Synthesis and Antitumor Activities of 4*H*-Pyrano[3,2-*h*]quinoline-3-carbonitrile, 7*H*-Pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline, and 14*H*-Pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*]quinoline Derivatives

Abdullah Megbas Al-Ghamdi[1], Ashraf Hassan Fekry Abd El-Wahab[2], Hany Mostafa Mohamed[2] and Ahmed Mohamed El-Agrody*,[1]

[1]*Chemistry Department, Faculty of Science, King Khalid University, 9004, Abha, Saudi Arabia*

[2]*Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11884, Egypt*

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Abstract: Several 4*H*-pyrano[3,2-*h*]quinoline 3,4,7-9, 7*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline 10a,b and 14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*]quinoline 11a-c derivatives were obtained by treatment of *(E)* 2-(4-chlorostyryl)-8-hydroxyquinoline 1, *(E)* 2-amino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitr-ile 3 or *(E)* 9-amino-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido-[4',5':6,5]pyrano [3,2-*h*]quinoline 10b with different electrophiles followed by nucleophilic reagents. Structures of these compounds were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT and MS data. The antitumor activity of the synthesized compounds was investigated and compared with that of the standard drug vinblastine, a well-known anticancer drug, using MTT colorimetric assay. Among them, compounds 10b and 3 showed the most potent activity against the human breast tumor cells (MCF-7) and the human lung carcinoma cells (HCT), while compound 10b exhibited the most potent activity against the human hepatocellular carcinoma cells (HepG-2). The structure-activity relationships are discussed.

Keywords: Antitumor, *(E)* 2-(4-chlorostyryl)-8-hydroxyquinoline, 4*H*-pyrano[3,2-*h*]quinoline, 7*H*-pyrimido[4',5':6,5]pyrano [3,2-*h*]quinoline, SAR.

INTRODUCTION

The quinoline nucleus is frequently found in bioactive compounds and plays an important role in biochemical processes [1-8]. In particular, styrylquinolines having inhibitory activity against HIV integrase have been reported recently [9-17]. In addition, the anti-proliferative effects of styrylquinolines derivatives on tumor cell lines have been observed and recently reported [18-24]. Other styrylquinoline derivatives have also gained strong attention recently due to their extensive biological activities [25-27].

In view of the above observations and in continuation of our program on the chemistry of 4*H*-pyran derivatives [28-43], we report herein the synthesis of a variety of 4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile, 7*H*-pyrimido[4',5': 6,5]pyrano[3,2-*h*]quinoline, and 14*H*-pyrimido[4',5':6,5] pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*]quinoline derivatives. A selection of these compounds was investigated for their antitumor activities. The chemical structures of the studied compounds and structure-activity relationships (SAR) are discussed in this work.

RESULTS AND DISCUSSION

The reaction of *(E)* 2-(4-chlorostyryl)-8-hydroxyquinoline (**1**) with *α*-cyano-*p*-chlorocinnamonitrile (**2**) in ethanolic piperidine under reflux afforded *(E)* 2-amino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile **3** (Scheme **1**).

Compound **3** was subjected for further reactions to produce fused heterotetracyclic or heteropentacyclic systems incorporating pyrimidine or pyrimido[1,2,4]triazolo nuclei in addition to pyranoquinoline moiety. Thus, condensation of **3** with benzaldehyde in ethanol and piperidine under reflux gave the 2-benzylideneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**4**) (Scheme **2**).

When 2-benzylideneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]-quinoline-3-carbonitrile (**4**) was treated with hydrazine hydrate or phenyl hydrazine in ethanol at room temperature or under reflux, the addition product **5** was formed (R = H or Ph, respectively). From the intermediate **5**, benzaldehyde hydrazone or benzaldehyde phenylhydrazone was eliminated to give *β*-enaminonitrile **3** [37] instead of the pyrimidopyranoquinoline derivative **6** (Scheme **2**).

Structure **4** was established on the basis of spectral data. The IR spectrum showed the presence of a CN stretch at υ 2212 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of **4** showed signals at δ 9.42 (s, 1H, N=CH) and 158.2 ppm (=CH) respectively. The mass spectrum of **4** showed m/z (%) peaks at 561 (M^{+}+4, 6), 559 (M^{+}+2, 40), 557 (M^{+}, 60) with the base peak at 89 (100). The *(E)* configuration of compound **4** was established from the coupling constant values (J = 16.5 Hz).

Treatment of **3** with triethyl orthoformate in acetic anhydride at reflux gave the corresponding *(E)* 2-

*Address correspondence to this author at the Chemistry Department, Faculty of Science, King Khalid University, 9004, Abha, Saudi Arabia; Tel: +966 530175825; Fax: +966 072417251; E-mail: elagrody_am@yahoo.com

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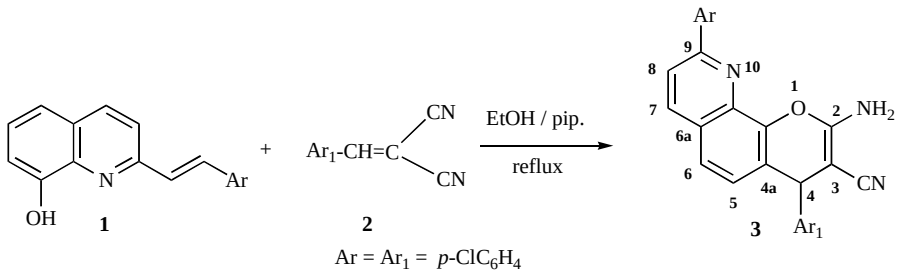
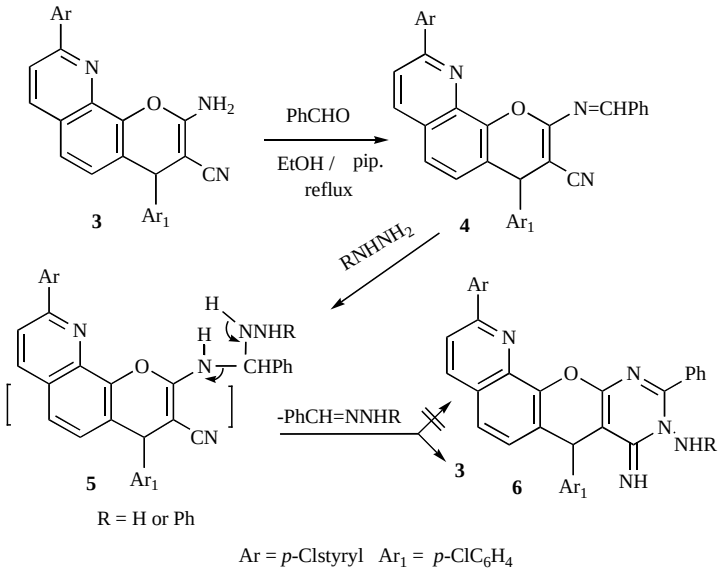
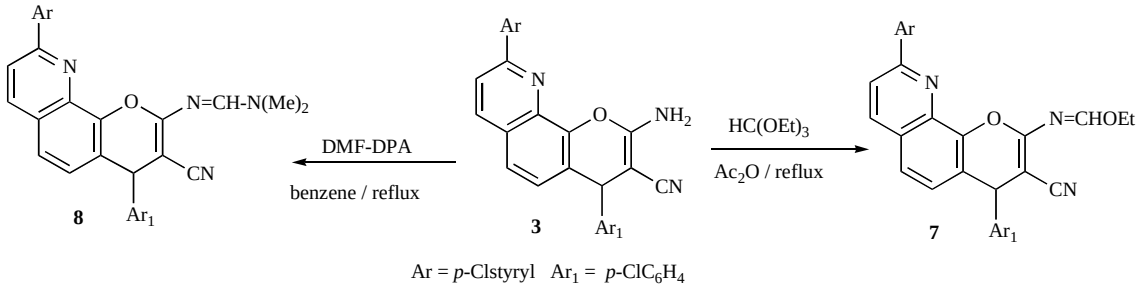


Scheme 1. Synthetic protocol of compound **3**.



