

(7*E*)-7-(4-Methoxybenzylidene)-3-(4-methoxyphenyl)-5-propyl-1,3,4,5,6,7-tetrahydro-2*H*-pyrazolo-[4,3-*c*]pyridine-2-carboxamide (3). Concentrated aqueous HCl (2 mL) was added to a mixture of compound **1** (3.77 g, 0.01 mol) and semicarbazide (0.75 g, 0.01 mol) in ethanol (10 mL). The mixture was refluxed for 6 h and cooled, and the precipitate was filtered off, dried, and recrystallized from methanol. Yield 78%, mp 172°C. IR spectrum, ν, cm^{-1}: 3326 (NH), 3250 (NH_2). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 8.65 s (1H, NH, D_2O exchangeable), 6.82–7.86 m (9H, H_{arom}, 7-CH), 5.56 s (1H, 3-H), 4.85 s (2H, NH_2, D_2O exchangeable), 3.85 s and 3.81 s (3H each, OCH_3), 3.26–3.52 m and 2.67–3.15 m (4H, 4-H, 6-H), 2.24 m (2H, 5-CH_2), 1.43 m (2H, $CH_3C\underline{H}_2$), 0.81 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 11.78, 20.30, 20.39, 54.70, 55.25, 55.30, 55.41, 55.88, 59.60, 60.81, 66.50, 113.91, 114.21, 114.26, 117.56, 117.78, 117.94, 128.88, 128.93, 128.96, 129.04, 159.34, 159.82, 161.45, 162.65. Mass spectrum: *m*/*z* 435 (I_{rel} 31%). Found, %: C 69.22; H 6.91; N 12.77. $C_{25}H_{30}N_4O_3$. Calculated, %: C 69.10; H 6.96; N 12.89. *M* 434.23.

(8*E*)-8-(4-Methoxybenzylidene)-1,2,5,6,7,8-hexahydro-4-(4-methoxyphenyl)-2-oxo-6-propyl-1,6-naphthyridine-3-carbonitrile (4). A mixture of compound **1** (3.77 g, 0.01 mol), ethyl cyanoacetate (0.12 g, 0.01 mol), and anhydrous ammonium acetate (7.04 g, 0.08 mol) in ethanol (30 mL) was refluxed for 2 h. After cooling, the precipitate was filtered off, dried, and recrystallized from dioxane. Yield 87%, yellow crystals, mp 265°C. IR spectrum, ν, cm^{-1}: 3447 (NH), 2219 (CN), 1698 (C=O). ^1H NMR spectrum, δ, ppm: 12.18 s (1H, NH, D_2O exchangeable), 7.04–7.70 m (9H, H_{arom}, 8-CH), 3.85 s and 3.81 s (3H each, OCH_3), 3.36–3.52 m and 2.67–3.15 m (4H, 5-H, 7-H), 2.25 m (2H, 6-CH_2), 1.44 m (2H, 6-$CH_2C\underline{H}_2$), 0.82 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.96, 20.11, 49.21, 52.57, 52.93, 57.12, 88.16, 93.13, 98.72, 114.61, 114.72, 115.72, 116.81, 123.44, 123.73, 127.04, 128.33, 129.87, 130.00, 132.00, 132.04, 148.35, 159.13, 159.95, 160.50, 160.92, 170.11. Mass spectrum, *m*/*z* (I_{rel}, %): 442 (30), 440 (100). Found, %: C 73.32; H 6.18; N 9.55. $C_{27}H_{27}N_3O_3$. Calculated, %: C 73.45; H 6.16; N 9.52. *M* 441.52.

