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ORIGINAL PAPER

A new and potent class of quinoline derivatives against cancer

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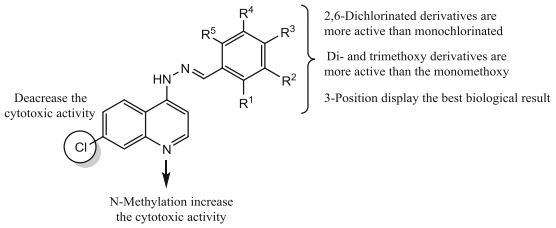
Abstract A new class of 4-quinolinylhydrazone derivatives has been synthesized and evaluated for their cytotoxic potential against three cancer cell lines using the MTT assay. Compounds displaying more than 90 % of growth inhibition were evaluated for in vitro anticancer activities against four human cancer cell lines. The results were expressed as the concentrations that induce 50 % inhibition of cell growth (IC_{50}) in μg/cm^3. These compounds exhibited good cytotoxic activity against at least three cancer cell lines, with IC_{50} values between 0.314 and 4.65 μg/cm^3. These derivatives are useful starting points for further study for new anticancer drugs and confirm the potential of quinoline derivatives as lead compounds in anticancer drug discovery.

Graphical Abstract



Keywords Drug research · Antitumor agents · Heterocycles · Hydrazones · Quinoline compounds

Introduction

Cancer is a growing public health problem that in 2012 was responsible for 8.2 million of deaths worldwide [1]. This disease accounted for nearly one-quarter of all deaths in the United States in 2012, exceeded only by heart diseases [2].

After 70 years of rapid advances in the identification and development of curative treatment for many malignant processes, the introduction of potential drugs, such as methotrexate and azathioprine, helped in the treatment of several tumors that were previously untreatable or only accessible through surgery and/or radiation [3]. Despite the introduction of new anticancer drugs in the market and new therapeutically approaches, nearly 10 million new cases occur each year worldwide [4]. The main disadvantage of conventional drugs is their nonspecific cytotoxicity to tumor cells, which lead to a narrow therapeutic window and low therapeutic index [5]. Furthermore, resistance to drugs such as imatinib and trastuzumab, used to target specific tyrosine kinases, is now evident [6, 7].

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Due to the high impact of this disease and the elevated cytotoxicity profiles of anticancer drugs, we urgently need new drugs and strategies to treat efficiently this disease. Moreover, new drugs must be less cytotoxic, safer, and more effective for the patient.

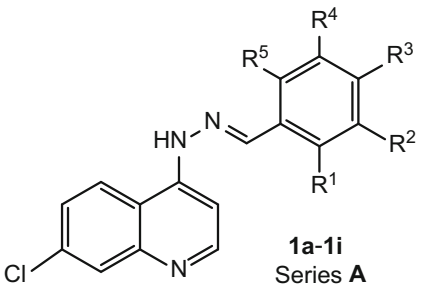
In our studies on the anticancer activities of heteroaromatic compounds, we identified a series of 7-chloro-4-quinolinylhydrazone derivatives (series **A**, **1a**-**1i**) with promising antitumoral activities (Table 1) [8, 9]. Following on from this earlier work, we have studied the anticancer activities of two new series of 4-quinolinylhydrazones, namely series **B** (**5a**–**5i**) and series **C** (**6a**–**6i**), see Fig. 1. These series **B** and **C** were synthesized to evaluate the importance of a 7-chloro substituent in the quinoline moiety and the effect of methylation on the activities of quinolinylhydrazone derivatives. Furthermore, the selection of the phenyl substituents in the series **B** and **C** compounds was based on the best biological results obtained with series **A** compounds (Fig. 1). Our results have enabled us to construct important structure–activity relationships.

Results and discussion

Synthesis and characterization

The preparation of series **B** (4-quinolinylhydrazones derivatives **6a**–**6i**) is shown in Scheme 1. First, 7-chloro-4-methoxyquinoline (**2**) was prepared from 4,7-dichloroquinoline on reaction with sodium methoxide (1 M) at 50 °C, and was subsequently dehalogenated to 4-methoxyquinoline (**3**) using hydrogen and Pd/C. 4-Hydrazinylquinoline (**4**) was prepared from **3** using hydrazine hydrate (80 %) in ethanol under reflux. Finally, the hydrazones **5a**–**5i** (Table 2) were obtained through reaction between 4-hydrazinoquinoline and appropriate benzaldehydes. Identification of the isolated series **B** compounds as hydrochloride salts, with protonated quinolinyl nitrogen atoms and with (*E*) geometries at the C=N center, was achieved by spectroscopic data in all cases, and specifically by X-ray crystallography for two hydrated salts, namely [(**5**: R^1=Cl; R^2=R^3=R^4=R^5=H)·2H_2O] [10] and [(**5**: R^1=R^3=Cl; R^2=R^4=R^5=H)·H_2O] [11], see later. Generally,

Table 1 Selected IC_{50} results for derivatives **1a**–**1i** of the series **A** against cancer cell lines



Compound series A	Substituents	Best IC_{50}/μg cm^{-3}	Cancer cell line
1a	R^1=R^2=R^3=R^4=R^5=H	2.085	SF-295[a]
1b	R^2=F; R^1=R^3=R^4=R^5=H	3.206	HTC-8[b]
1c	R^2=Cl; R^1=R^3=R^4=R^5=H	1.238	SF-295
1d	R^2=Br; R^1=R^3=R^4=R^5=H	1.588	HL-60[c]
1e	R^2=OMe; R^1=R^3=R^4=R^5=H	0.8018	HTC-8
1f	R^1=R^5=Cl; R^2=R^3=R^4=H	1.086	HTC-8
1g	R^2=R^3=OMe; R^1=R^4=R^5=H	0.6888	SF-295
1h	R^1=R^4=OMe; R^2=R^3=R^5=H	2.837	HL-60
1i	R^2=R^3=R^4=OMe; R^1=R^5=H	0.7483	HTC-8

[a] SF-295 (nervous system)

[b] HTC-8 (colon)

[c] HL-60 (leukemia)