Scheme 2. Synthetic protocol of compound **4**.

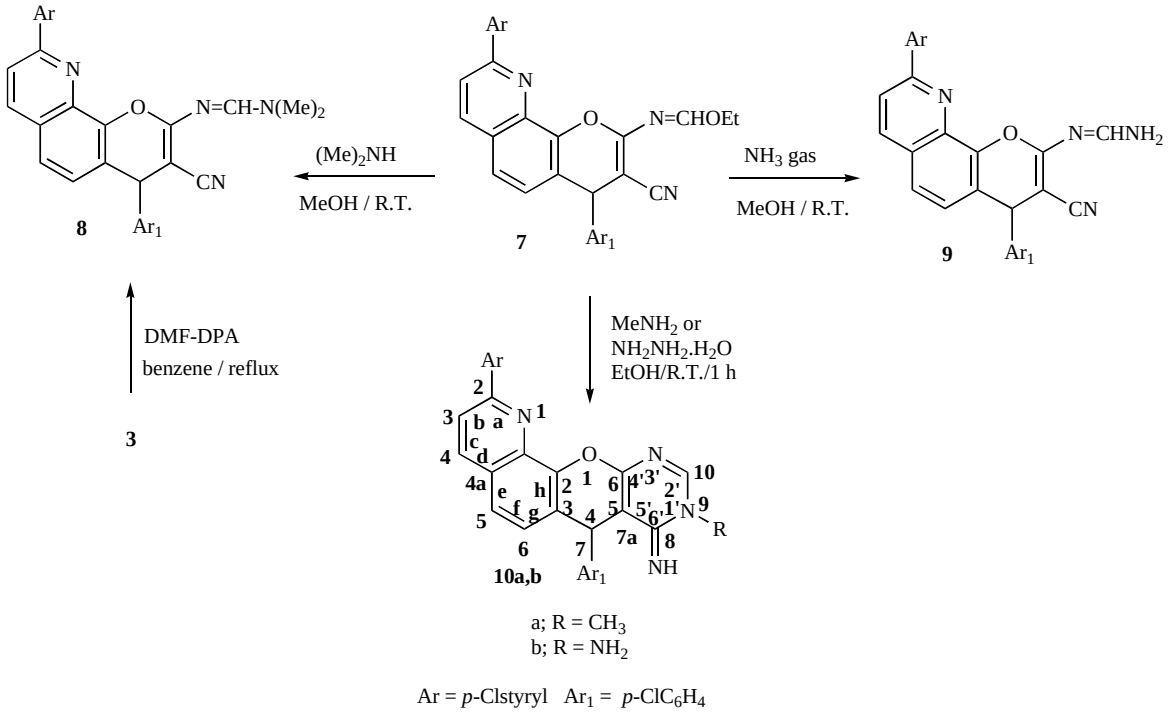


Scheme 3. Preparation of compounds **7** and **8**.

ethoxymethyleneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**7**), while reaction of **3** with dimethylformamide-dipentylacetal (DMF-DPA) in dry benzene under reflux gave *(E)* 4-(4-chlorophenyl)-9-(4-chlorostyryl)-2-dimethylaminomethyleneamino-4*H*-pyrano-[3,2-*h*]quinoline-3-carbonitrile (**8**) (Scheme **3**).

Structures of **7** and **8** were established on the basis of IR, ^{1}H NMR, ^{13}C NMR ^{13}C NMR-DEPT, ^{13}C NMR-APT and MS data. The IR spectra of compounds **7** and **8** showed the presence of CN stretches at υ 2207 and at υ 2199 cm^{-1}, respectively. The ^{1}H NMR and ^{13}C NMR spectra of compound **7** showed characteristic signals at δ 8.93 (N=CH), 4.54 (q, 2H, CH_2, J = 7 Hz), 1.46 (t, 3H, CH_3, J = 7 Hz) and at δ 161.8 (N=CH), 64.2 (CH_2), 13.9 (CH_3), respectively. Meanwhile, the ^{1}H NMR and ^{13}C NMR spectra of **8** showed signals at δ 8.71 (N=CH), 3.12 (s, 6H, $2CH_3$) and at δ 155.1 (N=CH), 34.4 ppm (CH_3), respectively. The ^{13}C NMR-DEPT spectra at 45°, 90°, 135° and ^{13}C NMR-APT for compounds **7** and **8** gave additional evidence for the proposed structures, in addition to the mass spectra. The relative *(E)* configurations of compounds **7** and **8** were established from the coupling constant values (J = 16-16.5 Hz).

Treatment of the imidate **7** with NH_3 gas in methanol at room temperature for 1h gave the open chain product, *(E)* 2-aminomethyleneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**9**), while reacton



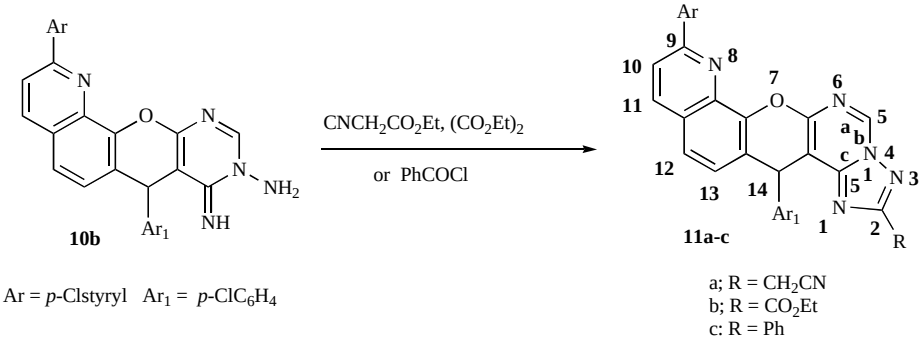
Scheme 4. Synthetic protocol of compounds **8-10**.

with methylamine or hydrazine hydrate gave the cyclic addition product *(E)* 9-methyl/amino-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido[4',5':6,5] pyrano[3,2-*h*]quinoline (**10a,b**), respectively (Scheme **4**).

Reaction of the imidate **7** with dimethylamine in methanol at room temperature for 1h afforded the amidine derivative **8 (**Scheme **4**), which can be obtained as described before from the reaction of **3** and dimethylformamide-dipentylacetal (DMF-DPA) (m.p. and mixed m.p.) (Scheme **3**).