(8*E*)-2-Chloro-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (5). Compound **4** (4.42 g, 0.01 mol) was added to a mixture of phosphorus pentachloride (0.21 g, 0.01 mol) and phosphoryl chloride (20 mL), and the mixture was refluxed for 2 h. After cooling, the mixture was poured into ice water with stirring, and the precipitate was filtered off, washed with water, dried, and recrystallized from methanol. Yield 71%, yellow–green powder, mp 186°C. IR spectrum: ν 2219 cm^{-1} (CN). ^1H NMR spectrum, δ, ppm: 6.73–8.14 m (9H, H_{arom}, 8-CH), 3.82 s and 3.81 s (3H each, OCH_3), 3.45–3.57 m and 2.71–3.32 m (4H, 5-H, 7-H), 2.41 m (2H, 6-CH_2), 1.47 m (2H, 6-$CH_2C\underline{H}_2$), 0.81 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.98, 12.09, 20.22, 22.05, 54.76, 55.77, 58.84, 58.99, 59.35, 60.04, 109.13, 113.94, 114.38, 114.81, 127.77, 127.81, 128.72, 128.85, 130.83, 132.30, 132.86, 134.84, 150.36, 152.54, 160.51, 161.42, 163.48. Mass spectrum: *m*/*z* 459 (I_{rel} 100%). Found, %: C 70.53; H 5.74; Cl 7.75; N 9.18. $C_{27}H_{26}ClN_3O_2$. Calculated, %: C 70.50; H 5.70; Cl 7.71; N 9.14. *M* 459.97.

***N*-[(8*E*)-3-Cyano-4-(8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl]hydrazinecarbothioamide (6).** A mixture of compound **5** (4.60 g, 0.01 mol) and thiosemicarbazide (0.91 g, 0.01 mol) in ethanol (30 mL) containing a few drops of triethylamine was refluxed for 5 h. The mixture was cooled and poured into ice water, and the precipitate was filtered off, washed with water, and recrystallized from methanol. Yield 82%, mp 278°C. IR spectrum, ν, cm^{-1}: 3447 (NH), 3348 (NH), 3265 (NH_2), 2219 (CN). ^1H NMR spectrum, δ, ppm: 12.64 s (1H, NH, D_2O exchangeable), 11.21 s (1H, NH, D_2O exchangeable), 6.94–7.96 m (9H, H_{arom}, 8-CH), 4.54 s (2H, NH_2, D_2O exchangeable), 3.83 s and 3.81 s (3H each, OCH_3), 3.35–3.67 m and 2.61–3.09 m (4H, 5-H, 7-H), 2.02 m (2H, 6-CH_2), 1.48 m (2H, 6-$CH_2C\underline{H}_2$), 0.85 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.14, 20.97, 54.82, 55.89, 56.51, 56.83, 58.33, 89.25, 114.62, 114.96, 114.98, 115.03, 117.45, 118.56, 125.95, 127.04, 127.08, 128.15, 128.40, 132.52, 139.02, 146.12, 150.92, 151.32, 160.85, 161.27, 162.87, 173.86. Mass spectrum, *m*/*z* (I_{rel}, %): 515 (18), 440 (100). Found, %: C 65.38; H 5.85; N 16.36; S 6.24. $C_{28}H_{30}N_6O_2S$. Calculated, %: C 65.35; H 5.88; N 16.33; S 6.23. *M* 514.64.

(8*E*)-2-Amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano-[3,2-*c*]pyridine-3-carbonitrile (7). A mixture of compound **1** (3.77 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), and piperidine (10 mL) in ethanol (30 mL) was stirred at room temperature for 5 h. The precipitate was filtered off and recrystallized from ethanol. Yield 92%, mp 175°C. IR spectrum, ν, cm^{-1}: 3407, 3328,

3262, 3214 (NH_2), 2192 (CN). ^{1}H NMR spectrum, δ, ppm: 6.75–7.40 m (9H, H_{arom}, 8-CH), 6.11 s (1H, 4-H), 4.62 s (2H, NH_2, D_2O exchangeable), 3.98 s and 3.77 s (3H each, OCH_3), 3.33–3.68 m and 2.60–3.05 m (4H, 5-H, 7-H), 2.23 m (2H, 6-CH_2), 1.25 m (2H, 6-$CH_2C\underline{H}_2$), 0.72 t (3H, $CH_3C\underline{H}_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.98, 12.19, 20.15, 52.76, 52.81, 55.53, 55.58, 56.74, 58.50, 107.13, 112.81, 114.32, 114.43, 121.02, 121.58, 124.66, 129.56, 129.59, 129.98, 130.04, 136.06, 139.70, 146.16, 146.31, 158.80, 158.83, 160.18. Mass spectrum, *m*/*z* (I_{rel}, %): 443 (10.7), 337 (100). Found, %: C 73.15; H 6.62; N 9.49. $C_{27}H_{29}N_3O_3$. Calculated, %: C 73.11; H 6.59; N 9.47. *M* 443.54.

