Synthesis, antitumor activity, and structure–activity relationship of some 4H-pyrano[3,2-h]quinoline and 7H-pyrimido[4′,5′:6,5]pyrano[3,2-h]quinoline derivatives

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Synthesis, antitumor activity, and structure–activity relationship of some 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline derivatives

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Abstract Several 4*H*-pyrano[3,2-*h*]quinoline (**3a–d**, **4a**, **7a**,**b**, **9a–c**, **10a**,**b**, **11a**,**b**, and **13a–c**) and 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline derivatives (**8a–c**) were obtained by treatment of 8-hydroxyquinoline (**1a**) and 8-hydroxy-2-methylquinoline (**1b**) with α-cyano-*p*-chloro/bromocinnamonitrile (**2a**,**b**) or 4*H*-pyrano[3,2-*h*]quinoline derivatives (**3a**,**c**,**d**) with different electrophilic reagents followed by nucleophilic reagents. Structures of these compounds were established on the basis of spectral data. The antitumor activity of the synthesized compounds was investigated in comparison with Vinblastine. Among them, compounds **3c**,**d**, **4a**, **8b**, **9b**,**c**, **11a**,**b**, and **13a**,**c** inhibited the growth of cancer cells compared to Vinblastine. The structure–activity relationships were discussed.

Keywords 8-Hydroxyquinoline · 8-Hydroxy-2-methylquinoline · 4*H*-pyrano[3,2-*h*]quinoline · 7*H*-pyrimido[4′,5′:6,5]pyrano[3, 2-*h*]quinoline · Antitumor · SAR

Introduction

Heterocyclic compounds hold a special place among pharmaceutically significant natural products and synthetic compounds (Desai and Dodiya, 2011). Nitrogen heterocyclic are abundant in nature and are of great significance to life; this is because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics, and alkaloid, as well as pharmaceuticals, herbicides, and many other compounds. Nitrogen heterocyclic containing a quinoline ring is often found in biologically active molecules (Al-Ghamdi *et al.*, 2012; Saugues *et al.*, 2011; Guo *et al.*, 2011; Broch *et al.*, 2010; Ramesh *et al.*, 2009; Righi *et al.*, 2008; Musiol *et al.*, 2007, 2006). 8-Hydroxyquinoline moieties have also been shown to possess diverse therapeutic activities such as antifungal (Thomas *et al.*, 2010), antibacterial (Chang *et al.*, 2010; Harris and Thorarensen, 2004; Musiol *et al.*, 2008), antiprotozoic, as well as antineoplastic (Badawey and Kappe, 1997) and antiproliferative (Mrozek-Wilczkiewicz *et al.*, 2010; Jampilek *et al.*, 2009) activities. In addition, the antiproliferative effects of styrylquinoline derivatives on tumor cell lines have been observed and recently reported (El-Agrody *et al.*, 2012; Polanski, 2010; Thomas and Roy, 2008; Andrew *et al.*, 2007; Zouhiri *et al.*, 2005; Pommier *et al.*, 2005). Other styrylquinoline derivatives have also gained strong attention recently due to their extensive biological activities (Larghi *et al.*, 2009; Xin-Hua *et al.*, 2009; Ganesh *et al.*, 2008; Narender *et al.*, 2006).

As a result of remarkable pharmacological efficiency of quinoline derivatives and in continuation of our program on the chemistry of 4*H*-pyran derivatives (Al-Ghamdi *et al.* 2012; El-Agrody, 1994; El-Agrody *et al.*, 1997a, b, 2000, 2001, 2002, 2011, 2012; El-Agrody and Al-Ghamdi, 2011; Sabry *et al.*, 2011; Abd-El-Aziz *et al.*, 2004, 2007; Eid *et al.*, 2003; Khafagy *et al.*, 2002; Bedair *et al.*, 2000, 2001; Sayed *et al.*, 2000), we report herein the synthesis of new 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline derivatives, and the evaluation of their antitumor activities. The chemical structures of the studied compounds and their structure–activity relationships (SAR) are discussed in this work.

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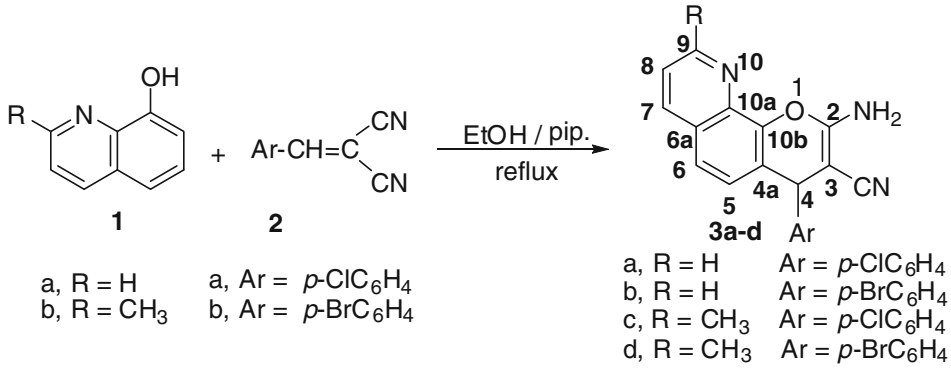
Scheme 1 Synthesis of 4*H*-pyrano[3,2-*h*]quinoline derivatives (**3a–d**)



Chemistry

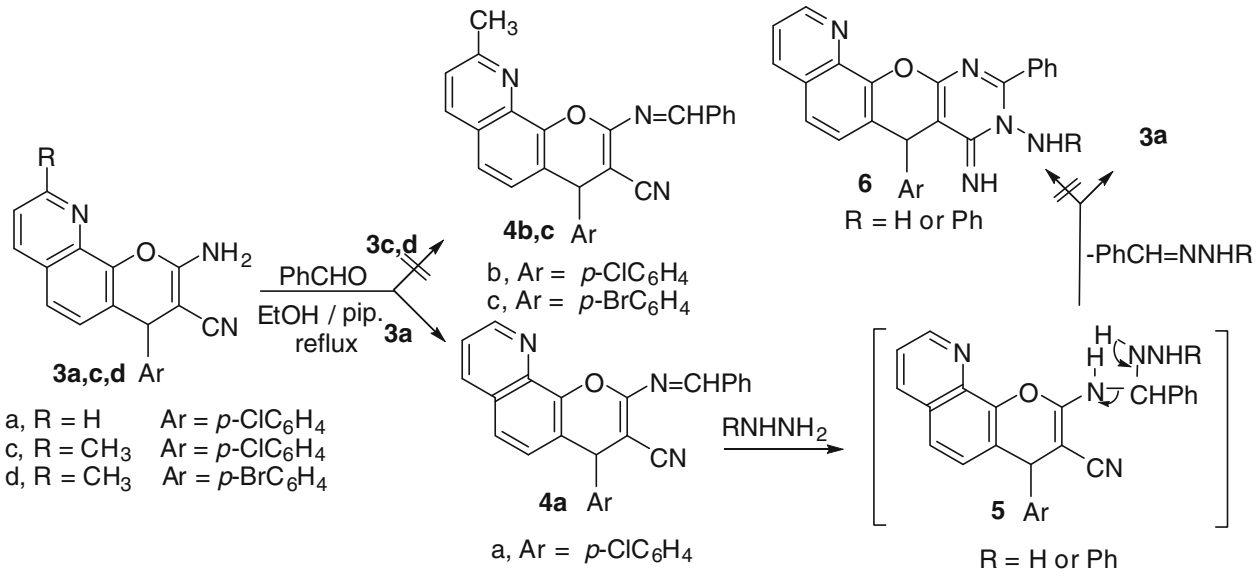
Treatment of 8-hydroxyquinoline (**1a**) and 8-hydroxy-2-methylquinoline (**1b**) with α-cyano-*p*-chloro/bromocinnamonitrile (**2a**,**b**) in ethanolic piperidine under reflux afforded 2-amino-4-(4-chloro/bromophenyl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**3a**,**b**) and 2-amino-4-(4-chloro/-bromophenyl)-9-methyl-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**3c**,**d**) (Scheme 1).

Compounds **3a**,**c**,**d** were subjected to electrophilic followed by nucleophilic reactions to produce fused heterotetracyclic systems incorporating pyrimidine nucleus in addition to pyranoquinoline moiety. Thus, condensation of **3a** with benzaldehyde in ethanol and piperidine under reflux gave the 2-benzylideneamino-4-(4-chlorophenyl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**4a**), while condensation of **3c**,**d** with benzaldehyde was unsuccessful; 2-benzylideneamino derivatives **4b**,**c** were not formed (Scheme 2).