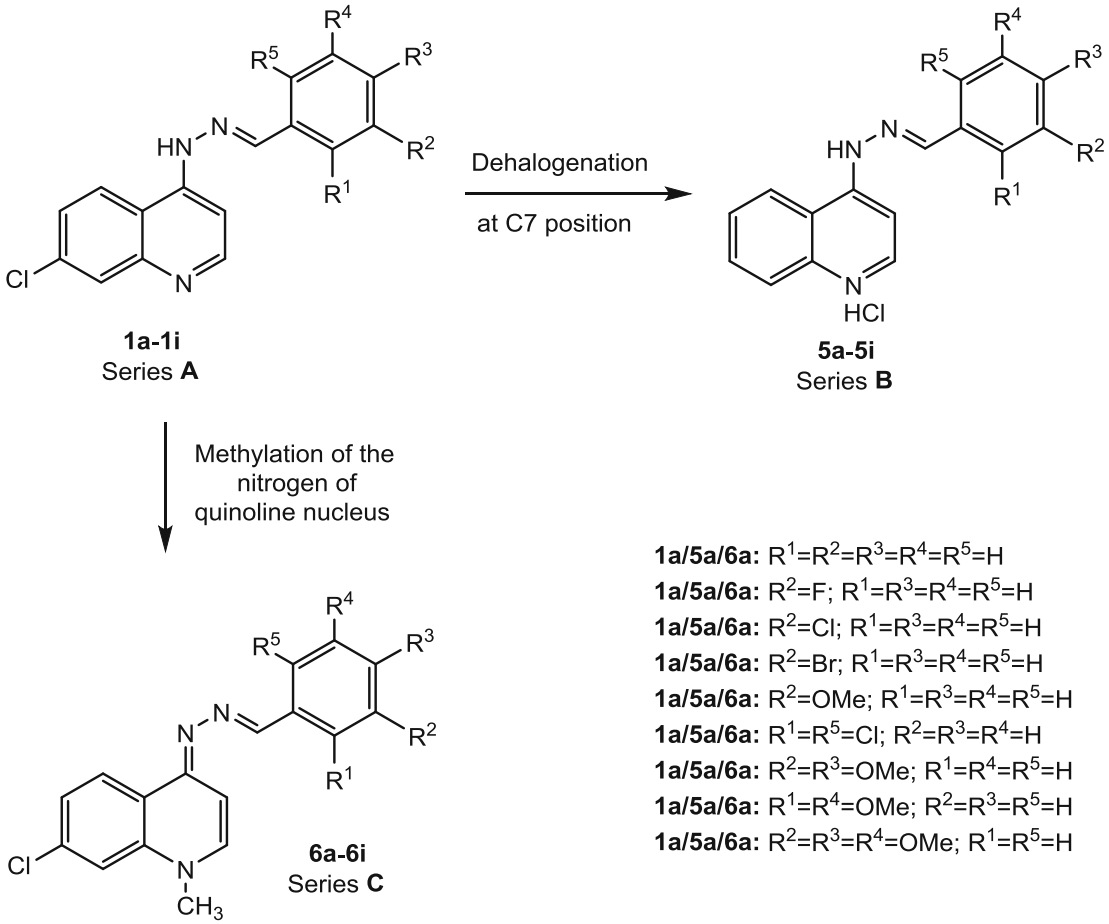


Fig. 1 Design concept for new series **B** (**5a**–**5i**) and series **C** (**6a**–**6i**) of 4-quinolinylhydrazones

in the ^{1}H NMR spectra of **5a**–**5i**, the chemical shifts of the N = CH protons are found in the range 8.37–8.81 ppm, while in the IR spectra, the N–H and N=C stretching vibrations occur in the ranges 3197–3247 and 1570–1585 cm^{-1}, respectively.

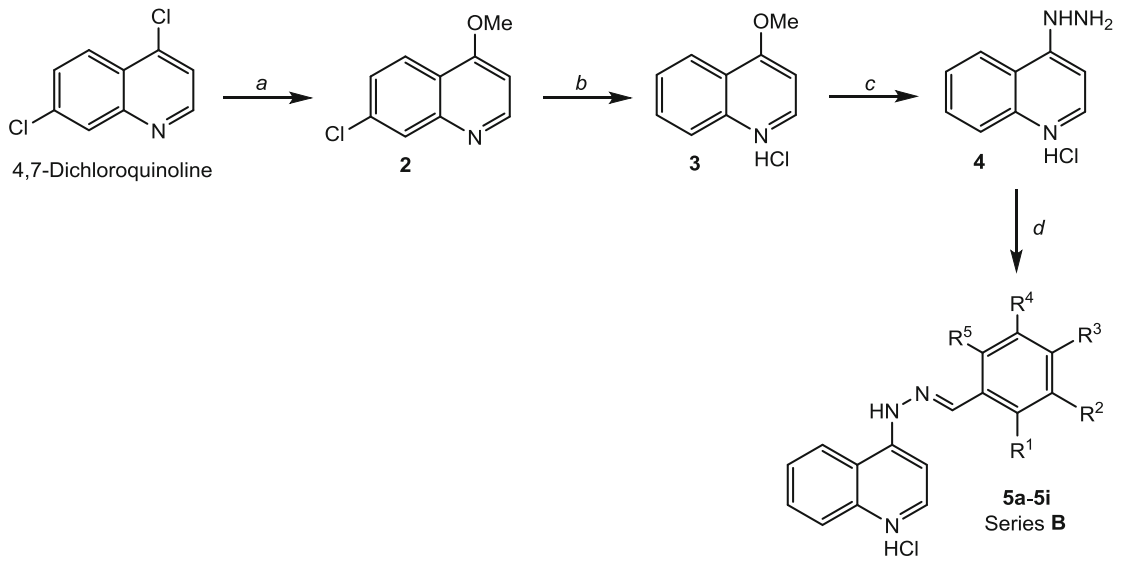
Series **C** derivatives were prepared by direct methylation of compounds **1a**-**1i** (series **A**) [8, 9], using MeI and K_2CO_3 in acetone (Scheme 2). The products were obtained as yellow or orange solids in moderate to excellent yields (Table 2). Identification of the isolated series **C** compounds **6** as substituted benzaldehyde 7-chloro-1-methyl-4*H*-quinolinyl-4-ylidene hydrazones, with methylated quinoline nitrogen atoms, was generally achieved by spectroscopic data, and specifically by X-ray crystallography for **6e**, see later. The NMe signal in the ^{1}H NMR spectra of **6a**-**6i** occurs as a singlet between 3.62 and 3.74 ppm. In addition, the IR spectra showed N–CH_3 and N=C stretching vibrations at 2855–2939 and 1626–1635 cm^{-1}, respectively.

X-ray structural studies

The structure of **6e**, an active compound, was confirmed in this study by X-ray crystallography: the triclinic space group, *P*-1, was assigned. Figure 1 shows the atom arrangements: there is a weak C5-H5–N2 intramolecular hydrogen bond. Selected bond lengths and angles are also listed in Fig. 2. The structure determination confirmed that methylation of the precursor, **1e**, had occurred on the quinoline nitrogen, with loss of the aromatic structure of the pyridine ring and the formation of a (*E*,*E*)-CH=N–N=CH-C_6H_4OMe-*m* fragment. The molecule overall is very near planar with only a small angle of 4° between the planes of the quinolinyl and phenyl groups. The only intermolecular interactions are weak C–H–O and C–H–N hydrogen bonds, and π–π stacking interactions.