The structures of **9** and **10** were supported by IR spectroscopy, which showed the presence of NH_2 absorptions at υ 3451, 3330 and a CN stretch at υ 2198 cm^{-1} for **9**, a NH absorption at υ 3257 for **10a** and a NH & NH_2 at υ 3331, 3275, 3184 cm^{-1} for **10b**, respectively. The ^1H NMR and ^{13}C NMR spectra of **9** showed signals at δ 8.69 (s, 1H, N=CH) and at δ 155.1 (N=CH). Characteristic resonances were observed at δ 7.78 (s, 1H, H-10), 3.31 (s, 3H, CH_3) and 136.5 (C-10), 35.3 (CH_3) for **10a** and at δ 7.83 (s, 1H, H-10) and 144.3 ppm (C-10) for **10b**. The ^{13}C NMR-DEPT spectra at 45°, 90°, 135° and ^{13}C NMR-APT for compound **9** and **10a** gave additional evidences for the proposed structures, in addition to the mass spectrum. The *(E)* configurations of compounds **9** and **10** were established from the coupling constant values (J = 16-16.5 Hz).

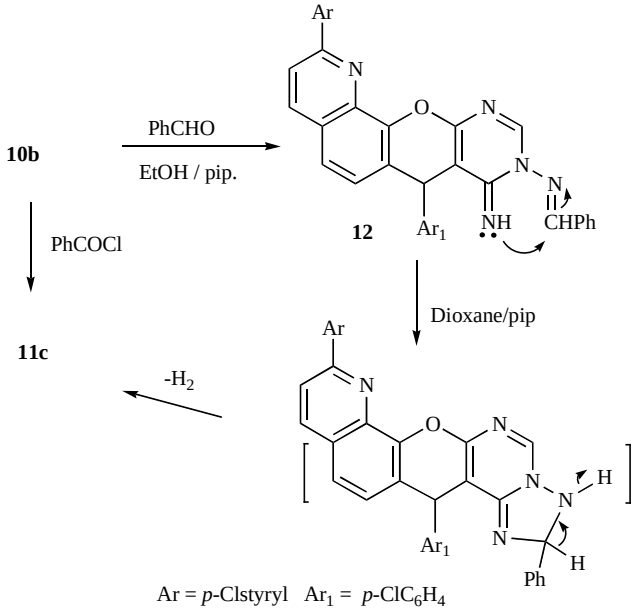
The imino compound **10b** proved to be a useful intermediate for the synthesis of a variety of 2-substituted-14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*] quinoline derivatives. Thus, treatment of **10b** with ethyl cyanoacetate and with diethyl oxalate in refluxing absolute ethanol afforded 14-(4-chlorophenyl)-9-(4-chlorotyryl)-2-cyanomethyl-14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*]quinoline (**11a**) and ethyl 14-(4-chlorophenyl)-9-(4-chlorotyryl)-14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4] triazolo[1,5-*c*]-quinoline-2-carboxylate (**11b**), respectively. Aroylation of **10b** with benzoyl chloride in refluxing dry benzene proceeded readily to give the 2-phenyl derivative **11c** (Scheme **5**).



Scheme 5. Synthetic protocol of compounds **11a-c**.

Structures **11a-c** were established on the basis of spectral data and in conjunction with our previous work [29–35, 42]. The IR spectra showed the presence of a CN band at υ 2257 cm^{-1} for **11a** and a CO band at υ 1745 cm^{-1} for **11b**. The ^1H and ^{13}C NMR spectra of **11a** showed signals at δ 3.60 (s, 2H, CH_2) and 23.8 ppm (CH_2). Characteristic resonances were observed at 4.36 (q, 2H, CH_2, J = 7.2 Hz) and 1.07 ppm (t, 3H, CH_3, J = 7.2 Hz) with the respective signals in the ^{13}C spectrum at 56.0 and 18.5 ppm for **11b**. The mass spectra of compounds **11a-c** gave additional evidence for the proposed structures. The *(E)* configurations of compounds **11a-c** were established from the coupling constant values (J = 16-16.5 Hz).

Condensation of **10b** with benzaldehyde under reflux in ethanolic piperidine afforded the open chain product 9-benzylideneamino-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline (**12**) [42] (Scheme **6**).



Scheme 6. Synthetic protocol of compounds **11c** and **12**.

Cyclization of **12** in 1,4-dioxane-piperdine solution under reflux [42] afforded the cycloaddition product **11c** (Scheme **6**), which can also be obtained as described above from the aroylation of **10b** with benzoyl chloride (m.p. and mixed m.p.) (Scheme **5**). The structure of **12** was established on the basis of its IR spectrum, which showed the presence of NH absorption at υ 3211 cm^{-1}. The ^1H and ^{13}C NMR spectra of **12** showed signals at δ 10.22 (s, 1H, N=CH) and 155.6 (N=CH). The mass data of compound **12** gave additional evidence for the proposed structure. The *(E)* configuration of compound **12** was established from the coupling constant values (J = 16.5 Hz).

ANTITUMOR ASSAYS

The quinoline derivatives were chosen for this study because it is known that quinoline and fused quinoline derivatives are important families of active compounds with a wide range of the biological and the pharmacological activities [1-27]. Compounds **3**,**4**, and **7-10** were evaluated for their human tumor cell growth inhibitory activity against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT) and hepatocellular carcinoma (HepG-2). The measurement of cell growth and viability were determined as described in the literature [44]. *In vitro* cytotoxicity evaluation using viability assays were performed by a Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University (Egypt), and using vinblastine as a standard drug. The inhibitory activity of the preceding compounds are given in Table **1** and Fig. (**1**).

The results from (Table **1**) indicated that compounds **10b** and **3** were the most active compounds against MCF-7 and HCT, while the remaining compounds exhibited lower activities as compared with the standard drug vinblastine. Finally, compound **10b** was the most active compound against HepG-2. It is worth mentioning that **10b** consistently produced low IC_{50} values much like vinblastine.

STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES

A panel of three different human cancer cell lines: MCF-7, HCT and HepG-2 was used for these experiments. The inhibitory concerntration (IC_{50}) of compound **3** and its analogs are summarized in (Table **1**).

The results from (Table **1**) indicated that compound **3** has the higher potent antitumor activity against MCF-7 than its analogs **9**, **8**, **7**, and **4**. By maintaining the *p*-chlorophenyl-4, *p*-chlorostyryl-9 and cyano-3 groups (electron-withdrawing groups) in 4*H*-pyrano[3,2-*h*]quinoline moiety, the SAR at the position-2 was explored. Replacing the amino-2 group with other electron-donating group such as (-N=$CHNH_2$/-N=$CHNMe_2$/-N=CHOEt/-N=CHPh) (more increasing in size) in compounds **4**, and **7-9** respectively, resulted in reduction of potency, suggesting that there might be a size limited pocket at position C-2. In addition, incorporating a pyrimidine nucleus at the position-2,3 with -NH-8/-NH_2-9 (electron-withdrawing groups) for compound **10b**, enhances potency than compound **3** and its analogs, while replacement of the -NH_2-9 group with the -CH_3-9 resulted in a large reduction of potency of compound **10a**, suggesting that a strong electron-donating group might be preferred at position C-9.