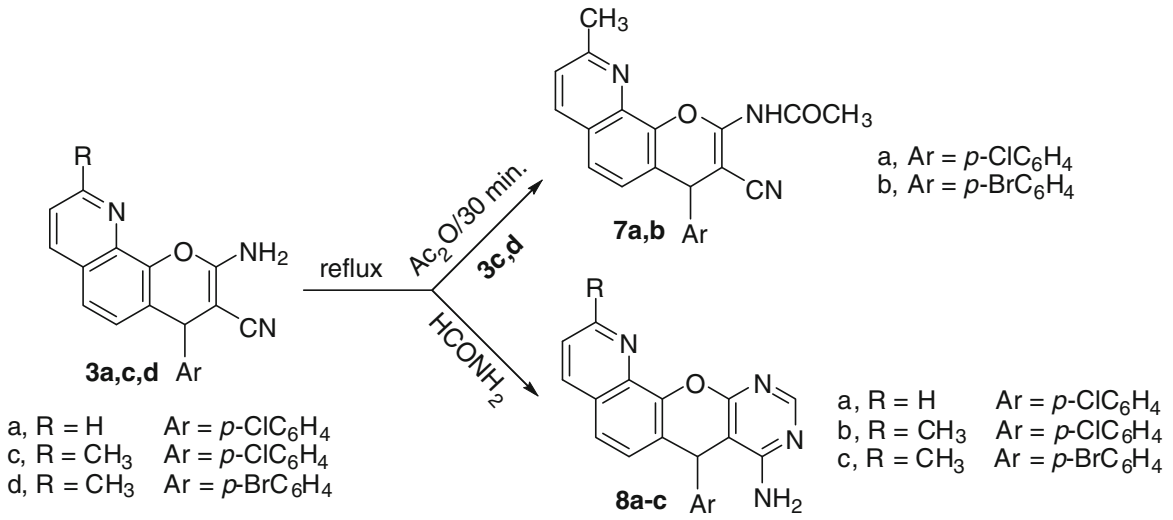
When 2-benzylideneamino-4-(4-chlorophenyl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**4a**) was treated with hydrazine hydrate or phenyl hydrazine in ethanol at room temperature or under reflux, the addition product **5** was formed (R = H or Ph, respectively). From the intermediate **5**, benzaldehyde hydrazone/phenylhydrazone were eliminated to give *β*-enaminonitrile **3a** (Khafagy *et al.*, 2002) instead of the pyrimidopyranoquinoline derivative **6** (Scheme 2). Structure **4a** was established on the basis of spectral data.

Treatment of **3c**,**d** with acetic anhydride under reflux for 30 min afforded the *N*-acetylamino derivative **7a**,**b**, while heating of **3a**,**c**,**d** with formamide under reflux provided the aminopyrimidopyranoquinoline derivative **8a–c** (Scheme 3).

Structures **7** and **8** were established on the basis of spectral data and in conjunction with our previous work (Abd-El-Aziz *et al.*, 2004, 2007; Bedair *et al.*, 2000, 2001; Eid *et al.*, 2003; El-Agrody, 1994; El-Agrody *et al.*, 1997a, b, 2000, 2001, 2002, 2011; Khafagy *et al.*, 2002; Sayed *et al.*, 2000; Sabry *et al.*, 2011).



Scheme 2 Preparation of compound (**4a**)



Scheme 3 Synthetic protocol of compounds (**7a**,**b**) and (**8a–c**)

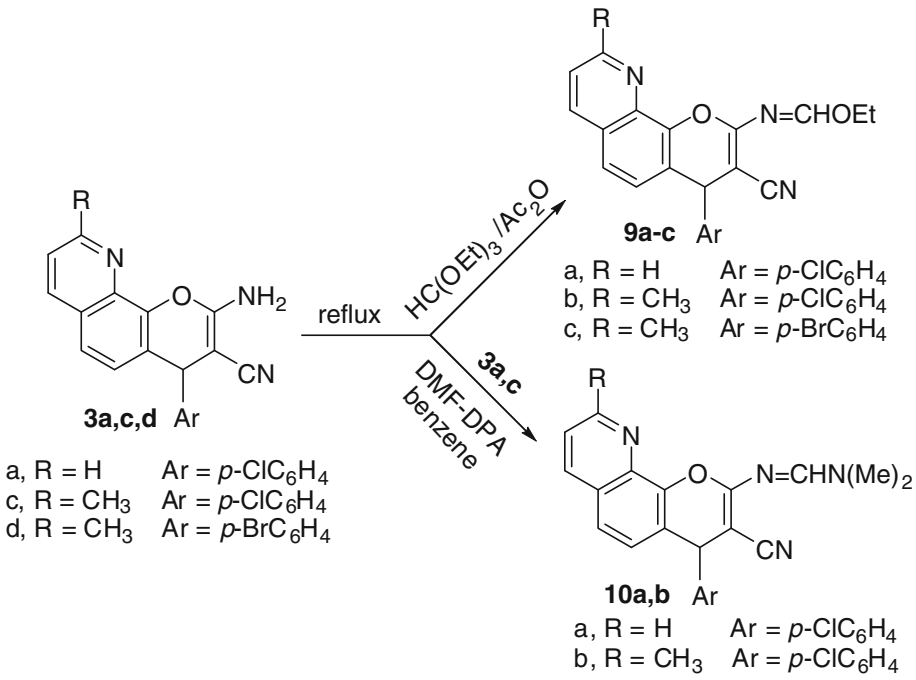
Treatment of **3a**,**c**,**d** with triethyl orthoformate in acetic anhydride at reflux gave the corresponding imidate derivatives **9a–c**, while reaction of **3a**,**c** with dimethylformaide-dipentylacetal (DMF-DPA) in benzene under reflux afforded the amidine derivatives **10a**,**b** (Scheme 4). Structures of **9** and **10** were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13} C NMR-DEPT, and MS data.

Hydrazinolysis of 4-(4-chlorophenyl)-2-ethoxymethylen-eamino-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**9a**) in ethanol at room temperature under stirring afforded the open form product 4-(4-chlorophenyl)-2-hydrazinomethy-leneamino-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**11a**) instead of the cyclized compound, aminoimino derivative **12a** (Scheme 5).

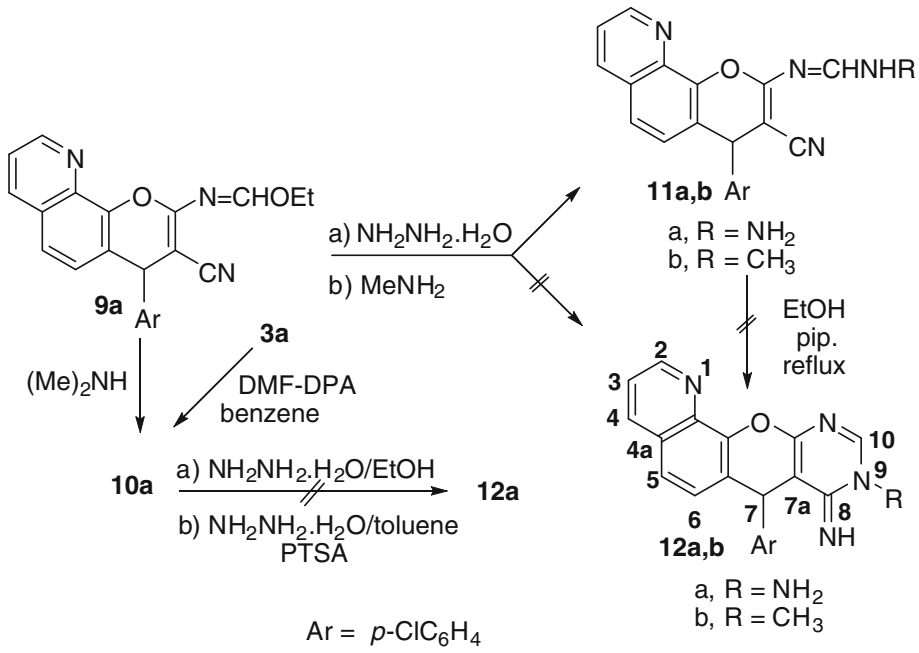
In a similar manner, reaction of **9a** with methylamine yielded the open form product 4-(4-chlorophenyl)-2-methylaminomethyleneamino-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**11b**), rather than the cyclized compound, imino derivative **12b** (Scheme 5). Attempts to cyclized compounds **11a**,**b** by reflux in ethanolic piperidine solution were unsuccessful, the aminoimino and imino derivatives **12a**,**b**, respectively, were not formed (Scheme 5).