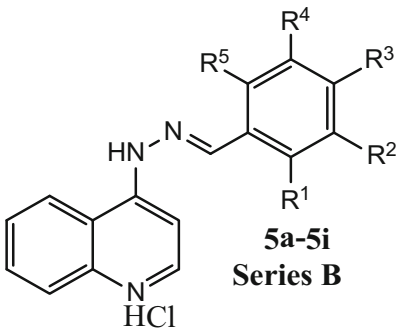
X-ray crystal structures have been previously reported for a number of anhydrous [12–14] and hydrated [15–18] series **A** compounds **1**. These include both active and non-

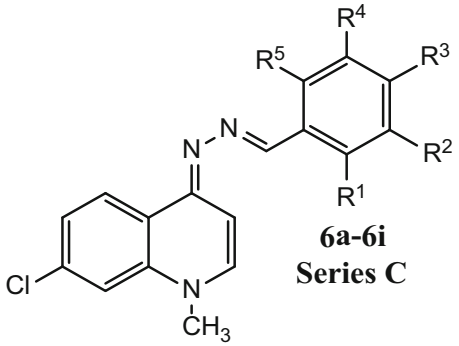
Scheme 1



(*a*) MeONa (1M), 50 °C, 3 h, 95%; (*b*) H_2, Pd/C, MeOH, r.t., 6 h, 85%; (*c*) $N_2H_4{\cdot}H_2O$ (80%), EtOH, 80 °C, 4 h, 80%; (*d*) corresponding benzaldehyde, EtOH, r.t., 4-24 h, 60-95%.

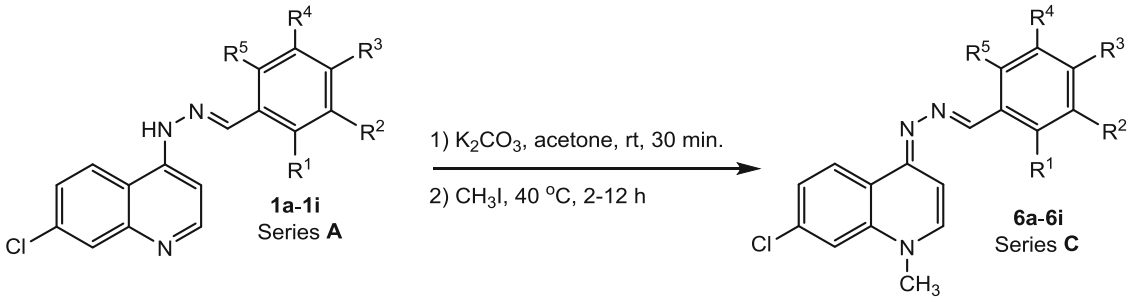
Table 2 Yields and melting points of series **B** and **C** compounds

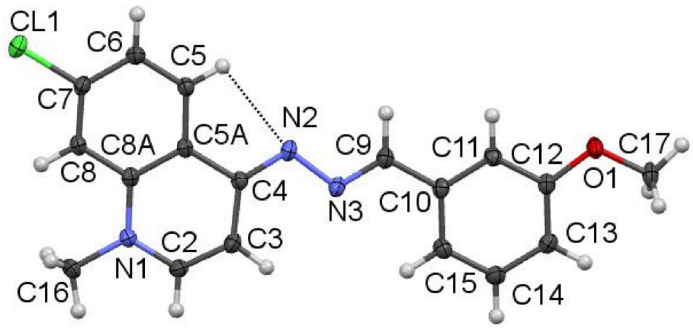




Series **B/C**	Substituents					Yield/%		M.p./°C	
	R^1	R^2	R^3	R^4	R^5	Series **B**	Series **C**	Series **B**	Series **C**
5a/6a	H	H	H	H	H	76	65	202–203	156–157
5b/6b	H	F	H	H	H	82	58	214–215	173–175
5c/6c	H	Cl	H	H	H	80	61	223–225	185–187
5d/6d	H	Br	H	H	H	65	62	304	189–190
5e/6e	H	OMe	H	H	H	77	68	261–262	144–145
5f/6f	Cl	H	H	H	Cl	89	62	227–229	142–143
5g/6g	H	OMe	OMe	H	H	82	82	240–241	190–191
5h/6h	OMe	H	H	OMe	H	85	90	284–286	151–152
5i/6i	H	OMe	OMe	OMe	H	68	77	288–289	214–215

Scheme 2





N1-C2	1.362(3)
C2-C3	1.355(3)
C3-C4	1.439(3)
C4-C5A	1.469(3)
C5A-C8A	1.413(3)
C4-N2	1.315(2)
N2-N3	1.403(2)
N3-C9	1.285(3)
C9-C10	1.472(3)
N1-C16	1.466(3)
C8A-N1	1.389(2)
C3-C4-N2-N3	2.4
C4-N2-N3-C9	179.39(17)
N2-N3-C9-C10	180.00(17)

(a) **(b)**

Fig. 2 **a** Atom arrangements and numbering scheme for compound **6e**, **b** selected bond lengths and angles (Å, °)

active compounds. All these compounds, including the hydrated derivatives, have (*E*)-stereochemistries at the C=N group and all show only slight distortions from overall planarity as indicated by the small angles, up to 13°, between the phenyl and quinoline groups.

Generally, series **B** salts were found to be difficult to obtain in suitable form for X-ray studies. However, crystal structures have been reported for two hydrates of two series **B** salts, namely [(**5**: R^1=Cl; R^2=R^3=R^4=R^5=H)·2H_2O] [10] and [(**5**: R^1=R^3=Cl; R^2=R^4=R^5=H)·H_2O] [11] as well as the non-hydrated 3-chlorobenzoate salt of (**5**: R^2=Cl; R^1=R^3=R^4=R^5=H) [19]. In all three cases, it was confirmed that the site of protonation in the cation was the quinolinyl nitrogen and that the C=N moiety had an (*E*)-stereochemistry. The cations exhibited a slight twist in the link between the phenyl group and the rest of the cation: the twist between phenyl and quinolinium rings are 7.51(12)° and 4.33(3)°, respectively, for [(**5**: R^1=Cl; R^2=R^3=R^4=R^5=H)·2H_2O] [10] and [(**5**: R^1=R^3=Cl; R^2=R^4=R^5=H)·H_2O] [11]. In the 3-chlorobenzoate salt, the cation exhibits a greater twist of 18.98(10)°.

Anticancer activity

Initially, all compounds were tested in vitro against three cancer cells at 25 μg/cm^3 using the MTT assay (Table 3) [20]. The compounds were classified by their growth inhibition (GI) percentage at least in one cell line as active if 95 < GI <100 %, moderately active if 70 < GI <90 %, or inactive if GI <50 %. For series **B**, compounds **5a** and **5c**-**5 h** were active against at least one cancer cell line with GI values greater than 90 %, while **5b** and **5i** only exhibited moderately active. For series **C**, compounds **6b**-**6e** and **6g**-**6i** were active, **6a** was moderately so and **6f** was inactive against all cancer cell lines at 25 μg/cm^3.

The active compounds were selected for in vitro anticancer activity evaluations against four human cancer cell lines (HCT-116, OVCAR-8, HL-60, and SF-295) using the MTT assay (Table 4) [19]. The concentrations that induced 50 % inhibition of cell growth (IC_{50}) in μg/cm^3 are presented in Table 4. None of the compounds was able to disrupt the cell membrane integrity of erythrocytes in the mouse model (data not shown) [21].