5,6,7,8-Tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile derivatives 8–11 *(general procedure).* A mixture of compound **7** (4.43 g, 0.01 mol) and pyromellitic dianhydride, phthalic anhydride, succinic anhydride, or morpholine (0.01 mol) in glacial acetic acid (30 mL) was refluxed for 6–10 h. The mixture was cooled and poured into ice water, and the solid was filtered off, washed with water, dried, and crystallized from methanol.

2,2′-(1,3,5,7-Tetraoxo-5,7-dihydropyrrolo[3,4-*f*]-isoindole-2,6(1*H*,3*H*)-diyl)bis[(8*E*)-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile] (8). Yield 69%, mp 292°C. IR spectrum, ν, cm^{-1}: 2235 (CN), 1723 (C=O). ^{1}H NMR spectrum, δ, ppm: 7.04–8.25 m (20H, 18H_{arom}, 2-CH), 6.98 s (2H, 4-H), 3.84 s and 3.83 s (6H each, OCH_3), 3.33–3.57 m and 3.10–3.18 m (8H, 5-H, 7-H), 2.98 m (4H, 6-CH_2), 1.58 m (4H, 6-$CH_2C\underline{H}_2$), 0.92 t (6H, CH_3CH_2). ^{13}C NMR spectrum, δ_C, ppm: 11.53, 21.82, 40.98, 50.12, 50.26, 50.32, 54.15, 55.89, 57.74, 59.36, 60.35, 106.36, 114.45, 114.56, 114.63, 117.39, 117.42, 124.16, 125.17, 127.13, 127.58, 129.84, 130.27, 132.13, 132.18, 135.32, 135.36, 136.79, 158.13, 160.59, 163.42, 165.41. Mass spectrum: *m*/*z* 1068 (I_{rel} 100%). Found, %: C 71.95; H 5.31; N 7.89. $C_{64}H_{56}N_6O_{10}$. Calculated, %: C 71.90; H 5.28; N 7.86. *M* 1069.16.

(8*E*)-2-(1,3-Dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (9). Yield 74%, mp 274°C. IR spectrum, ν, cm^{-1}: 2229 (CN), 1719 (C=O). ^{1}H NMR spectrum, δ, ppm: 6.98–8.04 m (13H, 12H_{arom}, CH), 6.81 s (1H, 4-H), 3.86 s and 3.82 s (3H each, OCH_3), 3.66–3.73 m and 2.61–3.25 m (4H, 5-H, 7-H), 2.52 m (2H, 6-CH_2), 1.92 m (2H, 6-$CH_2C\underline{H}_2$), 0.75 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.89, 21.82, 37.15, 38.88, 40.95, 40.98, 50.23, 50.43, 55.93, 55.97, 57.51, 60.25, 106.74, 114.19, 114.21, 114.25, 114.28, 117.43, 124.37, 127.62, 127.68, 127.75, 127.78, 129.93, 129.97, 132.35, 132.37, 132.54, 132.56, 146.42, 159.97, 160.32, 163.68, 166.25, 166.28. Mass spectrum: *m*/*z* 573 (I_{rel} 100%). Found, %: C 73.25; H 5.47; N 7.36. $C_{35}H_{31}N_3O_5$. Calculated, %: C 73.28; H 5.45; N 7.33. *M* 573.64.