In the case of HCT, an investigation of SAR revealed that compound **3** (*p*-chlorophenyl-4, *p*-chlorostyryl-9, cyano-3 and amino-2 groups) has the most potent activity against HCT compared to its analogs compounds **9**, **7**, **8**, and **4**. Replacing the amino-2 group with other electron-donating groups such as (-N=$CHNH_2$/-N=CHOEt/ -N=$CHNMe_2$/-N=CHPh) (more increasing in size) in compounds **9, 7, 8** and **4** respectively, resulted in reduction of potency, suggesting that there might be a size limited pocket at position C-2. Incorporating a pyrimidine nucleus at the position-2,3 with the electron-donating groups -NH-8/-NH_2-9 for compound **10b**, improve the antitumor activity over that of compound **3** and its analogs, while replacement of the -NH_2-9 group with the -CH_3-9 group resulted in a large reduction of the potency of compound **10a**, suggesting that a strong electron-donating group might be preferred at position C-9. Finally, compound

Table 1. Cytotoxicity of Various Concentrations of 3,4, and 7-10 on MCF-7, HCT, and HepG-2 Cells[a]

Compounds	Conc. (µmol/l)	MCF-7 Cell viability %	IC_{50} (µmol/l)	HCT Cell viability %	IC_{50} (µmol/l)	HepG-2 Cell viability %	IC_{50} (µmol/l)
Vinblastine	55.01	7.82		16.27		14.38	
	27.50	15.18		21.68		16.13	
	13.75	29.6		28.2		24.25	
	6.88	48.75	6.7	38.06	2.9	45.13	5.1
	3.44	60.35		47.54		55.00	
	1.72	76.24		53.42		72.13	
	0	100		100		100	
3	106.61	29.56		24.71		48.40	
	53.31	33.82		28.97		68.20	
	26.65	39.71		33.82		74.00	
	13.33	45.44	11.9	42.65	10.2	79.20	102.8
	6.66	61.91		58.97		85.80	
	3.33	86.62		69.12		95.40	
	0	100		100		100	
4	89.77	41.85		42.36		38.16	
	44.88	58.23		57.64		53.24	
	22.44	76.44		70.80		67.56	
	11.22	88.87	67.3	81.56	67.9	79.08	53
	5.61	97.02		90.02		88.44	
	2.81	100		98.68		97.52	
	0	100		100		100	
7	95.24	32.50		25.56		40.56	
	47.62	59.75		59.13		53.24	
	23.81	76.38		75.71		70.48	59.8
	11.91	84.75	63.2	88.10	59.4	81.16	
	5.95	91.00		97.94		96.22	
	2.97	100		100		100	
	0	100		100		100	
8	95.42	20.50		34.29		29.44	
	47.71	44.25		65.95		41.60	
	23.85	66.13		89.13		64.82	
	11.93	79.00	40.5	97.86	71.3	97.54	39.1
	5.96	94.75		100		88.66	
	2.98	99.88		100		97.12	
	0	100		100		100	
9	100.81	38.17		35.63		25.64	
	50.40	42.83		52.06		39.72	
	25.20	54.67		83.33		56.24	
	12.60	72.83	38.7	95.08	57.7	73.86	34.7
	6.30	87.17		98.65		86.22	
	3.15	96.50		100		94.18	
	0	100		100		100	

(Table 1). Contd…..

Compounds	Conc. (µmol/l)	MCF-7 Cell viability %	IC_{50} (µmol/l)	HCT Cell viability %	IC_{50} (µmol/l)	HepG-2 Cell viability %	IC_{50} (µmol/l)
10a	98.04	42.00		40.32		40.88	
	49.02	50.17		72.70		56.16	
	24.51	74.00		80.95		72.64	
	12.26	82.33	48.2	87.46	82	89.35	68.8
	6.13	95.17		95.40		96.28	
	3.06	99.67		99.29		100	
	0	100		100		100	
10b	97.85	16.14		18.57		17.98	
	48.92	23.00		23.93		23.72	
	24.46	27.93		30.60		31.18	
	12.23	41.43	9.6	36.55	5.9	40.36	5.3
	6.12	79.57		47.86		46.48	
	3.06	93.21		61.43		64.32	
	0	100		100		100	

[a]IC_{50} values expressed in µmol/l as the mean values of triplicate wells from at least three experiments.

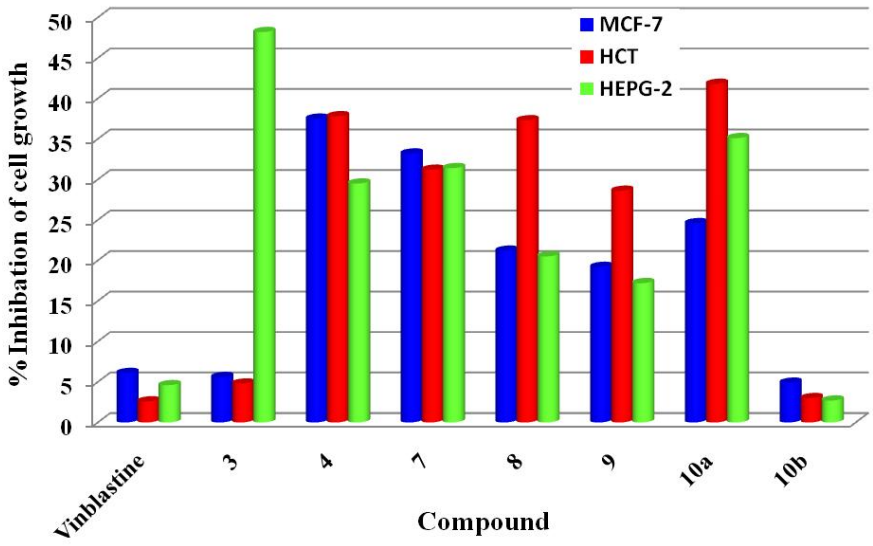


Fig. (1). IC_{50} values of some 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline derivatives aganist MCF-7, HCT, and HepG-2 tumor cells..

9 (*p*-chlorophenyl-4, *p*-chlorostyryl-9, cyano-3 and N=CHNH$_2$-2 groups) showed moderate antitumor activity against HepG-2 as compared to that of the standard drug vinblastine, while more decrease is shown in the antitumor activity with other electron-donating groups such as -N=CHNMe$_2$-2/-N=CHPh-2/-N=CHOEt-2/-NH$_2$-2 in compounds **8, 4, 7** and **3** respectively. In addition, incorporating a pyrimidine nucleus at the position-2,3 with the electron-donating groups -NH-8/-NH$_2$-9 for compound **10b**, rendered it more potent activity than compound **3** and its analogs. Thus compound **10b** exhibited higher antitumor activity against HepG-2 than the standard drug vinblastine, while replacement of the -NH$_2$-9 group with the -CH$_3$-9 group resulted in a large reduction of potency of compound **10a**, suggesting that a strong electron-donating group might be preferred at position C-9.

CONCLUSION

Eleven compounds of 4*H*-pyrano[3,2-*h*]quinoline, 7*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline and 14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*]quinoline derivatives were prepared. Structures of the synthesized compounds were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT, and MS data. Some of the newly synthesized compounds (**3**,**4**, **7-10**) were tested

against three tumor cell lines: MCF-7, HCT, and HepG-2. Compounds **10b** and **3** had the most potent antitumor activity against MCF-7 and HCT, while compound **10b** has the most potent antitumor activity against HepG2. This potency could be attributed to the incorporation of a pyrimidine nucleus with pyranoquinoline moiety in the presence of the electron-donating groups (-NH-8/-NH_2-9), in combination with the electron-withdrawing groups (*p*-ClC_6H_4-7; *p*-Clstyryl-2) or the presence of the electron-withdrawing groups (*p*-ClC_6H_4-4; *p*-Clstyryl-9; -CN-3) and the strong activating electron-donating group (-NH_2-2) in 4*H*-pyrano[3,2-*h*]quinoline moiety. A more extensive study is also warranted to determine additional antitumor parameters to give a deeper insight into structure activity relationship and to optimize the effectiveness of this series of molecules, which can then be used in bigger scenario such as drug design or development of antitumor therapeutics.