Table 3 Growth inhibition percentage (GI %) for three tumors cells line by the MTT assay

Entry		SF-295 GI %	SD %	HCT-116 GI %	SD %	OVCAR-8 GI %	SD %
Nr	Substituents						
Series **B**							
5a	H	**73.02**	**5.19**	**100.00**	**0.17**	**100.00**	**0.25**
5b	3-F	24.14	3.56	57.75	1.35	92.06	0.08
5c	3-Cl	**98.93**	**0.00**	**100.00**	**1.90**	**100.00**	**0.43**
5d	3-Br	**98.63**	**0.93**	**100.00**	**5.35**	**92.36**	**2.59**
5e	3-OMe	**100.00**	**1.35**	**100.00**	**4.46**	**100.00**	**2.88**
5f	2,6-Cl_2	**54.54**	**4.18**	**101.81**	**0.43**	**101.52**	**0.17**
5g	3,4-$(OMe)_2$	**100.00**	**0.76**	**52.29**	**1.08**	**90.33**	**1.97**
5h	2,5-$(OMe)_2$	**98.04**	**1.43**	**97.56**	**1.24**	**92.36**	**3.60**
5i	2,3,4-$(OMe)_3$	83.05	1.49	11.20	1.71	36.05	3.53
Series **C**							
6a	H	64.08	0.76	89.55	1.41	52.95	0.50
6b	3-F	**86.71**	**2.79**	**99.49**	**0.18**	**98.56**	**0.31**
6c	3-Cl	**87.68**	**0.00**	**100.38**	**0.54**	**100.67**	**0.16**
6d	3-Br	**55.88**	**2.95**	**94.27**	**1.03**	**100.00**	**0.00**
6e	3-OMe	**100.38**	**0.06**	**100.51**	**0.36**	**101.33**	**0.16**
6f	2,6-Cl_2	45.88	0.34	56.13	1.82	26.90	0.91
6g	3,4-$(OMe)_2$	**99.88**	**0.53**	**102.45**	**0.13**	**101.05**	**0.17**
6h	2,5-$(OMe)_2$	**101.69**	**0.06**	**101.82**	**0.00**	**100.93**	**0.17**
6i	2,3,4-$(OMe)_3$	**100.70**	**0.76**	**101.36**	**0.13**	**100.64**	**0.08**

The results in Tables 3 and 4 show that compounds of both series exhibit good cytotoxic activity against the four cancer cell lines. The results obtained for series **B** clearly indicate the importance of the degree and nature of the substitution in the phenyl group. Mono-*meta*-substituted compounds (**5c**–**5e**), irrespective of the substituent being electron donating or electron withdrawing, are more active than the non-substituted compound **5a**. Interestingly, the most active mono-substituted compound is the bromo derivative **5d** and, furthermore, the sequence of activity for the mono-halo derivatives is **5d** [3-Br] >**5c** [3-Cl] >**5b** [3-F]. The 3-OMe derivative **5e** is less reactive than the 3-Cl derivative **5c**. Considering just the mono-halogeno derivatives, the activity sequence correlates with their electronic donating effects, as well as the bulk of the substituent, but a correlation fails if the methoxy substituent is also included. The addition of another chloro group does increase the activity (compare results for **5c** and **5f**). In contrast, the addition of further methoxy groups does not improve the cytotoxic activity.

There are some interesting contrasts and similarities between the results of the two series **B** and **C**. First, the similarity: mono-substituted derivatives, no matter whether electron donating or withdrawing (**6b**–**6e**), are more active than the unsubstituted derivative, **6a**. However, in contrast, the sequence of activity is **6e** [3-OMe] >**6b** [3-F] >**6c** [3-Cl] >**6d** [3-Br]. This time the activity sequence of the halogeno substituents follows their mesomer donor abilities. Further differences between series **B** and **C** are (1) the 2,6-dichloro substituted compound **5f** is more active than the mono-chloro compound **5c** and (2) the di- and trimethoxy derivatives **6g**–**6i** are more active than the monomethoxy **6e** compound in the **C** series. Indeed, the trimethoxy derivative **6i** is the most active compound.

Series **C** derivatives showed the best activity against the HL-60 cancer cell line, the one exception being **6e**, which was most active against the HCT-116 cell line. This cell line specificity was not shown by compounds in series **B**.

Conclusion

Comparisons of the activities of selected members of the three series, series **A**, **B**, and **C** are available in Table 5. General conclusions can be made as follows: (1) the activity of the best compounds in series **B** and **C** is greater than those of the corresponding compounds in series **A** and (2) derivatives of series **C** showed greater cytotoxic activities than did the derivatives of series **B**.

Three compounds, **5d**, **5f**, and **6i**, in particular, show encouraging results and thus can be considered as useful starting points for further study. The structural modifications of series **A** made to form series **B** and **C** have led to

Table 4 Cytotoxic activity of quinoline derivatives of series **B** and **C** on tumor cells lines[a]

Entry Series **B**	Substituents	IC_{50}/μg cm^{-3}			
		HCT-116	OVCAR-8	HL-60	SF-295
5a	H	2.807	1.931	0.7914	1.789
		2.245–3.510	1.702–2.189	0.6837–0.9161	1.363–2.349
5c	3-Cl	1.874	2.655	0.9133	2.643
		1.672–2.101	2.414–2.919	0.8180–1.020	2.229–3.133
5d	3-Br	**1.023**	**0.5728**	**0.6808**	**0.5178**
		0.8716–1.200	**0.369–0.889**	**0.542–0.854**	**0.1643–1.632**
5e	3-OMe	2.165	3.573	0.8338	4.654
		1.901–2.466	3.136–4.071	0.4698–1.480	2.741–7.902
5f	2,6-Cl_2	**0.7148**	**0.6792**	**0.8270**	**1.084**
		0.4724–1.082	**0.4786–0.9638**	**0.6797–1.006**	**0.7747–1.518**
5g	3,4-$(OMe)_2$	2.169	1.346	2.751	1.351
		1.437–3.274	0.7149–2.536	1.437–3.274	0.7030–2.596
5h	2,5-$(OMe)_2$	2.828	3.628	2.091	3.894
		1.606–4.982	3.254–4.045	0.9105–4.803	1.660–9.134
Series **C**	Substituents	HCT-116	OVCAR-8	HL-60	SF-295
6b	3-F	0.8188	1.120	0.7325	2.564
		0.5596–1.198	0.8357–1.501	0.6273–0.8553	0.7498–8.769
6c	3-Cl	1.924	1.953	1.315	1.966
		1.655–2.236	1.666–2.290	0.8834–1.958	1.672–2.312
6d	3-Br	3.620	2.202	1.979	2.373
		2.883–4.545	1.728–2.806	1.655–2.366	2.084–2.701
6e	3-OMe	0.6963	0.7034	0.9317	2.167
		0.4607–1.052	0.4875–1.015	0.7603–1.142	0.4367–10.75
6g	3,4-$(OMe)_2$	1.507	0.9223	0.3318	1.661
		1.132–2.005	0.7493–1.135	0.2673 –0.4118	1.223–2.255
6h	2,5-$(OMe)_2$	0.8303	0.8408	0.6436	1.569
		0.7691 0.8963	0.6976–1.013	0.5717 –0.7247	1.331–1.850
6i	2,3,4-$(OMe)_3$	**0.5080**	**0.4333**	**0.3139**	**0.8190**
		0.3498–0.7375	**0.3469–0.5411**	**0.2866–0.3438**	**0.7071–0.9486**
Doxorubicin		0.01	0.265	0.02	0.23
		0.01–0.02	0.11–0.305	0.01–0.02	0.19–0.25