(8*E*)-2-(2,5-Dioxopyrrolidin-2-yl)-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (10). Yield 73%, mp 268°C. IR spectrum, ν, cm^{-1}: 2225 (CN), 1711 (C=O). ^{1}H NMR spectrum, δ, ppm: 6.87–7.51 m (9H, H_{arom}, 8-CH), 6.16 s (1H, 4-H), 4.06–4.57 m (4H, CH_2, pyrrole), 3.87 s and 3.82 s (3H each, OCH_3), 3.35–3.59 m and 2.61–3.18 m (4H, 5-H, 7-H), 2.37 m (2H, 6-CH_2), 1.44 m (2H, 6-$CH_2C\underline{H}_2$), 0.83 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.88, 21.79, 28.61, 28.65, 40.95, 50.11, 51.16, 55.16, 55.82, 56.12, 57.38, 60.25, 107.12, 114.24, 114.35, 114.39, 124.81, 127.71, 127.76, 128.12, 130.45, 130.59, 134.51, 137.14, 157.62, 157.95, 160.22, 161.16, 163.95, 175.21, 175.25. Mass spectrum, *m*/*z* (I_{rel}, %): 525 (0.39), 440 (100). Found, %: C 70.88; H 5.91; N 7.97. $C_{31}H_{31}N_3O_5$. Calculated, %: C 70.84; H 5.94; N 7.99. *M* 525.59.

(8*E*)-8-(4-Methoxybenzylidene)-4-(4-methoxyphenyl)-2-(piperazin-1-yl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (11). Yield 76%, mp 212°C. IR spectrum, ν, cm^{-1}: 2221 (CN), 3356 (NH). ^{1}H NMR spectrum, δ, ppm: 8.25 s (1H, NH, D_2O exchangeable), 6.86–7.91 m (9H, H_{arom}, 8-CH), 6.35 s (1H, 4-H), 4.10–4.28 m (8H, CH_2, piperazine), 3.87 s and 3.84 s (3H each, OCH_3), 3.56–3.59 m and 2.51–3.38 m (4H, 5-H, 7-H), 2.10 m (2H, 6-CH_2), 1.65 m (2H, 6-$CH_2C\underline{H}_2$), 0.84 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.85, 21.90, 41.63, 46.83, 46.88, 49.92, 49.95, 50.15, 50.23, 54.26, 55.95, 55.98, 57.61, 106.67, 114.27, 114.29, 114.35, 114.42, 117.35, 124.45, 127.53,127.64, 130.19, 130.22, 134.55, 137.15, 146.65, 157.82, 159.95, 163.85, 179.68. Mass spectrum: *m*/*z* 512 (I_{rel} 100%). Found, %: C 72.89; H 7.09; N 10.91. $C_{31}H_{36}N_4O_3$. Calculated, %: C 72.63; H 7.08; N 10.93. *M* 512.64.

Compounds 12a–12c *(general procedure).* A mixture of compound **7** (4.43 g, 0.01 mol), carbonyl compound (4-chlorobenzaldehyde, 3-methoxybenzaldehyde, or acetophenone; 0.01 mol), ethanol (30 mL), and glacial acetic acid (10 mL) was refluxed for 4 h. After cooling, the solid was filtered off and recrystallized from methanol.

(8*E*)-2-[(*E*)-4-Chlorobenzylideneamino]-8-(4-methoxy-benzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (12a). Yield 62%, mp 148°C. IR spectrum: ν 2232 cm^{-1} (C≡N). ^1H NMR spectrum, δ, ppm: 6.93–8.27 m (13H, H_{arom}, 12-CH, CH=N), 6.62 s (1H, 4-H), 3.88 s and 3.76 s (3H each, OCH_3), 3.35–3.59 m and 2.64–3.06 m (4H, 5-H, 7-H), 2.10 m (2H, 6-CH_2), 1.28 m (2H, 6-$CH_2C\underline{H}_2$), 0.79 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.82, 21.73, 41.43, 50.15, 50.18, 55.83, 55.88, 57.38, 75.94, 107.02, 114.21, 114.26, 114.36, 114.48, 117.63, 124.36, 127.38, 127.40, 127.84, 128.96, 128.98, 130.29, 130.32, 130.46, 130.63, 131.96, 134.56, 136.68, 137.12, 146.56, 157.81, 159.36, 163.75, 170.42. Mass spectrum, *m*/*z* (I_{rel}, %): 566 (3.2), 325 (100). Found, %: C 72.17; H 5.72; Cl 6.28; N 7.45. $C_{34}H_{32}ClN_3O_3$. Calculated, %: C 72.14; H 5.70; Cl 6.26; N 7.42. *M* 566.09.