The reaction of the imidate **9a** with dimethylamine in ethanol at room temperature yielded the amidine derivative

Scheme 4 Synthetic protocol of compounds (**9a–c**) and (**10a**,**b**)



Scheme 5 Preparation of compounds (**10a**) and (**11a**,**b**)

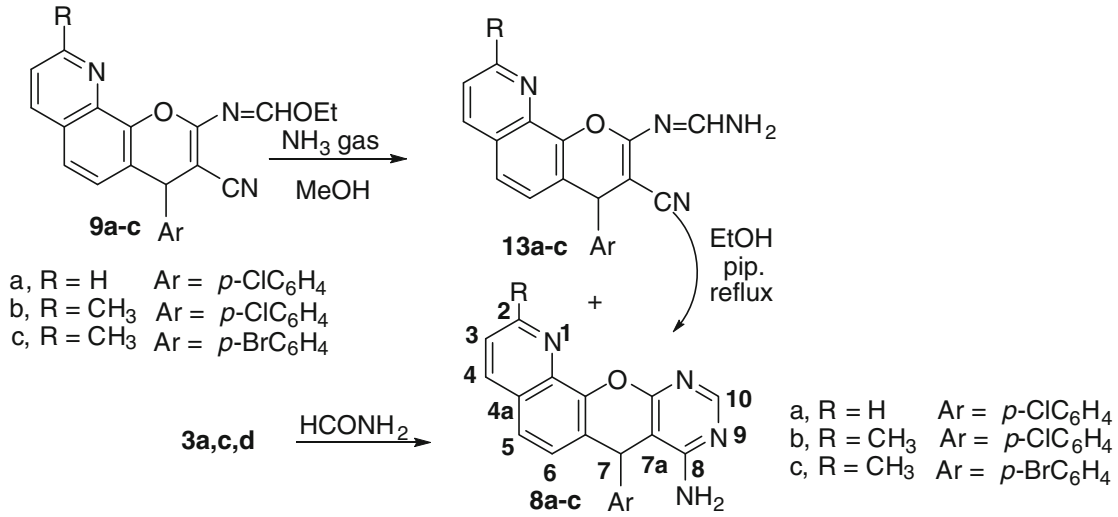


10a (Scheme 5), which can also be obtained as described before from the reaction of **3a** with DMF-DPA (m.p. and mixed m.p. are completely identical) (Scheme 4). The structure of **11** was supported by spectral data.

Hydrazinolysis of the amidine **10a** in ethanol at room temperature under stirring or reflux was unsuccessful, the aminoimino derivative **12a** was not formed (Scheme 5). Also, reaction of **10a** with hydrazine hydrate in toluene in the presence of *p*-toluenesulfonic acid under reflux failed (Salaheldin *et al.*, 2008), the aminoimino derivative **12a** was not formed (Scheme 5).

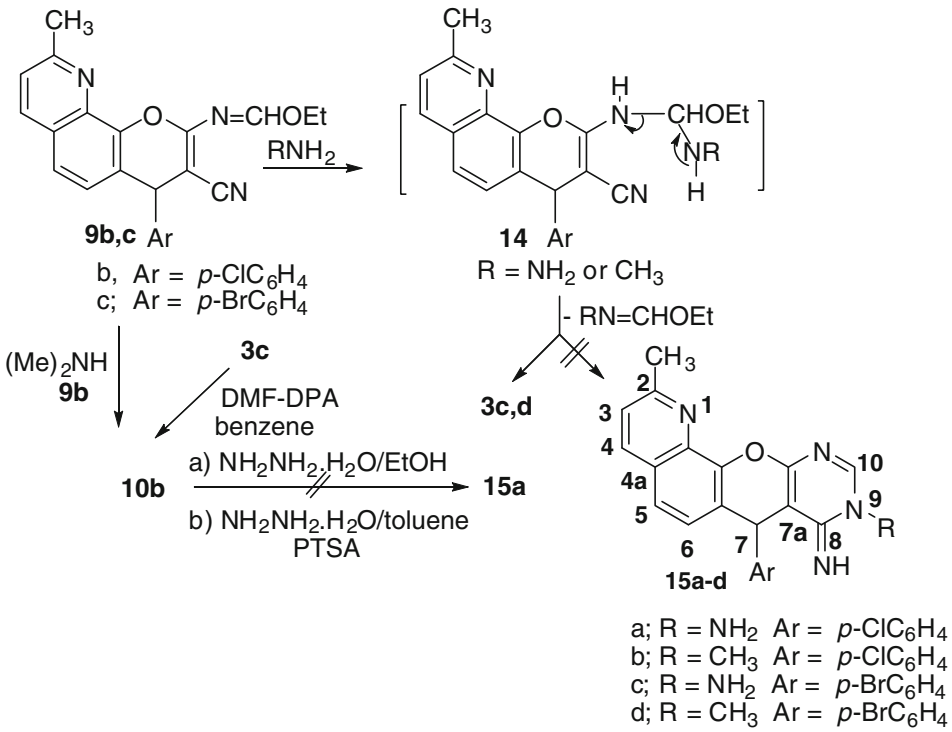
Treatment of the imidate **9a–c** with NH_3 gas bubbled in methanol at room temperature for 1 h yielded the 8-amino-7-(4-chlorophenyl)-7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline (**8a**) and 2-methyl derivatives **8b**,**c**, together with the open form product, 2-aminomethyleneamino-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**13a**) and 2-methyl derivatives **13b**,**c**. The open form product was separated from the filtrate of the reaction mixture (Scheme 6).

The tetracyclic structure **8** was supported by its independent synthesis from **3a**,**c**,**d** and formamide (Bedair *et al.*, 2000, 2001) as described before (Scheme 3) and also by cyclization of **13a–c** in ethanolic piperdine solution under reflux (Khafagy *et al.*, 2002) (m.p. and mixed m.p. are completely identical) (Scheme 6). The structure of **13** was supported on the basis of IR, ^{1}H NMR, ^{13}C NMR, and MS data.



Scheme 6 Synthetic protocol of compounds (**8a–c**) and (**13a–c**)

Scheme 7 Reaction of compounds (**9a**,**b**) with ammonia derivatives



When the imidate **9b**,**c** was treated with hydrazine hydrate or methylamine in ethanol at room temperature under stirring, the addition product **14** was formed (R = NH_2 or CH_3, respectively). From the intermediate **14**, ethyl *N*-methylformimidate or ethyl formohydrazonate was eliminated to give *β*-enaminonitrile **3c**,**d** (Khafagy *et al.*, 2002; Tacconi *et al.*, 1980) instead of the aminoimino derivatives **15a**,**c** or imino derivatives **15b**,**d**, respectively (Scheme 7).

Reaction of the imidate **9b** with dimethylamine in ethanol at room temperature under stirring afforded the amidine derivative **10b** (Scheme 7), which can be obtained as described before from the reaction of **3c** with DMF-DPA (m.p. and mixed m.p. are completely identical) (Scheme 4). Hydrazinolysis of the amidine **10b** in ethanol at room temperature under stirring or reflux was unsuccessful, the aminoimino derivative **15a** was not formed (Scheme 7). Also, reaction of **10b** with hydrazine hydrate in toluene in the presence of *p*-toluenesulfonic acid under reflux failed (Salaheldin *et al.*, 2008), the aminoimino derivative **15a** was not formed (Scheme 7).

Antitumor assays

Compounds **3a–d**, **4a**, **7a**,**b**, **8a**,**b**, **9a–c**, **10a**,**b** **11a**,**b**, and **13a–c** were evaluated for human tumor cell growth inhibitory activity against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT), and hepatocellular carcinoma (HepG-2). The measurements of cell growth and the viabilities were determined as described in the literature (Rahman *et al.*, 2001). In vitro cytotoxicity evaluation using viability assay was performed at the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University using Vinblastine as standard drug. The inhibitory activity of the synthetic compounds **3a–d**, **4a**, **7a**,**b**, **8a**,**b**, **9a–c**, **10a**,**b** **11a**,**b**, and **13a–c** against the three cell lines MCF-7, HCT, and HepG-2 are given in Table 1 and Fig. 1.

Results and discussion

Quinoline derivatives were selected for this study as their families are well-known to contain active compounds with a wide range of biological and pharmacological activities (Al-Ghamdi *et al.* 2012; El-Agrody *et al.*, 2012; Saugues *et al.*, 2011; Guo *et al.*, 2011; Desai *et al.*, 2011; Broch *et al.*, 2010; Thomas *et al.*, 2010; Chang *et al.*, 2010; Mrozek-Wilczkiewicz *et al.*, 2010; Jampilek *et al.*, 2009; Ramesh *et al.*, 2009; Larghi *et al.*, 2009; Xin-Hua *et al.*, 2009; Ganesh *et al.*, 2008; Righi *et al.*, 2008; Musiol *et al.*, 2008, 2007, 2006; Thomas and Roy, 2008; Andrew *et al.*, 2007; Narender *et al.*, 2006; Zouhiri *et al.*, 2005; Pommier *et al.*, 2005; Harris and Thorarensen, 2004; Badawey and Kappe, 1997). In the present study, twenty compounds of 4*H*-pyrano[3,2-*h*] quinoline and 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]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.