[a] Data are presented as IC_{50} values, with 95 % confidence intervals obtained by nonlinear regression for all cell lines leukemia (HL-60), ovarium (OVCAR-8), nervous system (SF-295), colon (HCT-116) from three independent experiments. Doxorubicin (Dox) was used as positive control. The trials were performed in triplicate; IC_{50}, concentrations that induce 50 % inhibition of cell growth in μg cm^{-3}

useful and interesting findings and suggest that further modifications are well worth making. Further studies on the activity and structure–activity relationships of modified quinoline derivatives are underway.

Experimental

Melting points were determined on a Büchi apparatus. Infrared spectra were recorded in a Thermo Nicolet Nexus 670 spectrometer, as potassium bromide pellets and frequencies are expressed in cm^{-1}. High-resolution mass spectrum was performed on a Bruker compact QTOF. NMR spectra were recorded in a Bruker Avance 400 operating at 400.00 MHz (^{1}H) and 100.0 MHz (^{13}C), and Bruker Avance 500 spectrometer operating at 500.00 MHz (^{1}H) and 125.0 MHz (^{13}C), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and *J*-coupling in Hertz (Hz). Proton and carbon spectra were typically obtained at room temperature. TLC plates, coated with silica gel, were run in a chloroform/methanol (9:1) mixture and spots were developed in ultraviolet.

*7-Chloro-4-methoxyquinoline (**2**)*

To a solution of sodium methoxide (1 M) in methanol were portionwise added 2.5 g of 4,7-dichloroquinoline

Table 5 Comparison of the best anticancer activities of series **A**, **B**, and **C** compounds

Series **A**	Best IC_{50}/μg cm^{-3} [cancer cell line]	Series **B**	Best IC_{50}/μg cm^{-3} [cancer cell line]	Series **C**	Best IC_{50}/μg cm^{-3} [cancer cell line]
1a	2.085 [SF-295]	**5a**	0.7914 [HL-60]	**6a**	–
1b	3.206 [HTC-8]	**5b**	–	**6b**	0.7325 [HL-60]
1c	1.238 [SF-295]	**5c**	0.9133 [HL-60]	**6c**	1.315 [HL-60]
1d	1.588 [HL-60]	**5d**	0.5178 [SF-295]	**6d**	1.979 [HL-60]
1e	0.8018 [HTC-8]	**5e**	0.8338 [HL-60]	**6e**	0.6963 [HCT-116]
1f	1.086 [HTC-8]	**5f**	0.6792 [OVCAR-8]	**6f**	–
1g	0.6888 [SF-295]	**5g**	1.346 [OVCAR-8]	**6g**	0.3318 [HL-60]
1h	2.837 [HL-60]	**5h**	2.091 [HL-60]	**6h**	0.6436 [HL-60]
1i	0.7483 [HTC-8]	**5i**	–	**6i**	0.3139 [HL-60]

(12.6 mmol). The mixture reaction was stirred at 40 °C for 4 h. After that, the solvent was removed under reduced pressure. Then, the crude product was extracted with 30 cm^3 water and ethyl acetate (3 × 30 cm^3) and the combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield the intermediate **2** as a white solid. Yield: 2.32 g (95 %); m.p.: 139–141 °C (Ref. [22] 141–143 °C).

*4-Methoxyquinoline hydrochloride (**3**)*

To a solution of 2.0 g intermediate **2** (10.3 mmol) in 30 cm^3 methanol catalytic amounts of 10 % Pd–C (5 mol %) were added, and the mixture was treated with H_2 for 6 h. The catalyst was filtered off and washed with methanol (2 × 10 cm^3), and the filtrate was concentrated to lead the intermediate **3** as a white solid. Yield: 1.17 g (89 %); m.p.: 165–167 °C (dec.) [Ref. [23] 165 °C (dec.)].

*4-Hydrazinylquinoline hydrochloride (**4**)*

Hydrazine hydrate (80 %, 7.6 cm^3, 126 mmol) in 25 cm^3 absolute ethanol was added to 1.0 g 4-methoxyquinoline hydrochloride (**3**, 6.3 mmol) dissolved in 20 cm^3 absolute ethanol. The mixture was refluxed for 6 h. After that, the mixture was allowed to cool at 5 °C for 12 h. The pallid yellow precipitate was filtered and washed with 5 cm^3 absolute ethanol and 20 cm^3 diethyl ether to give the compound **4** as a white solid. Yield: 0.851 g (85 %); m.p.: 301–303 °C (dec.) [Ref. [12] 300–301 °C (dec.)].

*General procedure for synthesis of 4-quinolinylhydrazone hydrochlorides **5a-5i***

The 4-quinolinylhydrazone derivatives **5a**–**5i** were obtained by the reaction between 4-hydrazinylquinoline (**4**, 1.03 mmol) and the appropriate benzaldehyde (1.24 mmol) in 5 cm^3 ethanol. After stirring for 4–24 h at room temperature, the resulting mixture was concentrated under reduced pressure and the residue purified by washing with cold Et_2O (3 × 10 cm^3), leading to the pure derivatives **5a**–**5i** as solids in 60–95 % yields.

4-(Benzylidenehydrazinyl)quinoline hydrochloride (**5a**, $C_{16}H_{14}ClN_3$)

Yield: 76 %; yellow solid; m.p.: 202–203 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.79 (1H, s), 8.49 (1H, s, H_{β}), 8.45 (1H, d, J = 5.8 Hz, H_2), 8.41 (1H, d, J = 8.0 Hz, H_5), 7.85–7.80 (3H, m, H_{δ}, H_{ϕ}, H_8), 7.73 (1H, t, J = 7.5 Hz, H_7), 7.54 (1H, t, J = 7.5 Hz, H_6), 7.49–7.42 (3H, m, $H_{\acute{o}}$, $H_{\acute{\gamma}}$, $H_{\acute{\delta}}$), 7.37 (1H, d, J = 5.8 Hz, H_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.2, 147.0, 145.5, 145.2, 134.7, 130.1, 129.5, 128.8, 126.8, 125.8, 124.7, 122.3, 117.1, 100.4 ppm; IR (KBr): $\bar{\nu}$ = 3178 (N–H), 2915 (N^+–H), 1616 (C=N) cm^{-1}; HRMS (ESI): m/z = 246.1113 ($[M+H]^+$).