(8*E*)-8-(4-Methoxybenzylidene)-2-[(*E*)-3-methoxybenzylideneamino]-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (12b). Yield 67%, mp 232°C. IR spectrum: ν 2229 cm^{-1} (C≡N). ^1H NMR spectrum, δ, ppm: 7.08–7.91 m (13H, H_{arom}, 12-CH, CH=N), 6.65 s (1H, 4-H); 4.15 s, 3.83 s, and 3.71 s (3H each, OCH_3); 3.35–3.59 m and 2.64–3.06 m (4H, 5-H, 7-H), 2.28 m (2H, 6-CH_2), 1.30 m (2H, 6-$CH_2C\underline{H}_2$), 0.86 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.85, 21.28, 41.45, 50.55, 50.69, 55.87, 55.90, 55.97, 57.45, 76.11, 106.72, 114.03, 114.29, 114.42, 114.63, 117.71, 124.82, 126.99, 127.49, 127.74, 129.22, 129.25, 130.34, 130.44, 130.76, 130.81, 132.25, 134.63, 136.62, 136.89, 146.86, 157.88, 160.15, 163.24, 170.65. Mass spectrum: *m*/*z* 561 (I_{rel} 100%). Found, %: C 74.82; H 6.29; N 7.47. $C_{35}H_{35}N_3O_4$. Calculated, %: C 74.84; H 6.28; N 7.48. *M* 561.67.

(8*E*)-8-(4-Methoxybenzylidene)-4-(4-methoxyphenyl)-2-[(*E*)-1-phenylethylideneamino]-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile (12c). Yield 71%, mp 207°C. IR spectrum: ν 2242 cm^{-1} (C≡N). ^1H NMR spectrum, δ, ppm: 6.91–7.97 m (13H, H_{arom}, 13-CH), 6.87 s (1H, 4-H), 3.88 s and 3.81 s (3H each, OCH_3), 3.35–3.59 m and 2.64-3.06 m (4H, 5-H, 7-H), 2.43 m (2H, 6-CH_2), 1.34 m (2H, 6-$CH_2C\underline{H}_2$), 0.87 s (3H, CH_3), 0.81 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 12.09, 17.12, 21.29, 41.69, 50.33, 50.56, 55.46, 55.81, 59.32, 76.06, 106.95, 114.21, 114.25, 114.83, 114.85, 117.71, 124.48, 127.22, 127.25, 127.90, 128.61, 129.15, 130.58, 130.67, 130.69, 130.85, 133.10, 133.26, 136.84, 137.29, 146.81, 158.01, 159.06, 160.83, 170.77. Mass spectrum, *m*/*z* (I_{rel}, %): 545 (0.48), 389 (100). Found, %: C 77.08; H 6.49; N 7.71. $C_{35}H_{35}N_3O_3$. Calculated, %: C 77.04; H 6.47; N 7.70. *M* 545.67.

***N*-[(8*E*)-3-Cyano-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridin-2-yl]acetamide (13).** A solution of compound **7** (4.43 g, 0.01 mol) in a 2 : 1 mixture of acetic acid and acetic anhydride (45 mL) was refluxed for 6 h. After cooling, the solid was filtered off and recrystallized from acetic acid. Yield 76%, mp 218°C. IR spectrum, ν, cm^{-1}: 3257 (NH), 2242 (CN), 1702 (C=O). ^1H NMR spectrum, δ, ppm: 12.27 s (1H, NH, D_2O exchangeable), 7.04–7.70 m (9H, H_{arom}, 8-CH), 6.86 s (1H, 4-H), 3.85 s and 3.81 s (3H each, OCH_3), 3.14–3.59 m and 2.51–3.04 m (4H, 5-H, 7-H), 2.30 m (2H, 6-CH_2), 2.01 s (3H, $COCH_3$), 1.24 m (2H, 6-$CH_2C\underline{H}_2$), 0.72 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.82, 21.72, 23.79, 40.93, 49.96, 49.98, 55.71, 55.84, 56.02, 56.05, 57.45, 60.48, 107.12, 114.23, 114.25, 114.36, 114.39, 117.45, 124.56, 127.56, 127.76, 127.89, 129.85, 129.94, 134.52, 137.62, 146.72, 157.81, 167.16. Mass spectrum: *m*/*z* 485 (I_{rel} 100%). Found, %: C 71.75; H 6.42; N 8.66. $C_{29}H_{31}N_3O_4$. Calculated, %: C 71.73; H 6.43; N 8.65. *M* 485.57.