Table 1 Effects of the treatment of MCF-7, HCT, and HepG-2 cells with various concentrations of the prepared compounds; cytotoxicity (IC_{50}) as measured by the MTT method

Compounds	Conc. (μg/ml)	MCF-7 cell viability (%)	IC_{50} (μg/ml)	HCT cell viability (%)	IC_{50} (μg/ml)	HepG-2 cell viability (%)	IC_{50} (μg/ml)
Vinblastine	50	07.82	6.1	16.27	2.6	14.38	4.6
	25	15.18		21.68		16.13	
	12.5	29.60		28.20		24.25	
	6.25	48.75		38.06		45.13	
	3.125	60.35		47.54		55.00	
	1.56	76.24		53.42		72.13	
	0	100.00		100.00		100.00	
3a	50	26.79	24.9	27.40	24	52.78	w
	25	49.82		48.20		57.22	
	12.5	72.86		67.40		63.52	
	6.25	82.50		76.80		68.15	
	3.125	91.61		92.60		68.52	
	1.56	100.00		100.00		79.63	
	0	100.00		100.00		100.00	
3b	50	39.11	34.8	42.40	43.7	91.80	NA
	25	58.93		78.40		96.27	
	12.5	70.00		91.20		98.35	
	6.25	75.71		97.80		100.00	
	3.125	83.93		100.00		100.00	
	1.56	94.82		100.00		100.00	
	0	100.00		100.00		100.00	
3c	50	28.00	27.9	27.60	15.8	23.15	1.8
	25	58.20		35.80		33.70	
	12.5	71.40		55.60		35.56	
	6.25	78.20		66.80		37.59	
	3.125	87.00		79.00		42.96	
	1.56	100.00		94.00		51.85	
	0	100.00		100.00		100.00	
3d	50	41.25	38.9	36.60	22.8	21.97	5.5
	25	62.32		48.80		29.34	
	12.5	71.79		58.20		39.61	
	6.25	75.18		66.60		44.87	
	3.125	82.32		88.00		63.95	
	1.56	93.93		100.00		77.50	
	0	100.00		100.00		100.00	
4a	50	26.50	2.8	09.13	0.6	18.62	2.8
	25	33.33		11.59		31.40	
	12.5	37.17		14.76		37.86	
	6.25	40.83		19.41		42.50	
	3.125	48.33		30.22		48.92	
	1.56	55.00		38.46		56.14	
	0	100.00		100.00		100.00	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 cell viability (%)	IC_{50} (μg/ml)	HCT cell viability (%)	IC_{50} (μg/ml)	HepG-2 cell viability (%)	IC_{50} (μg/ml)
7a	50	46.17	38.6	21.98	14.6	23.78	17.8
	25	54.00		42.54		35.85	
	12.5	68.67		53.33		60.22	
	6.25	76.83		77.46		78.56	
	3.125	86.50		85.16		89.70	
	1.56	96.33		96.43		100.00	
	0	100.00		100.00		100.00	
7b	50	23.38	13.5	22.54	21.3	32.56	26.5
	25	32.13		44.52		51.20	
	12.5	51.25		71.75		68.58	
	6.25	65.75		86.83		81.60	
	3.125	79.88		95.08		90.66	
	1.56	94.75		99.84		98.78	
	0	100.00		100.00		100.00	
8a	50	45.40	44.6	26.43	19.2	64.26	w
	25	68.80		42.00		67.59	
	12.5	75.80		62.14		73.33	
	6.25	81.20		74.57		83.33	
	3.125	94.50		78.57		90.37	
	1.56	100.00		83.14		94.44	
	0	100.00		100.00		100.00	
8b	50	70.24	w	41.63	26.4	28.33	6.3
	25	81.68		51.12		37.00	
	12.5	89.23		78.88		43.67	
	6.25	94.46		90.51		50.83	
	3.125	97.28		98.78		60.17	
	1.56	100.00		100.00		68.33	
	0	100.00		100.00		100.00	
9a	50	42.96	45.4	23.85	25.7	39.44	31.1
	25	67.07		51.42		57.12	
	12.5	74.18		67.08		72.28	
	6.25	81.37		73.26		86.63	
	3.125	92.96		81.71		91.04	
	1.56	98.31		93.44		97.56	
	0	100.00		100.00		100.00	
9b	50	37.54	23.8	15.21	3.6	45.21	44.5
	25	48.62		18.40		68.96	
	12.5	63.44		25.96		79.38	
	6.25	76.56		35.32		85.13	
	3.125	87.38		54.89		96.38	
	1.56	96.12		75.53		100.00	
	0	100.00		100.00		100.00	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 cell viability (%)	IC_{50} (μg/ml)	HCT cell viability (%)	IC_{50} (μg/ml)	HepG-2 cell viability (%)	IC_{50} (μg/ml)
9c	50	56.64	w	19.79	5.8	34.64	37.1
	25	67.42		23.30		62.86	
	12.5	79.16		34.04		76.43	
	6.25	87.58		46.49		83.29	
	3.125	95.39		62.87		94.57	
	1.56	98.10		81.91		100.00	
	0	100.00		100.00		100.00	
10a	50	37.74	29.3	49.52	49.4	48.06	47.9
	25	57.96		71.31		77.82	
	12.5	75.59		77.74		85.60	
	6.25	83.53		88.10		92.98	
	3.125	92.03		98.45		98.32	
	1.56	98.68		100.00		100.00	
	0	100.00		100.00		100.00	
10b	50	34.96	24.4	30.51	28.2	68.22	w
	25	49.62		53.47		81.46	
	12.5	68.18		69.69		94.72	
	6.25	76.94		74.18		97.84	
	3.125	87.22		78.67		100.00	
	1.56	97.04		82.03		100.00	
	0	100.00		100.00		100.00	
11a	50	21.43	8.4	24.80	7.4	24.87	8.7
	25	35.18		29.20		29.08	
	12.5	45.00		36.00		36.05	
	6.25	55.00		55.60		60.92	
	3.125	66.96		65.20		78.42	
	1.56	78.75		81.40		88.16	
	0	100.00		100.00		100.00	
11b	50	16.75	6.3	11.11	2.3	09.28	1.9
	25	21.38		13.57		17.90	
	12.5	29.25		22.22		30.44	
	6.25	50.13		29.44		39.02	
	3.125	65.00		41.43		45.68	
	1.56	76.75		58.25		52.76	
	0	100.00		100.00		100.00	
13a	50	23.12	10.5	18.69	6.8	35.76	23.6
	25	37.45		21.79		43.42	
	12.5	43.62		26.31		63.94	
	6.25	67.42		52.38		76.74	
	3.125	79.73		80.21		87.36	
	1.56	91.29		94.88		96.60	
	0	100.00		100.00		100.00	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 cell viability (%)	IC_{50} (μg/ml)	HCT cell viability (%)	IC_{50} (μg/ml)	HepG-2 cell viability (%)	IC_{50} (μg/ml)
13b	50	63.60	w	35.92	21.7	41.17	32.9
	25	74.22		44.18		53.83	
	12.5	81.54		65.20		57.67	
	6.25	89.32		70.71		68.00	
	3.125	96.51		82.24		76.67	
	1.56	98.38		91.63		80.50	
	0	100.00		100.00		100.00	
13c	50	56.24	w	24.80	6.2	52.33	w
	25	69.62		34.08		60.67	
	12.5	81.18		42.86		65.50	
	6.25	89.76		49.80		81.17	
	3.125	97.32		65.00		89.33	
	1.56	100.00		86.94		98.83	
	0	100.00		100.00		100.00	

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

NA not active

w weak activity (IC_{50} > μg/ml)

Compounds **3a–d**, **4a**, **7a**,**b**, **8a**,**b**, **9a–c**, **10a**,**b** **11a**,**b**, and **13a–c** were tested against three tumer cell lines: MCF-7, HCT, and HepG-2. The cytotoxicity evaluation using viability assays and inhibitory activities are given in Table 1 and Fig. 1.

The results from Table 1 indicate that compound **4a** was the most active compound against MCF-7 and compounds **11b**,**a** and **13a** showed very good activity, while compounds **7b**, **9b**,**10b**, **3a**,**c**, **10a**, **3b**, **7a**, and **3d** showed moderate activities. However, compounds **8a**, **9a**, **8b**, **9c**, and **13b**,**c** exhibited weak activities as compared with the standard drug Vinblastine. Furthermore, compounds **4a** and **11b** are the most active compounds against HCT, compounds **9b**,**c**, **13c**,**a**, and **11a** had very good activities, compounds **7a**, **3c**, **8a**, **7b**, **13b**, **3d**,**a**, **9a**, **8b**, and **10b** showed moderate activities, and compounds **3b** and **10a** showed weak activities as compared with the standard drug Vinblastine. Finally, compounds **3c**, **11b**, and **4a** are the most active compounds against HepG-2 and compounds **3d**, **8b**, and **11a** exhibited very good activities, while compounds **7a**, **13a**, and **7b** showed moderate activities. In addition, compounds **9a**, **13b**, **9c**,**b**, **10a**, **8a**, **10b**, **13c**, and **3a** showed weak activities, while compound **3b** was inactive as compared with the standard drug Vinblastine.