4-(3-Fluorobenzylidenehydrazinyl)quinoline hydrochloride (**5b**, $C_{16}H_{13}ClFN_3$)

Yield: 82 %; yellow solid; m.p.: 214–215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.46 (1H, s, H_{β}), 8.43–8.41

(1H, m, H_5, H_2), 7.84 (1H, d, $J = 8.2$ Hz, H_5), 7.74 (1H, t, $J = 7.5$ Hz, H_7), 7.67–7.63 (2H, m, H_6, $H_{5'}$), 7.57–7.48 (2H, m, $H_{7'}$, $H_{6'}$), 7.41 (1H, d, $J = 6.0$ Hz, H_3), 7.27–7.23 (1H, m, H_8) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 155.5$, 146.2, 145.5, 143.2, 135.9, 130.6, 129.5, 128.9, 127.8, 125.8, 124.9, 123.2, 117.2, 100.3 ppm; IR (KBr): $\bar{\nu} = 3189$ (N–H), 2908 (N^+–H), 1615 (C=N) cm^{-1}; HRMS (ESI): $m/z = 264.1015$ ($[M+H]^+$).

4-(3-Chlorobenzylidenehydrazinyl)quinoline hydrochloride (**5c**, $C_{16}H_{13}Cl_2N_3$)
Yield: 80 %; yellow solid; m.p.: 223–225 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.63$ (1H, s, $H_{3'}$), 8.60 (1H, d, $J = 8.4$ Hz, H_5), 8.52 (1H, d, $J = 6.0$ Hz, H_2), 7.94–7.92 (2H, m, $H_{5'}$, H_8), 7.86–7.78 (2H, m, H_6, H_7), 7.64 (1H, t, $J = 7.5$ Hz, $H_{8'}$), 7.56–7.46 (3H, m, H_3, $H_{7'}$, $H_{6'}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 150.4$, 145.7, 145.3, 142.7, 136.7, 133.8, 131.5, 130.6, 129.7, 126.3, 125.9, 125.6, 123.8, 123.1, 116.7, 100.6 ppm; IR (KBr): $\bar{\nu} = 3163$ (N–H), 2908 (N^+–H), 1608 (C=N) cm^{-1}; HRMS (ESI): $m/z = 282.0717$ ($[M+H]^+$).

4-(3-Bromobenzylidenehydrazinyl)quinoline hydrochloride (**5d**, $C_{16}H_{13}BrClN_3$)
Yield: 65 %; yellow solid; m.p.: 304 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 14.77$ (1H, s, NH), 13.10 (1H, s, NH), 8.91–8.89 (2H, m, $H_{3'}$, H_5), 8.69 (1H, d, $J = 6.9$ Hz, H_2), 8.11–8.09 (1H, m, H_8, $H_{5'}$), 8.03 (1H, t, $J = 7.7$ Hz, H_7), 7.87 (1H, d, $J = 7.8$ Hz, $H_{7'}$), 7.81 (1H, t, $J = 7.7$ Hz, H_6), 7.73 (2H, d, $J = 8.4$ Hz, $H_{5'}$, $H_{6'}$), 7.70–7.73 (2H, m, H_3, $H_{8'}$), 7.49 (1H, d, $J = 7.8$ Hz, $H_{6'}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 152.6$, 148.6, 172.5, 138.2, 135.9, 135.6, 133.8, 133.4, 131.1, 129.4, 126.9, 123.9, 122.4, 120.1, 115.1, 100.2 ppm; IR (KBr): $\bar{\nu} = 3152$ (N–H), 2684 (N^+–H), 1612 (C=N) cm^{-1}; HRMS (ESI): $m/z = 326.0217$ ($[M+H]^+$).

4-(3-Methoxybenzylidenehydrazinyl)quinoline hydrochloride (**5e**, $C_{17}H_{16}ClN_3O$)
Yield: 77 %; yellow solid; m.p.: 261–262 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 14.76$ (1H, s, NH), 13.12 (1H, s, NH), 9.01-8.99 (2H, m, $H_{3'}$, H_5), 8.63 (1H, d, $J = 7.0$ Hz, H_2), 8.10 (1H, d, $J = 8.0$ Hz, H_8), 7.98 (1H, t, $J = 8.0$ Hz, H_7), 7.75 (1H, t, $J = 8.0$ Hz, H_6), 7.61 (1H, d, $J = 7.0$ Hz, H_3), 7.42-7.37 (3H, m, $H_{5'}$ $H_{8'}$, $H_{6'}$), 7.06 (1H, d, $J = 7.5$ Hz, $H_{7'}$), 3.85 (3H, s, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 159.6$, 152.5, 150.3, 142.3, 138.2, 134.9, 133.7, 130.1, 126.8, 124.4, 120.4, 120.0, 116.9, 115.1, 111.8, 99.9, 55.3 ppm; IR (KBr): $\bar{\nu} = 3414$ (N–H), 2688 (N^+–H), 1589 (C=N) cm^{-1}; HRMS (ESI): $m/z = 278.1213$ ($[M+H]^+$).

4-(2,6-Dichlorobenzylidenehydrazinyl)quinoline hydrochloride (**5f**, $C_{16}H_{12}Cl_3N_3$)
Yield: 89 %; yellow solid; m.p.: 227–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.46$ (1H, s, NH), 8.70 (1H, s, $H_{3'}$), 8.43 (1H, s, H_2), 8.38 (1H, d, $J = 8.0$ Hz, H_5), 7.83 (1H, d, $J = 8.0$ Hz, H_8), 7.70 (1H, t, $J = 8.0$ Hz, H_7), 7.60–7.58 (2H, m, $H_{6'}$, $H_{8'}$), 7.52 (1H, t, $J = 8.0$ Hz, H_6), 7.40 (1H, t, $J = 8.0$ Hz, $H_{6'}$), 7.27 (1H, d, $J = 5.2$ Hz, H_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 153.2$, 152.3, 150.4, 144.5, 137.3, 132.8, 126.8, 123.5, 117.7, 115.1, 113.7, 110.2, 99.8 ppm; IR (KBr): $\bar{\nu} = 3485$ (N–H), 1579 (C=N) cm^{-1}; HRMS (ESI): $m/z = 314.0332$ ($[M+H]^+$).

4-(3,4-Dimethoxybenzylidenehydrazinyl)quinoline hydrochloride (**5g**, $C_{18}H_{18}ClN_3O_2$)
Yield: 82 %; yellow solid; m.p.: 240–241 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 14.70$ (1H, s, NH), 12.88 (1H, s, NH), 9.16 (1H, s, $H_{3'}$), 8.84 (1H, d, $J = 7.2$ Hz, H_5), 8.68 (1H, d, $J = 6.8$ Hz, H_2), 8.10 (1H, d, $J = 8.4$ Hz, H_8), 8.03 (1H, dd, $J = 8.4$, 7.6 Hz, H_7), 7.81 (1H, dd, $J = 7.6$, 7.2 Hz, H_6), 7.55 (2H, m, H_3, $H_{5'}$), 7.19 (1H, d, $J = 8.3$ Hz, $H_{8'}$, $H_{6'}$), 3.87 (6H, s, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 152.7$, 152.4, 148.5, 146.4, 142.2, 138.2, 133.6, 127.0, 126.7, 124.5, 123.9, 120.0, 117.1, 115.0, 99.8, 61.4, 55.8 ppm; IR (KBr): $\bar{\nu} = 2766$ (N^+–H), 1612 (C=N) cm^{-1}; HRMS (ESI): $m/z = 308.1321$ ($[M+H]^+$).