(9*E*)-4-Amino-9-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-7-propyl-1,5,6,7,8,9-hexahydro-2*H*-pyrano[2,3-*b*:5,6-*c*′]dipyridin-2-one (14). A solution of compound **13** (4.86 g, 0.01 mol) in dioxane (50 mL) containing 5 mL of triethylamine was refluxed for 8 h. After cooling, the solid was filtered off and recrystallized from dioxane. Yield 64%, mp 247°C. IR spectrum, ν, cm^{-1}: 3348 (NH), 3297 (NH_2), 1682 (C=O). ^1H NMR spectrum, δ, ppm: 12.39 s (1H, NH, D_2O exchangeable), 6.97–7.68 m (9H, H_{arom}, 9-CH), 6.84 s (1H, 5-H), 6.25 s (1H, 3-H), 4.38 s (2H, NH_2, D_2O exchangeable), 3.88 s and 3.86 s (3H each, OCH_3), 3.14–3.42 m and 2.51–3.04 m (4H, 6-H, 8-H), 2.27 m (2H, 7-CH_2), 1.37 m (2H, 7-$CH_2C\underline{H}_2$), 0.87 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.86, 21.81, 28.93, 50.03, 50.96, 55.81, 55.82, 57.39, 90.38, 90.46, 106.65, 114.16, 114.17, 114.22, 114.23, 127.40, 127.46, 127.49, 127.53, 130.19, 130.21, 134.65, 136.96, 142.53, 146.75, 157.72, 160.05, 161.31, 163.82. Mass spectrum: *m*/*z* 485 (I_{rel} 100%). Found, %: C 71.78; H 6.45; N 8.67. $C_{29}H_{31}N_3O_4$. Calculated, %: C 71.73; H 6.43; N 8.65. *M* 485.57.

(8*E*)-8-(4-Methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydropyrazolo[4′,3′:5,6]-pyrano[3,2-*c*]pyridin-3(4*H*)-imine (15). A mixture of compound **7** (4.43 g, 0.01 mol) and hydrazine hydrate

(5.12 g, 0.16 mol) in ethanol (30 mL) was refluxed for 10 h. After cooling, the mixture was poured dropwise into ice water, and the solid was filtered off and recrystallized from methanol. Yield 68%, mp 196°C. IR spectrum: ν 3384 cm^{-1} (NH). ^1H NMR spectrum, δ, ppm: 12.46 s (1H, NH, D_2O exchangeable), 6.84–7.88 m (9H, H_{arom}, 8-CH), 6.74 s (1H, 4-H), 3.82 s and 3.79 s (3H each, OCH_3), 3.28–3.48 m and 2.63–3.07 m (4H, 5-H, 7-H), 2.37 m (2H, 6-CH_2), 1.24 m (2H, 6-$CH_2C\underline{H}_2$), 0.85 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.85, 21.81, 25.43, 50.62, 51.16, 51.89, 56.13, 56.15, 57.35, 104.26, 107.55, 114.27, 114.29, 114.35, 114.43, 127.30, 127.35, 127.42, 127.56, 130.26, 130.28, 134.77, 140.95, 146.85, 157.82, 160.83, 164.35. Mass spectrum: *m*/*z* 456 (I_{rel} 100%). Found, %: C 71.06; H 6.19; N 12.28. $C_{27}H_{28}N_4O_3$. Calculated, %: C 71.03; H 6.18; N 12.27. *M* 456.54.