EXPERIMENTAL SECTION

Melting points were determined with a Stuart Scientific Co. Ltd apparatus. IR spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV 500 MHz spectrometer. ^{13}C NMR spectra were obtained using distortionless enhancement by polarization transfer (DEPT), with this technique, the signals of CH & CH_3 carbon atoms appears normal (up) and the signal of carbon atoms in CH_2 environments appears negative (down). ^{13}C NMR spectra were obtained using attached proton test (APT), with this technique, the signals of CH and CH_3 carbon atoms appear normal (up) and the signals of CH_2 and Cq environments appear negative (down).The MS was measured on a Shimadzu GC/MS-QP5050A spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser.

(*E*) 2-Amino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (3)

Prepared according to the literature procedure [43].

(E) 2-Benzylideneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-h]quinoline-3-carbonitrile (4)

A mixture of **3** (0.469 g, 10 mmol), benzaldehyde (0.01 mmol), ethanol (20 mL) and piperidine (0.5 mL) was refluxed for 2 h. The solid product was collected by filtration and crystallized from ethanol give **4** as pale yellow crystals from benzene m.p. 281-282 ^{o}C; yield 81%; IR (KBr) ν (cm^{-1}): 3067, 3060, 3022, 2970, 2915 (CH stretching), 2212 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.42 (s, 1H, N=CH), 8.39-7.17 (m, 17H, aromatic), 8.01 (d, 1H, =CH, *J* = 16.5 Hz), 7.71 (d, 1H, =CH, *J* = 16.5 Hz), 5.47 (s, 1H, H-4); ^{13}C NMR (125 MHz) ($CDCl_3$) *δ* (ppm): 164.1 (C-2), 158.2 (=CH), 155.7 (C-9), 142.6 (C-10b), 142.4, 137.8 (C-10a), 136.6 (C-7), 135.12, 134.4 (=CH), 133.7, 133.6, 133.3, 132.5, 130.3 (=CH), 129.3 (C-5), 129.1, 129.1, 129.0, 127.2 (C-6a), 126.2 (aromatic), 124.3 (C-4a), 121.0 (C-8), 120.2 (C-6), 117.4 (CN), 86.6 (C-3), 41.9 (C-4); MS m/z (%): 561 (M^++4, 6), 559 (M^++2, 40), 557 (M^+, 60), 89 (100); Anal. Calcd for $C_{34}H_{21}Cl_2N_3O$: C, 73.12; H, 3.79 N, 7.52. Found: C, 73.15; H, 3.82; N, 7.55 %.

Reaction of 4 with Hydrazine Derivatives

A mixture of 2-benzylideneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano-[3,2-*h*]quinoline-3-carbonitrile **4** (0.557 g, 10 mmol), hydrazine hydrate or phenyl hydrazine (0.01 mmol) in EtOH (20 mL) was stirred at room temperature or refluxed for 2 h to give **3** (m.p. and mixed m.p.) yield (83%).

Reaction of β-Enaminonitrile 3 with Triethyl Orthoformate

General Procedure

A mixture of *β*-enaminonitrile **3** (0.469 g, 10 mmol), triethyl orthoformate (10 mmol) and acetic anhydride (30 mL) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from proper solvent to give **7**. The physical and spectral data of the compound **7** was as follows.

(E) 2-Ethoxymethyleneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (7)

Colorless needles from benzene; m.p. 272-273 ^{o}C; 81%; IR (KBr) ν (cm^{-1}): 3050, 3035, 2984, 2960, 2900, 2865, 2850 (CH stretching), 2207 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.93 (s, 1H, N=CH), 8.14-7.07 (m, 12H, aromatic), 7.72 (d, 1H, =CH, *J* = 16.5 Hz), 7.31(d, 1H, =CH, *J* = 16.5 Hz), 5.04 (s, 1H, H-4), 4.54 (q, 2H, CH_2, *J* = 7 Hz), 1.46 (t, 3H, CH_3, *J* = 7 Hz); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 161.8 (N=CH), 160.3 (C-2), 156.0 (C-9), 155.1 (C-10b), 144.6, 142.8 (C-10a), 137.4, 136.6 (C-7), 135.2, 133.4 (=CH), 131.6, 129.6 (=CH), 129.5, 129.1, 129.0 (C-5), 128.9, 127.1 (C-6a), 126.3 (aromatic), 124.1 (C-4a), 123.4 (C-6), 120.8 (C-8), 120.4 (CN), 79.2 (C-3), 64.2 (CH_2), 41.4 (C-4), 13.9 (CH_3); ^{13}C NMR-DEPT spectrum at 135^o CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 161.8 (N=CH), 136.5 (C-7), 133.4 (=CH), 129.6 (=CH), 129.5 (aromatic), 129.1 (aromatic), 129.0 (C-5), 128.9 (aromatic), 126.4 (aromatic); 123.4 (C-6), 120.8 (C-8), 64.2 (CH_2), 41.4 (C-4), 13.9 (CH_3). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 161.8 (N=CH), 136.5 (C-7), 133.4 (=CH), 129.6 (=CH), 129.5 (aromatic), 129.1 (aromatic), 129.0 (C-5), 128.9 (aromatic), 126.3 (aromatic); 123.4 (C-6), 120.8 (C-8), 41.4 (C-4), 13.9 (CH_3). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 161.76 (N=CH), 136.5 (C-7), 133.4 (=CH), 129.6 (=CH), 129.5 (aromatic), 129.1 (aromatic), 129.0 (C-5), 128.9 (aromatic), 126.3 (aromatic); 123.4 (C-6), 120.8 (C-8), 64.2 (CH_2), 41.4(C-4), 13.9 (CH_3). ^{13}C NMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 161.8 (N=CH), 160.3 (C-2), 155.0 (C-9), 155.1 (C-10b), 144.6 (aromatic), 142.8 (C-10a), 137.4 (aromatic), 136.5 (C-7), 135.2 (aromatic), 133.4 (=CH), 131.6 (aromatic), 129.6 (=CH), 129.5 (aromatic), 129.1 (aromatic), 129.0 (C-5), 128.9 (aromatic), 127.1 (C-6a), 126.3 (aromatic), 124.1 (C-4a), 123.4 (C-6), 120.8 (C-8), 120.4 (CN), 79.2 (C-3), 64.1 (CH_2), 41.4 (C-4), 13.9 (CH_3); MS *m/z* (%): 529 (M^++4, 1), 527 (M^++2, 7), 525 (M^+) (10), 358 (100); Anal. Calcd for

$C_{30}H_{21}Cl_2N_3O_2$: C, 68.45; H, 4.02; N, 7.98. Found: C, 68.35; H, 3.64; N, 7.92 %.

Reaction of β-enaminonitrile 3 with Dimethylformamide-Dipentylacetal (DMF-DPA)

General Procedure

A mixture of *β*-enaminonitrile **3** (0.469 g, 10 mmol), dimethylformamide-dipentylacetal (DMF-DPA) (0.231 g, 10 mmol) and benzene (30 mL) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from proper solvent to give **8**. The physical and spectral data of the compound **8** was as follows.