4-(2,5-Dimethoxybenzylidenehydrazinyl)quinoline hydrochloride (**5h**, $C_{18}H_{18}ClN_3O_2$)
Yield: 85 %; yellow solid; m.p.: 284–286 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 14.61$ (1H, s, NH), 12.75 (1H, s, NH), 9.13 (1H, s, $H_{3'}$), 8.78 (1H, d, $J = 8.3$ Hz, H_5), 8.65 (1H, d, $J = 6.8$ Hz, H_2), 8.08 (1H, d, $J = 8.3$ Hz, H_8), 8.02 (1H, dd, $J = 8.3$, 7.2 Hz, H_7), 7.80 (1H, dd, $J = 8.3$, 7.2 Hz, H_6), 7.68 (1H, d, $J = 6.8$ Hz, H_3), 7,56 (1H, s, $H_{6'}$), 7.14-7.09 (2H, m, $H_{6'}$, $H_{7'}$), 3.87 (3H, s, OCH_3), 3.81 (3H, s, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 153.4$, 152.9, 152.3, 145.9, 142.6, 138.3, 133.9, 127.1, 123.5, 122.3, 120.3, 118.7, 115.1, 113.7, 109.8, 100.1, 56.4, 55.7 ppm; IR (KBr): $\bar{\nu} = 2771$ (N^+–H), 1589 (C=N) cm^{-1}; HRMS (ESI): $m/z = 308.1319$ ($[M+H]^+$).

4-(3,4,5-Trimethoxybenzylidenehydrazinyl)quinoline hydrochloride (**5i**, $C_{19}H_{20}ClN_3O_3$)
Yield: 68 %; *E*/*Z* ratio = 67:33; yellow solid; m.p.: 288–289 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 14.52$ (1H, s, NH), 12.97 (1H, s, NH), 8.83 (1H, s, $H_{3'}$), 8.89 (1H, d, $J = 8.4$ Hz, H_5), 8.67–8.65 [1.6H, d, $J = 6.8$ Hz, H_2, $H_{3'}$ (*Z*)], 8.05 (1H, d, $J = 8.4$ Hz, H_8), 8.03 (1H, t, $J = 8.4$ Hz, H_7), 7.80 (1H, t, $J = 8.4$ Hz, H_6), 7.69 (1H, d, $J = 6.8$ Hz, H_3), 7.22 [1H, s, $H_{5'}$, $H_{6'}$(*Z*)], 7.19 (2H, s, $H_{5'}$, $H_{6'}$), 3.90 (6H, s, OCH_3), 3.84 (6H, s, OCH_3), 3.75–3.73 [4.7H, m, OCH_3

(*Z*)] ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 161.1, 153.3, 153.1, 152.4, 150.4, 142.4, 140.2, 140.1, 138.1, 133.7, 129.2, 128.9, 126.8, 123.9, 120.1, 115.1, 105.6, 105.0, 99.9, 60.2, 60.1, 56.1, 55.9 ppm; IR (KBr): $\bar{\nu}$ = 2770 (N^+–H), 1589 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 338.1422 ($[M + H]^+$).

Synthesis of quinoline derivatives of series ***C*** *(**6a–6i**)*

To a solution of 4-chloro-4-quinolinylhydrazone derivatives [8, 9] (1 eq., 0.20 g) in 10 cm^3 acetone was added potassium carbonate (4.0 eq.). The reaction mixture was stirred at room temperature for 30 min, methyl iodide (4.0 eq.) was added, and the reaction mixture was heated to 40 °C for 3–36 h. The mixture was evaporated and 20 cm^3 water was added to the residue. The mixture was extracted with ethyl acetate (3 × 10.0 cm^3), the combined organic phases were dried over anhydrous $MgSO_4$, and rotary evaporated to yield impure solids. These were purified by column chromatography using a mixture of hexane and ethyl acetate (50 %) as eluent to leave the desired products **6a–6i** as yellow solids in 61–90 % yield.

Benzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6a**, $C_{17}H_{14}ClN_3$)

Yield: 65 %; yellow solid; m.p.: 156–157 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.48 (1H, s, $H_{3'}$), 8.41 (1H, d, *J* = 8.7 Hz, H_5), 7.82 (2H, d, *J* = 7.0 Hz, $H_{5'}$, $H_{6'}$), 7.51 (1H, d, *J* = 1.6 Hz, H_8), 7.45–7.39 (4H, m, H_2, H_6 $H_{7'}$, $H_{8'}$), 7.33 (dd, *J* = 8.7, 1.6 Hz, 1H, H_6), 6.87 (1H, d, *J* = 7.8 Hz, H_3), 3.64 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 155.8, 152.9, 140.4, 140.3, 135.8, 135.7, 129.5, 128.6, 127.3, 126.0, 123.2, 120.7, 115.3, 99.6, 39.5 ppm; IR (KBr): $\bar{\nu}$ = 2855 (N–CH_3), 1635 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 296.0872 ($[M+H]^+$).

3-Fluorobenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6b**, $C_{17}H_{13}ClFN_3$)

Yield: 58 %; yellow solid; m.p.: 173–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 (1H, d, *J* = 8.6 Hz, H_5), 8.49 (1H, s, $H_{3'}$), 7.67–7.63 (2H, m, $H_{5'}$, $H_{6'}$), 7.49–7.45 (2H, m, H_8, $H_{8'}$), 7.35 (1H, d, *J* = 7.8 Hz, H_2), 7.29 (dd, *J* = 8.6, 1.6 Hz, 1H, H_6), 7.15 (1H, dd, *J* = 8.4, 1.6 Hz, $H_{7'}$), 7.02 (1H, d, *J* = 7.8 Hz, H_3), 3.75 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 173.5, 167.5, 162.4, 151.4, 150.5, 149.7, 146.9, 141.0, 137.1, 134.4, 133.7, 131.9, 126.3, 125.3, 123.3, 110.7, 48.9 ppm; IR (KBr): $\bar{\nu}$ = 2835 (N–CH_3), 1626 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 314.0783 ($[M+H]^+$).

3-Chlorobenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6c**, $C_{17}H_{13}Cl_2N_3$)

Yield: 61 %; yellow solid; m.p.: 185–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.46 (1H, s, $H_{3'}$), 8.42 (1H, d, *J* = 8.7 Hz, H_5), 7.87 (1H, s, $H_{5'}$), 7.78–7.77 (1H, m, $H_{7'}$), 7.55 (1H, d, *J* = 1.6 Hz, H_8), 7.48-7.44 (3H, m, H_2, $H_{8'}$, $H_{6'}$), 7.34 (dd, *J* = 8.7, 1.6 Hz, 1H, H_6), 6.91 (1H, d, *J* = 7.8 Hz, H_3), 3.66 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 156.6, 151.4, 140.7, 140.5, 138.2, 135.9, 133.5, 130.5, 128.9, 126.4, 126.2, 125.9, 123.4, 120.7, 115.3, 99.7 ppm; IR (KBr): $\bar{\nu}$ = 2864 (N–CH_3), 1631 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 329.0491 ($[M+H]^+$).