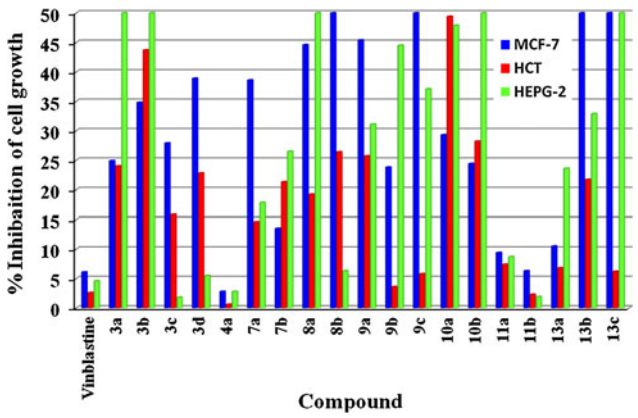


Fig. 1 Antitumor activity of 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido[4′,5′:6,5]pyrano-[3,2-*h*]quinoline derivatives

SAR studies

The SAR studies of **3a** and its analogs revealed that compound **4a** has the highest potent antitumor activity against MCF-7 compared to other compounds **11b**,**a**, **13a**, **3a**, **10a**, **3b**, and **9a**. These data indicate that the activity of compound **4a** was considerably attributed to the presence of the electron-donating group (–N=CHPh-2) in 4*H*-pyrano-[3,2-*h*]quinoline moiety, suggesting that blocking of the ($–NH_2$-2) group with a bulky electron-donating group might be preferred at position C-2. The blocking of the ($–NH_2$-2) group with other electron-donating group such as (–N=CHNHMe-2/–N=CHNHNH$_2$-2/–N=CHNH$_2$-2) in compounds **11b**,**a**, and **13a**, resulted in slight reduction of potency. In addition, the presence of other electron-donating group such as ($–NH_2$-2/–N=CHNMe$_2$-2/$–NH_2$-2/–N=CHOEt-2) for compounds **3a**, **10a**, **3b**, and **9a**, in combination with the electron-withdrawing groups (*p*-Cl/

BrC_6H_4-4; –CN-3), resulted in more reduction of potency. The introduction of an electron-donating group ($–CH_3$-9) in combination with the electron-withdrawing groups (*p*-Cl/BrC_6H_4-4; –CN-3) and the electron-donating groups (–NHCOMe-2/–N=CHOEt-2/–N=$CHNMe_2$-2/$–NH_2$-2/–N=CHNHNH_2-2) resulted in reduction of potency of compounds **7b**, **9b**, **10b**, **3c**, **7a**, **3d**, **13b**,**c**, and **9c**, less than its hydrogen-substituted analog, suggesting that an electron-donating group might not be preferred at position C-9. Incorporating a pyrimidine nucleus with pyranoquinoline moiety in the presence of electron-withdrawing group (*p*-Cl/BrC_6H_4-7) and electron-donating group ($–NH_2$-8) for compound **8a** resulted in reduction of potency. More reduction of potency with the introduction of an electron-donating group ($–CH_3$-2) in compound **8b** was observed.

In the case of HCT, investigation of (SAR) revealed that compounds **4a** and **11b** had the most potent activity against HCT compared to compound **3a** and its analogs. This potency could be attributed to the presence of the electron-donating groups (–N=CHPh-2 or –N=CHNHMe-2) in 4*H*-pyrano[3,2-*h*]quinoline moiety, while the blocking of the ($–NH_2$-2) group with an electron-donating group such as (–N=$CHNH_2$-2/–N=$CHNHNH_2$-2) in compounds **13a** and **11a** resulted in slight reduction of potency. The presence of the electron-donating group ($–NH_2$-2) resulted in the reduction of potency for compound **3a**, while blocking ($–NH_2$-2) group with other electron-donating group such as (–N=CHOEt-2/N=$CHNMe_2$-2) resulted in more reduction of potency for compounds **9a**, **3b**, and **10a**, suggesting that there might be a size-limited pocket at position C-2. The introduction of an electron-donating group ($–CH_3$-9) in 4*H*-pyrano[3,2-*h*]quinoline nucleus, in combination with the electron-withdrawing groups (*p*-Cl/BrC_6H_4-4; –CN-3) and the electron-donating groups (–N=CHOEt-2/–N=$CHNHNH_2$-2), resulted in slight reduction of potency of compounds **9b**,**c** and **13c** compared to its hydrogen-substituted analog. The presence of other electron-donating groups resulted in more reduction of potency of compounds **7a**, **3c**, **7b**, **13b**, **3d**, and **10b**. The presence of electron-withdrawing group (*p*-ClC_6H_4-7) and the electron-donating group ($–NH_2$-8) in 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline moiety for compound **8a** resulted in reduction of potency, while replacement of the hydrogen atom at position C-2 with an electron-donating group ($–CH_3$-2) resulted in more reduction of potency for compound **8b**.

Furthermore, compounds **11b** and **4a** showed higher antitumor activities against HepG-2 than the standard drug Vinblastine. This could be attributed to the presence of the electron-donating groups (–N=CHNHMe-2) or (–N=CHPh-2) in 4*H*-pyrano[3,2-*h*]quinoline moiety, suggesting that there might be a size-limited pocket at position C-2. Blocking the ($–NH_2$-2) with (–N=$CHNHNH_2$-2) in compound **11a** resulted in slight reduction of potency and more reduction of potency with other electron-donating groups such as (–N=$CHNH_2$-2/–N=CHOEt-2/–N=$CHNMe_2$-2/-NH_2-2) in compounds **13a**, **9a**, **10a**, and **3a**,**b**. The introduction of an electron-donating group ($–CH_3$-9) in 4*H*-pyrano[3,2-*h*]quinoline nucleus, in combination with the electron-withdrawing groups (*p*-Cl/BrC_6H_4-4; –CN-3) and the electron-donating group ($–NH_2$-2) for compounds **3c**,**d**, showed antitumor activity against (HepG-2) higher than or closer to that of the standard drug Vinblastine, suggesting that there might be a size-limited pocket at position C-4. The presence of other electron-donating groups such as (–NHCOMe-2/–N=CHNH_2-2/–N=CHOEt-2/–N=$CHNMe_2$-2/–N=$CHNH_2$-2) in compounds **7a**,**b**, **13b**, **9c**,**b**, **10b**, and **13c**, resulted in more reduction of potency. In addition, the presence of an electron-withdrawing group (*p*-ClC_6H_4-7) and the electron-donating group ($–NH_2$-8) in 7*H*-pyrimido[4′,5′:6,5]-pyrano[3,2-*h*]quinoline moiety for compound **8a** resulted in strong reduction of potency, while replacement of the hydrogen atom at position C-2 with the electron-donating group ($–CH_3$) resulted in strong improvement of potency for compound **8b**, suggesting that an electron-donating group might be preferred at position C-2.

Conclusions

Our interest in the synthesis of quinoline derivatives is to focus on their antitumor activities as a part of our recent research line that aims at the development of new heterocyclic compounds as strong potent antitumor agents (Al-Ghamdi *et al.*, 2012; El-Agrody *et al.*, 2012). Thus, in this paper we revealed the synthesis of some 4*H*-pyrano-[3,2-*h*]quinoline and 7*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*]quinoline derivatives, followed by antitumor evaluation for all of the novel compounds. Twenty compounds of 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido[4′,5′:6,5]-pyrano[3,2-*h*]quinoline derivatives were prepared and their structures were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, and MS data. Compounds **4a**, **11b**,**a**, and **13a** had the most potent antitumor activities against MCF-7 and compounds **4a**, **11b**, **9b**,**c**, **11a**, and **13a**,**c** had the most potent activities against HCT, while compounds **3c**, **11b**, **4a** **3d**, **8b**, and **11a** had the most potent antitumor activities against HepG-2. A more extensive study is also warranted to determine additional antitumor parameters in order to give a deeper insight to its structure–activity relationship and to optimize the effectiveness of this series of molecules, which can then be used in bigger scenarios such as drug design or development of antitumor therapeutics.

Experimental

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), where the signals of CH & CH_3 carbon atoms appear normal (up) and the signals of carbon atoms in CH_2 environments appear negative (down). The MS were measured on a Shimadzu GC/MS-QP5050A spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser.

Synthesis of 4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile derivatives (**3a-d**)

Prepared as previously described (El-Agrody and Al-Ghamdi, 2011).

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

A mixture of **3a** (0.01 mol), benzaldehyde (0.01 mol), ethanol (20 ml), and piperidine (0.5 ml) was refluxed for 2 h. The solid product was collected by filtration and crystallized from ethanol giving **4a** as yellow crystals; m.p. 185–186 °C; IR (KBr) υ (cm^{-1}): 3097, 3065, 3033, 2941, 2915 (CH), 2227 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.28 (s, 1H, N=CH), 8.10–7.05 (m, 14H, aromatic), 5.08 (s, 1H, H-4); ^{13}C NMR (125 MHz) ($CDCl_3$) δ (ppm): 163.87 (C-2), 159.91 (=CH), 158.25 (C-10b), 152.10 (C-9), 134.63 (C-10a), 135.35 (C-7), 129.37 (C-5), 128.92 (C-6a), 122.29 (C-4a), 119.65 (CN), 113.43 (C-8), 112.33 (C-6), 83.42 (C-3), 43.70 (C-4), 141.18, 131.84, 130.73, 130.57, 130.10, 129.85, 129.56, 126.74; MS *m/z* (%): 423 (M^++2, 8.02), 421 (M^+, 23.35), 310 (100), 207 (3), 177 (14), 111 (26), 50 (21); Anal. Calcd for $C_{26}H_{16}ClN_3O$: C, 74.02; H, 3.82; N, 9.96. Found: C, 74.08; H, 3.88; N, 10.02 %.