Ethyl *N*-[(8*E*)-3-cyano-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-6-propyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridin-2-yl]formimidate (16). A mixture of compound **7** (4.43 g, 0.01 mol) and triethyl orthoformate (5.27 g, 0.04 mol) in acetic anhydride (20 mL) was refluxed for 12 h. After cooling, the solid was filtered off and recrystallized from ethanol. Yield 76%, mp 134°C. IR spectrum: ν 2233 cm^{-1} (C≡N). ^1H NMR spectrum, δ, ppm: 6.80–8.72 m (10H, H_{arom}, 8-CH, N=CH), 6.75 s (1H, 4-H), 4.34 q (2H, OCH_2), 3.85 s and 3.81 s (3H each, OCH_3), 3.14–3.59 m and 2.51–3.04 m (4H, 5-H, 7-H), 2.30 m (2H, 6-CH_2), 1.91 t (3H, $C\underline{H}_3CH_2O$), 1.25 m (2H, 6-$CH_2C\underline{H}_2$), 0.80 t (3H, $C\underline{H}_3CH_2CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.98, 14.33, 15.19, 20.19, 41.52, 50.09, 50.18, 55.71, 55.86, 57.52, 61.57, 64.14, 76.25, 106.72, 114.30, 114.42, 114.70, 114.78, 117.39, 124.42, 127.36, 129.55, 131.17, 137.21, 140.25, 146.68, 156.12, 157.83, 161.69, 170.25. Mass spectrum, *m*/*z* (I_{rel}, %): 499 (27.81), 377 (100). Found, %: C 72.15; H 6.67; N 8.44. $C_{30}H_{33}N_3O_4$. Calculated, %: C 72.12; H 6.66; N 8.41. *M* 499.60.

(9*E*)-4-Imino-9-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-7-propyl-6,7,8,9-tetrahydro-4*H*-pyrido[3′,4′:5,6]pyrano[2,3-*d*]pyrimidin-3(5*H*)-amine (17). A mixture of compound **16** (5.00 g, 0.01 mol) and hydrazine hydrate (5.12 g, 0.16 mol) in ethanol (40 mL) was refluxed for 6 h. After cooling, the solid was filtered off and recrystallized from ethanol. Yield 72%, mp 224°C. IR spectrum, ν, cm^{-1}: 3395 (NH), 3232 (NH_2). ^1H NMR spectrum, δ, ppm: 8.76 s (1H, NH, D_2O exchangeable), 6.98–8.45 m (9H, H_{arom}, 9-CH), 6.91 s (1H, 2-H), 6.88 s (1H, 5-H), 4.84 s (2H, NH_2, D_2O exchangeable), 3.84 s and 3.82 s (3H each, OCH_3), 3.15–3.59 m and 2.51–3.10 m (4H, 6-H, 8-H), 2.34 m (2H, 7-CH_2), 1.24 m (2H, 7-$CH_2C\underline{H}_2$), 0.80 t (3H, $C\underline{H}_3CH_2$). ^{13}C NMR spectrum, δ_C, ppm: 11.99, 20.21, 28.82, 51.20, 51.82, 55.51, 55.59, 57.45, 94.56, 106.53, 114.25, 114.28, 114.44, 114.47, 125.27, 127.71, 127.86, 127.88, 130.43, 130.48, 134.73, 137.72, 143.56, 146.93, 156.31, 157.35, 161.16, 163.35. Mass spectrum: *m*/*z* 485 (I_{rel} 100%). Found, %: C 69.28; H 6.42; N 14.43. $C_{28}H_{31}N_5O_3$. Calculated, %: C 69.26; H 6.43; N 14.42. *M* 485.58.

Evaluation of anticancer activity *in vitro*. The test cultures, HepG-2 (human liver carcinoma), PC-3 (human prostate adenocarcinoma), and HCT116 (human colorectal carcinoma) cell lines, were purchased from the American Type Culture Collection (Rockville, MD) and were maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/mL penicillin, and 100 U/mL streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2. The antitumor activity against HepG-2, PC-3, and HCT-116 human cancer cell lines was estimated using the 3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, which is based on cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [28–32]. The cells were dispensed in a 96-well sterile microplate (5×10^4 cells per well) and incubated at 37°C with series of each tested compound or Doxorubicin® (positive control) in DMSO at different concentrations for 48 h in a serum-free medium prior to the MTT assay. After incubation, the media were carefully removed, 40 μL of MTT (2.5 mg/mL) was added to each well, and the plate was incubated for an additional 4 h. The purple formazan dye crystals were solubilized by addition of 200 μL of DMSO, and the absorbance was measured at λ 590 nm using a SpectraMax® Paradigm® Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated in three different days. The IC_{50} values were determined by probit analysis using SPSS software (SPSS Inc., Chicago, IL).

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