***(E)* 4-(4-chlorophenyl)-9-(4-chlorostyryl)-2-dimethylaminomethyleneamino-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (8)**

Pale yellow crystals from benzene; m.p. 270-271 °C; 85%; IR (KBr) ν (cm^{-1}): 3065, 3027, 2922, 2855, 2810 (CH stretching), 2199 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.71 (s, 1H, N=CH), 8.34-7.15 (m, 12H, aromatic), 7.97 (d, 1H, =CH, *J* = 16 Hz), 7.60 (d, 1H, =CH, *J* = 16 Hz), 5.16 (s, 1H, H-4), 3.12 (s, 6H, $2CH_3$); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 159.9 (C-2), 155.1 (C-9), 155.1 (N=CH), 144.0 (C-10b), 143.1, 137.8 (C-10a), 136.5 (C-7), 135.2, 133.2 (=CH), 133.2, 131.8, 129.7 (=CH), 129.2, 129.0, 128.9, 128.8 (C-5), 127.0 (C-6a), 126.3 (aromatic), 123.4 (C-6), 121.2 (C-8), 121.2 (C-4a), 120.0 (CN), 71.5 (C-3), 41.7 (C-4), 34.4 (CH_3),; ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 155.1(N=CH), 136.5 (C-7), 133.2 (=CH), 129.7 (=CH), 129.2 (aromatic), 129.0 (aromatic), 129.0 (aromatic), 128.8 (C-5), 126.3 (aromatic), 123.4 (C-6), 121.2 (C-8), 41.8 (C-4), 34.4 (CH_3). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 155.1 (N=CH), 136.5 (C-7), 133.2 (=CH), 129.7 (=CH), 129.2 (aromatic), 129.0 (aromatic), 129.0 (aromatic), 128.8 (C-5), 126.3 (aromatic), 123.4 (C-6), 121.2 (C-8), 41.7 (C-4). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 155.2 (N=CH), 136.5 (C-7), 133.2 (=CH), 129.7 (=CH), 129.2 (aromatic), 129.0 (aromatic), 129.0 (aromatic), 128.8 (C-5), 126.3 (aromatic), 123.4 (C-6), 121.2 (C-8), 41.7 (C-4), 34.4 (CH_3). ^{13}C NMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 159.9 (C-2), 155.1 (C-9), 155.1 (N=CH), 144.0 (C-10b), 143.1 (aromatic), 137.8 (C-10a), 136.5(C-7), 135.2 (aromatic), 133.2 (CH), 133.2 (aromatic), 131.8 (aromatic), 129.7 (=CH), 129.2 (aromatic), 129.0 (aromatic), 129.0 (aromatic), 128.8(C-5), 127.0 (C-6a), 126.3 (aromatic), 123.4 (C-6), 121.2 (C-8), 121.2 (C-4a), 120.0 (CN), 71.5 (C-3), 41.7 (C-4), 34.4 (CH_3); MS *m/z* (%): 524 (M^+) (36), 50 (100); Anal. Calcd for $C_{30}H_{22}Cl_2N_4O$: C, 68.58; H, 4.22; N, 10.66. Found: C, 68.68; H, 3.93; N, 10.53 %.

Reaction of 7 with Ammonia

General Procedure

Method (a)

A mixture of imadate **7** (0.525 g, 10 mmol) and NH_3 gas bubbled in methanol (30 mL) was stirred for 1 h and then the mixture was left overnight. The solid product was collected and recrystallized from proper solvent to give **9**. The physical and spectral data of compound **9** was as follows.

***(E)* 2-Aminomethyleneamino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (9)**

Colorless needles from benzene; m.p. 290-291 °C; 83%; IR (KBr) ν (cm^{-1}): 3451, 3330 (NH_2), 3055, 3030, 2950 (CH stretching), 2198 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.69 (s, 1H, N=CH), 8.40-7.15 (m, 12H, aromatic), 8.09 (bs, 1H, NH_2, exchangeable by D_2O), 7.88 (d, 1H, =CH, *J* = 16.5 Hz), 7.54 (d, 1H, =CH, *J* = 16.5 Hz), 5.14 (s, 1H, H-4); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 155.1 (N=CH), 155.0 (C-9), 143.9 (C-10b), 143.1, 137.8 (C-10a), 136.5 (C-7), 135.1, 133.2, 133.0 (=CH), 131.8, 129.8 (=CH), 129.4, 129.1, 128.9, 128.8 (C-5), 127.0 (C-6a), 126.3 (aromatic), 121.2 (C-4a), 123.4 (C-8), 121.0 (C-6), 120.0 (CN), 71.6 (C-3), 41.7 (C-4); ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 155.1 (N=CH), 136.5 (C-7), 133.0 (=CH), 129.8 (=CH), 129.4 (aromatic), 129.1 (aromatic), 128.9 (aromatic), 128.8 (C-5), 126.3 (aromatic) 123.4 (C-8), 121.0 (C-6), 41.7 (C-4). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 155.1 (N=CH), 136.5 (C-7), 133.0 (=CH), 129.8 (=CH), 129.4 (aromatic), 129.1 (aromatic), 128.9 (aromatic), 128.8 (C-5), 126.3 (aromatic) 123.4 (C-8), 121.0 (C-6), 41.7(C-4). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 155.1 (N=CH), 136.5 (C-7), 133.0 (=CH), 129.8 (=CH), 129.4 (aromatic), 129.1(aromatic), 128.9 (aromatic), 128.8 (C-5), 126.3 (aromatic) 123.4 (C-8), 121.0 (C-6), 41.7 (C-4). In the ^{13}C NMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 160.5 (C-2), 155.1 (N=CH), 155.0 (C-9), 143.9 (C-10b), 143.1 (aromatic), 137.9 (C-10a), 136.5 (C-7), 135.1 (aromatic), 133.2 (aromatic), 133.0 (=CH), 131.8 (aromatic), 129.8 (=CH), 129.4 (aromatic), 129.1 (aromatic), 128.9 (aromatic), 128.8 (C-5), 127.0 (C-6a), 126.3 (aromatic) 121.2 (C-4a), 123.4 (C-8), 121.0 (C-6), 120.0 (CN), 71.6 (C-3), 41.7 (C-4); MS *m/z* (%): 500 (M^++4, 8), 498 (M^++2, 66) with a base peak at 496 (M^+, 100); Anal. Calcd for $C_{28}H_{18}Cl_2N_4O$: C, 67.61; H, 3.65; N, 11.26. Found: C, 67.48; H, 3.30; N, 11.05.

Reaction of 7 with Hydrazine Hydrate and Methylamine

General Procedure

A mixture of imadate **7** (0.525 g, 10 mmol) and hydrazine hydrate or methylamine (0.031 g, 10 mmol) in ethanol (30 mL) was stirred at room temperature for 1h. The solid product was collected and recrystallized from proper solvent to give **10a,b**. The physical and spectra data of the compounds **10a,b** are as follows.