Reaction of **4a** with hydrazine derivatives

A mixture of 2-benzylideneamino-4-(4-chlorophenyl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**4a**) (0.01 mol) and hydrazine hydrate or phenyl hydrazine (0.01 mol) in EtOH (20 ml) was stirred at room temperature or refluxed for 2 h to give **3a** (m.p. and mixed m.p. are completely identical) yielding (83 %).

Reaction of β-enaminonitrile (**3c**,**d**) with acetic anhydride

A solution of β-enaminonitrile **3c** or **3d** (0.01 mol) in Ac_2O (20 ml) was heated under reflux for 30 min. The solid product was filtered, washed with cooled EtOH, dried and recrystallized from proper solvent to give **7a**,**b**. The physical and spectral data of compounds **7a**,**b** are as follows:

*2-Acetylamino-4-(4-chlorophenyl)-9-methyl-4H-pyrano [3,2-h]quinoline-3-carbonitrile (**7a**)*

Light brown crystals from ethanol; m.p. 225–226 °C; 83 %; IR (KBr) υ (cm^{-1}): 3383 (NH), 3060, 3023, 2940, 2820 (CH stretching), 2214 (CN), 1657 (CO); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 10.30 (bs, 1H, NH), 8.08-7.10 (m, 8H, aromatic), 5.07 (s, 1H, H-4), 2.84 (s, 3H, CH_3), 2.27 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 169.21 (CO), 160.57 (C-2), 151.40 (C-9), 143.02 (C-10b), 137.32 (C-10a), 136.71 (C-7), 129.42 (C-5), 128.82 (C-6a), 124.62 (C-8), 123.72 (C-4a), 120.42 (C-6), 116.41 (CN), 61.57 (C-3), 43.19 (C-4), 25.19 (CH_3), 23.48 (CH_3), 140.94, 133.95, 129.61, 125.70 (aromatic); MS *m/z* (%): 391 (M^++2, 7), 389 (M^+), (21), 278 (100), 236 (8), 209 (12), 111 (45), 75 (25); Anal. Calcd for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.80; H, 4.17; N, 10.81 %.

*2-Acetylamino-4-(4-bromophenyl)-9-methyl-4H-pyrano [3,2-h]quinoline-3-carbonitrile (**7b**)*

Light brown crystals from ethanol m.p. 249–250 °C; 80 %; IR (KBr) υ (cm^{-1}): 3320 (NH), 3050, 2975, 2861 (CH), 2189 (CN), 1657 (CO); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.60 (bs, 1H, NH), 7.93-7.04 (m, 8H, aromatic), 5.24 (s, 1H, H-4), 2.72 (s, 3H, CH_3), 2.33 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 165.12 (CO), 162.48 (C-2), 160.00 (C-9), 158.28 (C-10b), 135.87 (C-10a), 131.52 (C-7), 126.55 (C-5), 126.06 (C-6a), 124.03 (C-8), 123.80 (C-4a), 121.82 (C-6), 120.95 (CN), 63.20 (C-3), 39.23 (C-4), 25.52 (CH_3), 21.37 (CH_3), 144.21, 138.18, 130.30, 123.02 (aromatic); MS *m/z* (%): 435 (M^++2, 17), 433 (M^+, 15), 278 (100), 209 (14), 184 (15), 149 (31), 79 (70); Anal. Calcd for $C_{22}H_{16}BrN_3O_2$: C, 60.84; H, 3.71; N, 9.68. Found: C, 60.85; H, 3.73; N, 9.70 %.

Reaction of β-enaminonitrile (**3a**,**c**,**d**) with formamide

A mixture of β-enaminonitrile **3a**,**c**,**d** (0.01 mol) and formamide (20 ml) was refluxed for 2 h. The solid product was filtered, washed with cooled EtOH, dried and recrystallized from proper solvent to give **8a–c**. The physical and spectral data of compounds **8a–c** are as follows:

*8-Amino-7-(4-chlorophenyl)-7H-pyrimido [4′,5′:6,5]pyrano[3,2-h]quinoline (**8a**)*

Colorless needles from benzene; m.p. 298–299 °C; 69 %; IR (cm^{-1}) in KBr: 3433, 3290, 3195, (NH_2), 3097, 2950, 2897,(CH), 1638 (C=N); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.00 (s, 1H, H-10), 8.37–7.34 (m, 9H, aromatic), 6.85 (bs, 2H, NH_2), 5.50 (s, 1H, H-4); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 162.70 (C-11a), 162.46 (C-8), 156.93 (C-10), 150.49 (C-1b), 144.14 (C-2), 138.09 (C-1a), 135.95 (C-4), 127.83 (C-6), 126.75 (C-4a), 123.76 (C-6a), 122.76 (C-3), 122.20 (C-5), 95.22 (C-7a), 37.86 (C-7), 143.01, 131.69, 129.47, 128.68 (aromatic); MS *m/z* (%): 362 (M^++2, 8), 360 (M^+, 26), 249 (100), 222 (25), 194 (12), 111 (8), 75 (12), Anal. Calcd for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.03; H, 3.63; N, 15.21 %.

*8-Amino-7-(4-chlorophenyl)-2-methyl-7H-pyrimido[4′,5′:6,5]pyrano[3,2-h]quinoline (**8b**)*

Colorless needles from benzene; m.p. 288–289 °C; 67 %; IR (cm^{-1}) in KBr: 3420, 3320, 3220, (NH_2), 3091, 2945, 2897,(CH), 1654 (C=N); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.20 (s, 1H, H-10), 8.24–7.28 (m, 8H, aromatic), 6.94 (bs, 2H, NH_2, cancelled by D_2O), 5.46 (s, 1H, H-4), 2.74 (s, 3H, CH_3); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 162.67 (C-11a), 162.51 (C-8), 159.05 (C-10), 156.84 (C-2), 143.70 (C-1b), 137.57 (C-1a), 136.05 (C-4), 126.13 (C-6), 125.70 (C-4a), 123.51 (C-6a), 122.83 (C-3), 120.20 (C-5), 95.29 (C-7a), 37.85 (C-7), 24.97 (CH_3), 143.13, 131.63, 129.41, 128.88 (aromatic); MS *m/z* (%): 376 (M^++2, 8.25), 374 (M^+, 20.30), 263 (100), 236 (33), 209 (18), 154 (6), 111 (46), 74 (55), 50 (28); Anal. Calcd for $C_{21}H_{15}ClN_4O$: C, 67.29; H, 4.03; N, 14.95. Found: C, 67.48; H, 3.99; N, 14.98 %.

*8-Amino-7-(4-bromophenyl)-2-methyl-7H-pyrimido [4′,5′:6,5]pyrano[3,2-h]quinoline (**8c**)*

Colorless crystals from benzene; m.p. 292–293 °C; 71 %; IR (cm^{-1}) in KBr: 3095, 2947, 2899 (CH), 3391, 3318, 3193 (NH_2), 1638 (C=N); MS *m/z* (%): 420 (M^++2, 20.6), 418 (M^+, 18.6), 263 (100), 263 (20.6), 126 (8.2), 77 (6.2); Anal. Calcd for $C_{21}H_{15}BrN_4O$: C, 60.16; H, 3.61; N, 13.36. Found: C, 60.32; H, 3.72; N, 13.46 %.

Reaction of β-enaminonitrile (**3a**,**c**,**d**) with triethyl orthoformate

A mixture of β-enaminonitrile **3a**,**c**,**d** (0.01 mol), triethyl orthoformate (0.01 mol), 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 **9a–c**. The physical and spectral data of the compounds **9a–c** are as follows:

*4-(4-Chlorophenyl)-2-ethoxymethyleneamino-4H-pyrano [3,2-h]quinoline-3-carbonitrile (**9a**)*

Colorless needles from benzene; m.p. 228–229 °C; 83 %; IR (KBr) υ (cm^{-1}): 3044, 2987, 2936, 2903, 2864 (CH), 2205 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.92 (s, 1H, N=CH), 8.68–7.00 (m, 9H, aromatic), 4.98 (s, 1H, H-4), 4.40 (q, 2H, CH_2, $J = 7.5$ Hz), 1.33 (t, 3H, CH_3, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.68 (N=CH), 157.94 (C-2), 150.77 (C-10b), 143.93 (C-9), 138.55 (C-10a), 135.93 (C-7), 129.58 (C-5), 128.46 (C-6a), 124.77 (C-4a), 122.22 (C-8), 119.96 (C-6), 117.89 (CN), 80.19 (C-3), 64.29 (CH_2), 43.24 (C-4), 13.95 (CH_3), 141.80, 133.75, 129.74, 126.74 (aromatic); MS *m/z* (%): 391 (M^++2, 10), 389 (M^+, 29), 278 (100), 222 (84), 140 (26), 75 (33); Anal. Calcd for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: 68.61; H, 4.82; N, 11.46 %.