***(E)* 9-Methyl-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido-[4',5':6,5]pyrano[3,2-*h*]quinoline (10a)**

Colorless needles from benzene; m.p. 215-217 °C; 85%; IR (KBr) ν (cm^{-1}): 3257 (NH), 3044, 2965 (CH stretching), 1647 (C=N); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.34-7.15 (m, 12H, aromatic), 8.00 (d, 1H, =CH, *J* = 16 Hz), 7.78 (s, 1H, H-10), 7.54 (d, 1H, =CH, *J* = 16 Hz), 6.60 (bs, 1H, NH,

exchangeable by D_2O), 5.02 (s, 1H, H-7), 3.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.3 (C-8), 155.5 (C-11a), 155.2 (C-2), 144.6 (C-1b), 142.8, 137.4 (C-1a), 136.5 (C-10), 136.5 (C-4), 135.2, 133.4 (=CH), 133.2, 131.6, 129.6 (=CH), 129.3, 129.0, 128.9, 128.7 (C-6), 126.9 (C-4a), 126.6 (aromatic), 121.7 (C-6a), 121.1 (C-5), 120.3 (C-3), 97.5 (C-7a), 40.4 (C-4), 35.3 (CH_3); ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.5 (C-10), 136.5 (C-4), 133.4 (=CH), 129.6 (=CH), 129.0 (aromatic), 129.0 (aromatic), 128.9 (aromatic), 128.7 (C-6), 126.6 (aromatic), 121.0 (C-5), 120.3 (C-3), 40.4 (C-4), 35.3 (CH_3). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.5 (C-10), 136.5 (C-4), 133.4 (=CH), 129.6 (=CH), 129.0 (aromatic), 129.0 (aromatic), 128.9 (aromatic), 128.7 (C-6), 126.6 (aromatic), 121.0 (C-5), 120.3 (C-3), 40.4 (C-4). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 136.5 (C-10), 136.5 (C-4), 133.4 (=CH), 129.6 (=CH), 129.0 (aromatic), 129.0 (aromatic), 128.9 (aromatic), 128.7 (C-6), 126.6 (aromatic), 121.0 (C-5), 120.3 (C-3), 40.4 (C-4), 35.3 (CH_3). In the ^{13}C NMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 160.3 (C-8), 155.5 (C-11a), 155.1 (C-2), 144.6 (C-1b), 142.8 (aromatic), 137.4 (C-1a), 136.5 (C-10), 136.5 (C-4), 135.2 (aromatic), 133.4 (=CH), 133.2 (aromatic), 131.6 (aromatic), 129.6 (=CH), 129.03 (aromatic), 129.0 (aromatic), 128.9 (aromatic), 128.7 (C-6), 126.9 (C-4a), 126.6 (aromatic), 121.7 (C-6a), 121.0 (C-5), 120.3 (C-3), 97.5 (C-7a), 40.4 (C-4), 35.3 (CH_3); MS *m/z* (%): 514 (M^++4, 3), 512 (M^++2, 15), 510 (M^+, 25), 77 (100); Anal. Calcd for $C_{29}H_{20}Cl_2N_4O$: C, 68.11; H, 3.94; N, 10.96. Found: C, 67.72; H, 3.58; N, 10.63.

(E) 9-Amino-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*]quinoline (10b)

Colorless needles from benzene; m.p. 260-261 °C; 88%; IR (KBr) ν (cm^{-1}): 3331, 3275, 3184 (NH & NH_2), 3061, 3020, 2970, 2915 (CH stretching), 1656 (C=N); ^1H NMR (500 MHz) ($CDCl_3$) δ: 8.34-7.33 (m, 12H, aromatic), 7.83 (s, 1H, H-10), 7.86 (d, 1H, =CH, *J* = 16.5 Hz), 7.60 (d, 1H, =CH, *J* = 16.5 Hz), 6.76 (bs, 1H, NH, cancelled by D_2O), 5.73 (bs, 2H, NH_2, exchangeable by D_2O), 5.42 (s, 1H, H-7); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.1 (C-8), 159.9 (C-11a), 157.1 (C-2), 155.5 (C-1b), 144.3 (C-10), 139.1, 137.9 (C-1a), 136.5 (C-4), 135.2 (=CH), 133.2, 133.1, 130.5 (=CH), 130.3, 129.8, 129.6, 128.9 (C-6), 127.6 (C-4a), 126.9, 126.2 (C-6a), 123.5 (C-5), 122.3 (aromatic), 120.3 (C-3), 96.3 (C-7a), 40.1 (C-7); MS *m/z* (%): 499 (M^++4, - NH_2, 1), 497 (M^++2, - NH_2, 6), 495 (M^+, - NH_2, 10), 406 (100); Anal. Calcd for $C_{28}H_{19}Cl_2N_5O$: C, 65.63; H, 3.74; N, 13.67. Found: C, 65.49; H, 3.57; N, 12.57.

Preparation of (E) 4-(4-chlorophenyl)-9-(4-chlorostyryl)-2-dimethylaminomethylene-amino-4H-pyrano[3,2-h]quinoline-3-carbonitrile (8)

General Procedure

Method (a)

A mixture of imadate **7** (0.525 g, 10 mmol) and dimethylamine (0.045 g, 10 mmol) in methanol (30 mL) was stirred at room temperature for 1h. The solid product was collected and recrystallized from proper solvent to give **8** (m.p. and mixed m.p.) yield (87%).

Method (b)

A mixture of *β*-enaminonitrile **3** (0.469 g, 10 mmol), dimethylformamide-dipentylacetal (DMF-DPA) (0.231 g, 10 mmol) and benzene (30 mL) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from proper solvent to give **8** (m.p. and mixed m.p.) yield (81%).

(E) 14-(4-Chlorophenyl)-9-(4-chlorotyryl)-2-cyanomethyl-14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo-[1,5-*c*]quinoline (11a)

A solution of **10b** (0.511 g, 10 mmol) and ethyl cyanoacetate (0.13 g, 10 mmol) in dry ethanol (30 mL) was refluxed for 3 h to give **11a** as colorless crystals which were collected by filtration and recrystallized from benzene; m.p. 280–281 °C; 6%; IR (KBr) ν (cm^{-1}): 3079, 2975, 2937, 2898 (CH stretching), 2257 (CN); ^1H NMR (500 MHz) ($CDCl_3$) δ: 9.23 (s, 1H, H-5), 8.34-7.15 (m, 12H, aromatic), 7.86 (d, 1H, =CH, *J* = 16.5 Hz), 7.60 (d, 1H, =CH, *J* = 16.5 Hz), 5.73 (s, 1H, H-14), 3.60 (s, 2H, CH_2), 23.81 (CH_2); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 165.1 (C-6a), 155.7 (C-9), 155.5 (C-7a), 145.2 (C-14b), 142.5 (C-2), 142.2, 139.1, 136.5 (C-7b), 135.3 (C-5), 135.2 (C-11), 133.2, 132.5, 133.2 (=CH), 131.9, 130.5 (=CH), 130.3, 129.8 (C-13), 129.6, 128.9 (aromatic), 127.6 (C-11a), 124.6 (C-13a), 123.2 (C-12), 122.3 (C-10), 120.3 (C-14a), 117.5 (CN), 48.7 (C-14), 23.8 (CH_2); MS *m/z* (%): 564 (M^++4, 1), 562 (M^++2, 7), 560 (M^+, 11), 351 (M^++2, 2), 349 (M^+, 6), 312 (30), 233 (8), 179 (17), 111 (100), 75 (49) Anal. Calcd for $C_{31}H_{18}Cl_2N_6O$: C, 66.32; H, 3.23; N, 14.97. Found: C, 66.42; H, 3.34; N, 15.06.