*4-(4-Chlorophenyl)-2-ethoxymethyleneamino-9-methyl-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**9b**)*

Colorless needles from benzene; m.p. 225–226 °C; 82 %; IR (KBr) υ (cm^{-1}): 3049, 2985, 2935, 2867 (CH), 2207 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.87 (s, 1H, N=CH), 8.06–7.05 (m, 8H, aromatic), 5.03 (s, 1H, H-4), 4.51 (q, 2H, CH_2, $J = 7.5$ Hz), 2.85 (s, 3H, CH_3), 1.44 (t, 3H, CH_3, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.82 (N=CH), 159.99 (C-2), 157.80 (C-9), 143.30 (C-10b), 141.86 (C-10a), 136.28 (C-7), 129.23 (C-5), 126.71 (C-6a), 124.00 (C-8), 123.11 (C-6), 120.20 (C-4a), 117.92 (CN), 80.46 (C-3), 64.28 (CH_2), 43.15 (C-4), 25.54 (CH_3), 13.95 (CH_3), 133.71, 129.66, 129.51, 125.87 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 160.82 (N=CH ↑), 136.28 (C-7 ↑), 129.51 (aromatic ↑) 129.23 (C-5 ↑), 125.87 (aromatic ↑) 124.00 (C-8 ↑), 123.11 (C-6 ↑), 64.28 (CH_2 ↓), 43.15 (C-4 ↑), 25.54 (CH_3 ↑), 13.95 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 160.82 (N=CH ↑), 136.28 (C-7 ↑), 129.51 (aromatic ↑) 129.23 (C-5 ↑), 125.87 (aromatic ↑) 124.00 (C-8 ↑), 123.11 (C-6 ↑), 43.15 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 160.82 (N=CH ↑), 136.28 (C-7 ↑), 129.51 (aromatic ↑) 129.23 (C-5 ↑), 125.87 (aromatic ↑) 124.00 (C-8 ↑), 123.11 (C-6 ↑), 64.28 (CH_2 ↑), 43.15 (C-4 ↑), 25.54 (CH_3 ↑), 13.95 (CH_3 ↑); MS *m/z* (%): 405 (M^++2, 11), 403 (M^+, 28), 292 (100), 236 (80), 154 (11), 75 (14); Anal. Calcd for $C_{23}H_{18}ClN_3O_2$: C, 68.40; H, 4.49; N, 10.40. Found: C, 69.33; H, 4.47; N, 10.65 %.

*4-(4-Bromophenyl)-2-ethoxymethyleneamino-9-methyl-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**9c**)*

Colorless needles from benzene; m.p. 217–218 °C; 81 %; IR (KBr) υ (cm^{-1}): 3035, 2933, 2931, 2864 (CH), 2208 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.84 (s, 1H, N=CH), 8.05–7.03 (m, 8H, aromatic), 5.01 (s, 1H, H-4), 4.50 (q, 2H, CH_2, $J = 7$ Hz), 2.83 (s, 3H, CH_3), 1.43 (t, 3H, CH_3, $J = 7$ Hz); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.76 (N=CH), 159.98 (C-2), 157.80 (C-9), 143.42 (C-10b), 142.39 (C-10a), 136.18 (C-7), 126.71 (C-5), 125.80 (C-6a), 124.01 (C-8), 123.09 (C-6), 120.02 (C-4a), 117.97 (CN), 80.38 (C-3), 64.28 (CH_2), 43.22 (C-4), 25.59 (CH_3), 13.95 (CH_3), 137.79, 132.18, 130.01, 121.83 (aromatic); ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 160.76 (N=CH ↑), 136.18 (C-7 ↑), 132.18 (aromatic ↑) 130.01 (aromatic ↑), 126.71 (C-5 ↑), 124.01 (C-8 ↑), 123.09 (C-6 ↑), 64.28 (CH_2 ↓), 43.22 (C-4 ↑), 25.59 (CH_3 ↑), 13.95 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 160.76 (N=CH ↑), 136.18 (C-7 ↑), 132.18 (aromatic ↑) 130.01 (aromatic ↑), 126.71(C-5 ↑), 124.01 (C-8 ↑), 123.09 (C-6 ↑), 43.22 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ 160.76 (N=CH ↑), 136.18 (C-7 ↑), 132.18 (aromatic ↑) 130.01 (aromatic ↑), 126.71(C-5 ↑), 124.01 (C-8 ↑), 123.09 (C-6 ↑), 64.28 (CH_2 ↑), 43.22 (C-4 ↑), 25.59 (CH_3 ↑), 13.95 (CH_3 ↑); MS *m/z* (%): 449 (M^++2, 23), 447 (M^+, 29), 292 (100), 264 (14), 236 (82), 157 (7), 66 (15); Anal. Calcd for $C_{23}H_{18}BrN_3O_2$: C, 61.62; H, 4.05; N, 9.37. Found: C, 61.44; H, 4.45; N, 9.77 %.

Preparation of compounds **10a**,**b**

A mixture of β-enaminonitrile **3a** or **3c** (0.01 mol), DMF-DPA (0.01 mol), 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 **10a**,**b**. The physical and spectral data of the compounds **10a**,**b** are as follows:

*4-(4-Chlorophenyl)-2-dimethylaminomethyleneamino-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**10a**)*

Colorless needles from benzene; m.p. 210–211 °C; 83 %; IR (KBr) υ (cm^{-1}): 3015, 2921, 2850, 2808 (CH), 2196 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.89 (s, 1H, N=CH), 8.88–7.02 (m, 9H, aromatic), 4.91 (s, 1H, H-4), 3.14, 3.08 (s, 6H, $2CH_3$); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.00 (N=CH), 154.54 (C-2), 150.99 (C-10b), 144.27 (C-9), 138.72 (C-10a), 136.16 (C-7), 129.16 (C-5), 128.34 (C-6a), 124.07 (C-4a), 122.18 (C-8), 121.87 (C-6), 120.11 (CN), 73.43 (C-3), 43.24 (CH_3), 41.17 (CH_3), 34.91 (C-4), 142.93, 133.24, 129.65, 127.00 (aromatic); MS *m/z* (%): 390 (M^++2, 7), 388 (M^+, 20), 277 (100), 222 (6), 178 (7), 75 (9); Anal. Calcd for $C_{22}H_{17}ClN_4O$: C, 67.95; H, 4.41; N, 14.41. Found: C, 68.01; H, 4.22; N, 14.40 %.

*4-(4-Chlorophenyl)-2-dimethylaminomethyleneamino-9-methyl-4H-pyrano-[3,2-h]quinoline-3-carbonitrile (**10b**)*

Colorless needles from benzene; m.p. 260–261 °C; 81 %; IR (KBr) υ (cm^{-1}): 3049, 2997, 2893, 2912, 2810 (CH), 2195 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.56 (s, 1H, N=CH), 7.93-6.95 (m, 8H, aromatic), 4.88 (s, 1H, H-4), 3.14, 3.08 (s, 6H, $2CH_3$), 2.73 (s, 3H, CH_3); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 160.01 (N=CH), 159.48 (C-2), 154.64 (C-9), 144.27 (C-10b), 143.04 (C-10a), 138.17 (C-7), 129.56 (C-5), 126.57 (C-6a), 126.01 (C-8), 123.28 (C-4a), 122.72 (C-6), 120.22 (CN), 73.44 (C-3), 43.26 (CH_3), 41.18 (CH_3), 34.86 (C-4), 25.78 (CH_3), 143.7; MS *m/z* (%): 404 (M^++2, 8), 402 (M^+, 26), 291 (100), 236 (5), 188 (6), 111 (91), 75 (65); Anal. Calcd for $C_{23}H_{19}ClN_4O$: C, 68.57; H, 4.75; N, 13.91. Found: C, 68.22; H, 4.43; N, 13.85 %.

Reaction of **9a** with hydrazine hydrate and methylamine

A mixture of imadate **9a** (0.01 mol) and hydrazine hydrate or methylamine (0.01 mol) in ethanol (30 ml) was stirred at room temperature for 1 h. The solid product was collected and recrystallized from proper solvent to give **11a**,**b**. The physical and spectra data of the compounds **11a**,**b** are as follows:

*4-(4-Chlorophenyl)-2-(hydrazinomethyleneamino)-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**11a**)*

Colorless needles from benzene; m.p. 203–204 °C; 81 %; IR (KBr) υ (cm^{-1}): 3400, 3316, 3200 (NH & NH_2), 3051, 2940, 2815 (CH), 2178 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.14 (s, 1H, N=CH), 8.91–7.01 (m, 9H, aromatic), 5.99 (bs, 2H, NH_2), 5.00 (s, 1H, H-4), 4.87 (bs, 1H, NH); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 159.94 (N=CH), 158.18 (C-2), 156.94 (C-10b), 151.10 (C-9), 138.45 (C-10a), 136.13 (C-7), 129.44 (C-5), 128.28 (C-6a), 123.81 (C-4a), 122.19 (C-8), 121.24 (C-6), 120.04 (CN), 97.56 (C-3), 41.25 (C-4), 143.57, 133.66, 129.77, 126.59 (aromatic); MS *m/z* (%): 375 (M^+, 7), 268 (100), 232 (6), 175 (27), 101 (41), 75 (3); Anal. Calcd for $C_{20}H_{14}ClN_5O$: C, 63.92; H, 3.75; N, 18.64. Found: C, 63.51; H, 3.17; N, 18.41 %.

*4-(4-Chlorophenyl)-2-(methylaminomethyleneamino)-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**11b**)*

Colorless needles from benzene; m.p. 201–202 °C; 81 %; IR (KBr) υ (cm^{-1}): 3338 (NH), 3123, 3076, 2868 (CH),

2185 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 9.15 (s, 1H, N=CH), 8.95-7.03 (m, 9H, aromatic), 5.05 (s, 1H, NH), 4.85 (s, 1H, H-4), 3.36 (s, 3H, CH_3); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 159.70 (N=CH), 157.50 (C-2), 150.95 (C-10b), 150.52 (C-9), 138.65 (C-10a), 136.12 (C-7), 128.34 (C-5), 127.04 (C-6a), 123.93 (C-4a), 122.15 (C-8), 121.20 (C-6), 119.80 (CN), 97.80 (C-3), 41.24 (C-4), 143.76, 133.59, 129.42, 126.86 (aromatic); MS *m/z* (%): 376 (M^++2, 7), 374 (M^+, 21), 268 (10), 222 (100), 184 (23), 139 (31), 75 (35); Anal. Calcd for $C_{21}H_{15}ClN_4O$: C, 67.29; H, 4.03; N, 14.95. Found: C, 66.44; H, 3.57; N, 14.33 %.

Reaction of **9a–c** with ammonia

Method (a)

A mixture of imadate **9a–c** (0.01 mol) 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 **8a–c**. Compounds **13a–c** were separated from the reaction filtrate and recrystallized from ethanol. The physical and spectral data of compounds **13a–c** are as follows:

*2-(Aminomethyleneamino)-4-(4-chlorophenyl)-4H-pyrano [3,2-h]quinoline-3-carbonitrile (**13a**)* Colorless needles from ethanol; m.p. 230–232 °C; 38 %; IR (KBr) υ (cm^{-1}): 3335, 3300, 3178 (NH_2), 3046, 3009, 2876 (CH), 2194 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.94 (s, 1H, N=CH), 8.09–7.02 (m, 9H, aromatic), 5.04 (bs, 2H, NH_2), 4.85 (s, 1H, H-4); ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 159.32 (N=CH), 150.67 (C-2), 144.95 (C-10b), 142.77 (C-9), 136.13 (C-10a), 135.12 (C-7), 129.17 (C-5), 128.37 (C-6a), 124.06 (C-4a), 122.14 (C-8), 121.15 (C-6), 119.34 (CN), 97.56 (C-3), 41.19 (C-4), 138.08, 133.49, 129.48, 127.00 (aromatic); MS *m/z* (%): 362 (M^++2, 1), 360 (M^+, 3), 344 (3), 342 (6), 249 (5), 222 (100), 195 (29), 117 (14), 75 (16); Anal. Calcd for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.03; H, 3.63; N, 15.21 %.

*2-(Aminomethyleneamino)-4-(4-chlorophenyl)-9-methyl-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**13b**)* Colorless needles from ethanol; m.p. 260–261 °C; 39 %; IR (KBr) υ (cm^{-1}): 3463, 3340, 3200 (NH_2), 3060, 3025, 2836 (CH), 2188 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.24 (s, 1H, N=CH), 8.20–7.12 (m, 8H, aromatic), 7.18 (bs, 2H, NH_2, cancelled by D_2O), 4.99 (s, 1H, H-4), 2.71 (s, 3H, CH_3); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 162.51 (N=CH), 160.32 (C-2), 159.08 (C-9), 156.85 (C-10b), 137.56 (C-10a), 136.11 (C-7), 128.65 (C-5), 125.77 (C-6a), 123.51 (C-8), 122.79 (C-4a), 121.41 (C-6), 120.13 (CN), 95.28 (C-3), 40.42 (C-4), 24.97 (CH_3), 142.60, 131.63, 129.52, 126.12 (aromatic); MS *m/z* (%): 376 (M^++2, 3.84), 374 (M^+, 10.05), 263 (56), 236 (21), 208 (7), 180 (6), 152 (6), 111 (100), 74 (89), 50 (16); Anal. Calcd for $C_{21}H_{15}ClN_4O$: C, 67.29; H, 4.03; N, 14.95. Found: C, 67.34; H, 3.91; N, 14.83 %.

*2-(Aminomethyleneamino)-4-(4-bromophenyl)-9-methyl-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**13c**)* Colorless needles from ethanol; m.p. 252–253 °C; 40 %; IR (KBr) υ (cm^{-1}): 3350, 3322, 3195 (NH_2), 3050, 3010, 2974, 2879 (CH), 2189 (CN); ^{1}H NMR (500 MHz) ($CDCl_3$) δ: 8.21 (s, 1H, N=CH), 8.24–7.12 (m, 8H, aromatic), 7.17 (bs, 2H, NH_2), 4.98 (s, 1H, H-4), 2.74 (s, 3H, CH_3); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 162.51 (N=CH), 159.05 (C-2), 156.85 (C-10b), 143.55 (C-9), 136.12 (C-10a), 136.12 (C-7), 129.78 (C-5), 126.13 (C-6a), 125.77 (C-8), 123.51 (C-4a), 120.29 (C-6), 120.18 (CN), 95.22 (C-3), 40.08 (C-4), 137.57, 131.63, 131.85, 122.71 (aromatic); MS *m/z* (%): 420 (M^++2, 9.21), 418 (M^+, 10.31), 236 (84), 209 (23), 180 (10), 155 (38), 127 (4), 75 (100), 50 (16); Anal. Calcd for $C_{21}H_{15}BrN_4O$: C, 60.16; H, 3.61; N, 13.36. Found: 60.21; H, 3.73; N, 13.42 %.

Method (b)

A mixture of **3a**,**c**,**d** (0.01 mol) and formamide (20 ml) was stirred at reflux for 3 h. The solvent was removed under vacuum. The solid obtained was recrystallized from benzene to give **8a–c** (m.p. and mixed m.p. are completely identical) yield (71–67 %).

Method (c)

Compound **13a–c** (0.01 mol) was heated under reflux in ethanol (20 ml) and piperidine (0.5 ml) for 3 h to give **8a–c** (m.p. and mixed m.p. are completely identical) yield (58–60 %).

Reaction of the imadate (**9a**,**b**) with dimethylamine

Method (a)

A mixture of imadate **9a**,**b** (0.01 mol) and dimethylamine (0.01 mol) in ethanol (30 ml) was stirred at room temperature for 1 h. The solid product was collected and recrystallized from proper solvent to give **10a**,**b** (m.p. and mixed m.p. are completely identical) yield (88–87 %).

Method (b)

A mixture of β-enaminonitrile **3a**,**c** (0.01 mol), DMF-DPA (0.01 mol), 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 **10a**,**b** (m.p. and mixed m.p. are completely identical) yield (83–81 %).

Reaction of **10a**,**b** with hydrazine hydrate

Method (a)

A mixture of imidine **10a**,**b** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 ml) was stirred at room temperature or reflux for 1 h. The solid product was collected and recrystallized from proper solvent to give **10a**,**b** (m.p. and mixed m.p. are completely identical) yield (80–81 %).

Method (b)

A mixture of imidine **10a**,**b** (0.01 mol), hydrazine hydrate (0.01 mol), and *p*-toluenesulfonic acid (0.01 mol) in toluene (30 ml) was heated under reflux for 7 h. The solid product was collected and recrystallized from proper solvent to give **10a**,**b** (m.p. and mixed m.p. are completely identical) yield (78–80 %).

Antitumor screening

Cell culture

MCF-7, HCT, and HepG-2 cells were grown on RPMI-1640 medium supplemented with 10 % inactivated fetal calf serum and 50 μg/ml gentamycin. Vero cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal calf serum, 1 % L-glutamine, HEPES buffer, and 50 μg/ml gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2 and were subcultured two to three times a week.

Cytotoxicity evaluation using viability assay

The in vitro cytotoxicity activity was studied against three cell lines: MCF-7, HCT, and HepG-2 using the colorimetric MTT assay (Mossman, 1983). The cells were seeded in 96-well microtiter plate at a cell concentration of 1×10^4 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 twofold dilutions of the metabolites were added to confluent cell monolayers. The microtiter 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 not found 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, crystal violet solution (1 %) was added to each well for 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain was 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 cytotoxic effect of each tested compound was calculated.

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