(E) Ethyl 14-(4-chlorophenyl)-9-(4-chlorotyryl)-14*H*-pyrimido[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-*c*] quinoline-2-carboxylate (11b)

A solution of **10b** (0.511 g, 10 mmol) and diethyl oxalate (0.146 g, 10 mmol) in absolute ethanol (30 mL) was refluxed for 3 h to give **11b** as colorless crystals which were collected by filtration and recrystallized from benzene; m.p. 290–291 °C, 82%; IR (KBr) ν (cm^{-1}): 3089, 2975, 2938, 2899 (CH stretching), 1745 (CO); ^1H NMR (500 MHz) ($CDCl_3$) δ: 8.34 (s, 1H, H-5), 8.32-7.15 (m, 12H, aromatic), 7.89 (d, 1H, =CH, *J* = 16 Hz), 7.54 (d, 1H, =CH, *J* = 16 Hz), 5.43 (s, 1H, H-14), 4.36 (q, 2H, CH_2, *J* = 7.2 Hz), 1.07 (t, 3H, CH_3, *J* = 7.2 Hz); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 170.5 (C-6a), 160.3 (CO), 155.5 (C-2), 155.1 (C-9), 144.6 (C-7a), 142.8 (C-14b), 140.4, 137.4, 136.5 (C-7b), 135.5 (C-5), 135.2 (C-11), 133.4 (=CH), 133.2, 131.6 (=CH), 129.6, 129.0, 129.0, 128.9, 128.7 (C-13), 126.9 (aromatic), 126.4 (C-11a), 123.4 (C-13a), 121.7 (C-12), 121.0 (C-14a), 120.3 (C-10), 56.0 (CH_2), 40.4 (C-14), 18.5 (CH_3); MS *m/z* (%): 597 (M^++4, 1), 595 (M^++2, 4), 593 (M^+, 7), 412 (M^++2, 7), 410 (M^+, 10), 273 (9), 240 (8), 176 (7), 111 (69), 50 (100); Anal. Calcd for $C_{32}H_{21}Cl_2N_5O_3$: C, 64.66; H, 3.56; N, 11.78. Found: C, 64.48; H, 3.44; N, 11.58.

***(E)* 14-(4-Chlorophenyl)-9-(4-chlorotyryl)-2-phenyl-14*H*-pyrimido-[4',5':6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-c] quinoline (11c)**

Method (a)

A solution of **10b** (0.511 g, 10 mmol) and benzoyl chloride (0.13 g, 10 mmol) in dry benzene was refluxed for 6 h to give **11c** as colorless crystals which were collected by filtration and recrystallized from benzene; m.p. 393–394 °C, 78%; IR (KBr) υ (cm^{-1}): 3057, 3030, 2977 (CH stretching), 1627 (C=N); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.77 (s, 1H, H-10), 8.41-7.33 (m, 17H, aromatic), 7.84 (d, 1H, =CH, *J* = 16 Hz), 7.62 (d, 1H, =CH, *J* = 16 Hz), 6.30 (s, 1H, H-7); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 170.0 (C-6a), 165.0 (C-2), 155.7 (C-9), 154.1 (C-7a), 153.0 (C-14b), 144.1, 143.0 (C-7b), 140.5 (C-5), 137.7 (C-11), 136.7, 135.1 (=CH), 133.7, 133.3, 131.8, 131.0 (=CH), 130.3, 129.6, 129.3, 129.1, 129.0 (C-13), 128.9, 128.6 (aromatic) 127.2 (C-11a), 124.1 (C-13a), 121.5 (C-12), 120.4 (C-10), 101.2 (C-14a), 40.0 (C-14); MS *m/z* (%): 601 (M^++4, 3), 599 (M^++2, 14), 597 (M^+, 28), 111 (100); Anal. Calcd for $C_{35}H_{21}Cl_2N_5O$: C, 70.24; H, 3.54; N, 11.70. Found: C, 70.33; H, 3.76; N, 11.87.

Method (b)

Compound **12** (0.01 mmol) was heated under reflux in 1,4-dioxane (20 mL) and piperidine (0.5 mL) for 3 h to give **11c** (m.p. and mixed m.p.) yield (68%).

***(E)* 9-Benzylideneamino-7-(4-chlorophenyl)-2-(4-chlorostyryl)-8-imino-8,9-dihydro-7*H*-pyrimido[4',5':6,5]pyrano-[3,2-*h*]quinoline (12)**

A mixture of **10b** (0.511 g, 10 mmol), benzaldehyde (0.106 g, 10 mmol), ethanol (20 mL) and piperidine (0.5 mL) was refluxed for 2 h. The solid product, formed, was collected by filtration and recrystallized from ethanol give **12** as yellow crystals; m.p. 395–396 °C; 87%; IR (KBr) ν (cm^{-1}): 3211 (NH), 3058, 2970, 2932, 2891 (CH stretching), 1639 (C=N); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 11.11 (bs, 1H, NH, exchangeable by D_2O), 10.22 (s, 1H, N=CH), 8.48 (s, 1H, H-10), 8.38-7.29 (m, 17H, aromatic), 7.88 (d, 1H, =CH, *J* = 16.5 Hz), 7.62 (d, 1H, =CH, *J* = 16.5 Hz), 6.30 (s, 1H, H-7); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 164.0 (C-8), 160.0 (C-10), 158.5 (C-11a), 156.8 (C-2), 155.7 (N=CH), 144.6 (C-1b), 143.7, 137.9 (C-1a), 136.5 (C-4), 135.2, 134.2 (=CH), 133.4, 133.2, 131.7, 129.7 (=CH), 129.6, 129.1, 129.0, 128.9 (C-6), 128.8, 128.3, 127.0 (C-4a), 126.8, 126.2 (aromatic), 123.8 (C-6a), 123.4 (C-5), 120.3 (C-3), 96.8 (C-7a), 38.4 (C-7); MS *m/z* (%): 603 (M^++4, 2), 601 (M^++2, 13), 599 (M^+, 23), 55 (100); Anal. Calcd for $C_{35}H_{23}Cl_2N_5O$: C, 70.00; H, 3.86; N, 11.66. Found: C, 69.49; H, 3.57; N, 11.47.

PHARMACOLOGY

Antitumor Screening

(a) Cell Culture

MCF-7, HCT, and HepG-2 were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µmol/l gentamycin. Vero cell was propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, HEPES buffer and 50 µmol/l gentamycin.

All cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultures two to three times a week.

(b) Cytotoxicity Evaluation Using Viability Assay

The *in-vitro* cytotoxicity activity was studied against three cell lines: MCF-7 (breast adenocarcinoma), HCT (lung carcinoma) and HepG-2 (hepatocellular carcinoma) using the colorimetric MTT assay as described and modified by Tim Mossman [45]. The cells were seeded in 96-well microtitre plate at a cell concentration of 1 x 10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding.

Serial two-fold dilutions of the metabolites were added confluent cell monolayer. The microtitre plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample.

Control cells were incubated without the test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment.

After incubation of the cells for 24 h at 37 °C, various concentrations of sample were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric MTT method.

In brief, after the end of the incubation period, the crystal violet solution (1%) was added to each well for 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid was then added to all wells and mixed thoroughly, and the plates were read on ELISA reader, using a test wavelength of 490 nm. Treated samples were compared with the control in the absence of the tested samples. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated.

CONFLICT OF INTEREST

Declared none.

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