3-Bromobenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6d**, $C_{17}H_{13}BrClN_3$)

Yield: 62 %; yellow solid; m.p.: 189–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (1H, s, $H_{3'}$), 8.42 (1H, d, *J* = 8.7 Hz, H_5), 8.01 (1H, s, $H_{5'}$), 7.81 (1H, d, *J* = 7.8 Hz, $H_{7'}$), 7.58–7.55 (2H, m, $H_{6'}$, H_8), 7.47 (1H, d, *J* = 7.9 Hz, H_2), 7.37 (1H, t, *J* = 7.8 Hz, $H_{8'}$), 7.35 (1H, dd, *J* = 8.7, 1.8 Hz, H_6), 6.91 (1H, d, *J* = 7.9 Hz, H_3), 3.67 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 156.5, 151.3, 140.7, 140.4, 138.9, 135.9, 130.8, 128.6, 129.3, 126.3, 126.2, 123.4, 122.1, 120.7, 115.2, 99.7 ppm; IR (KBr): $\bar{\nu}$ = 2918 (N–CH_3), 1629 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 373.9989 ($[M+H]^+$).

3-Methoxybenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6e**, $C_{18}H_{16}ClN_3O$)

Yield: 68 %; yellow solid; m.p.: 144–145 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (1H, s, $H_{3'}$), 8.41 (1H, d, *J* = 8.7 Hz, H_5), 7.51 (1H, d, *J* = 1.8 Hz, H_8), 7.43–7.31 (5H, m, H_2, H_6, $H_{5'}$, $H_{7'}$, $H_{8'}$), 6.96 (1H, d, *J* = 8.0 Hz, $H_{6'}$), 6.87 (1H, d, *J* = 7.9 Hz, H_3), 3.82 (3H, s, OCH_3), 3.64 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 159.4, 155.9, 152.9, 140.4, 137.3, 135.7, 129.7, 126.1, 123.2, 120.7, 120.1, 115.2, 115.1, 111.8, 99.6, 55.1 ppm; IR (KBr): $\bar{\nu}$ = 2924 (N–CH_3), 1630 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 326.0980 ($[M+H]^+$).

2,6-Dichlorobenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6f**, $C_{17}H_{12}Cl_3N_3$)

Yield: 62 %; yellow solid; m.p.: 142–143 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.66 (1H, s, $H_{3'}$), 8.47 (1H, d, *J* = 8.7 Hz, H_5), 8.02 (1H, d, *J* = 1.7 Hz, H_8), 7.56 (2H, d, *J* = 8.0 Hz, $H_{6'}$, $H_{8'}$), 7.51 (1H, d, *J* = 7.9 Hz, H_3), 7.39–7.35 (2H, m, H_6, $H_{7'}$), 6.81 (1H, d, *J* = 7.9 Hz, H_3), 3.67 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 151.7, 150.0, 147.5, 140.4, 133.6, 132.7, 129.3, 127.8, 125.8, 123.7, 120.2, 115.4, 110.9, 99.9 ppm; IR (KBr): $\bar{\nu}$ = 2922 (N–CH_3), 1627 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 364.0093 ($[M+H]^+$).

3,4-Dimethoxybenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6g**, $C_{19}H_{18}ClN_3O_2$)

Yield: 82 %; yellow solid; m.p.: 190–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.40–8.37 (2H, m, $H_{3'}$, H_5), 7.48 (2H, m, H_8, $H_{5'}$), 7.39 (1H, d, *J* = 7.9 Hz, H_2), 7.32–7.27 (2H, m, H_6, $H_{8'}$), 7.01 (1H, d, *J* = 8.3 Hz, $H_{7'}$), 6.86 (1H, d, *J* = 7.9 Hz, H_3), 3.84 (3H, s, OCH_3), 3.81 (3H, s,

OCH_3), 3.62 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 155.0, 153.2, 150.4, 148.9, 140.5, 140.2, 135.6, 128.6, 126.0, 123.2, 121.8, 120.8, 115.1, 111.5, 109.0, 99.7, 55.6, 55.4 ppm; IR (KBr): $\bar{\nu}$ = 2929 (N–CH_3), 1630 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 356.1090 ($[M+H]^+$).

2,5-Dimethoxybenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6h**, $C_{19}H_{18}ClN_3O_2$)
Yield: 90 %; yellow solid; m.p.: 151–152 °C; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.70 (1H, s, $H_{\acute{3}}$), 8.41 (1H, d, *J* = 8.7 Hz, H_5), 7.54 (1H, d, *J* = 2.9 Hz, $H_{\acute{6}}$), 7.50 (1H, d, *J* = 1.6 Hz, H_8), 7.41 (1H, d, *J* = 7.9 Hz, H_2), 7.31 (1H, dd, *J* = 8.7, 1.6 Hz, H_6), 7.03 (1H, d, *J* = 9.0 Hz, $H_{\acute{6}}$), 6.97 (1H, dd, *J* = 9.0, 2.9 Hz, H_6), 6.87 (1H, d, *J* = 7.9 Hz, H_3), 3.81 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.64 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 155.7, 153.2, 152.3, 148.2, 140.5, 140.3, 135.7, 126.1, 124.5, 123.2, 120.8, 116.6, 115.1, 113.2, 110.0, 99.7, 56.2, 55.4 ppm; IR (KBr): $\bar{\nu}$ = 2930 (N–CH_3), 1632 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 356.1089 ($[M+H]^+$).

3,4,5-Trimethoxybenzaldehyde (E)-(7-chloro-1-methyl-4(1H)-quinolinylidene)hydrazone (**6i**, $C_{20}H_{20}ClN_3O_3$)
Yield: 77 %; yellow solid; m.p.: 214–215 °C; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.41 (2H, m, H_5, $H_{\acute{3}}$), 7.50 (1H, d, *J* = 1.6 Hz, H_8), 7.41 (1H, d, *J* = 7.9 Hz, H_2), 7.31 (1H, dd, *J* = 8.7, 1.6 Hz, H_6), 7.15 (2H, s, $H_{\acute{5}}$, $H_{\acute{6}}$), 6.87 (1H, d, *J* = 7.9 Hz, H_3), 3.85 (6H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.64 (3H, s, NCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 156.6, 153.2, 148.2, 140.2, 136.3, 125.1, 123.6, 121.6, 115.1, 113.3, 110.2, 99.9, 56.5, 55.6 ppm; IR (KBr): $\bar{\nu}$ = 2938 (N–CH_3), 1630 (C=N) cm^{-1}; HRMS (ESI): *m/z* = 385.1197 ($[M+H]^+$).

X-ray crystallographic analysis

Data were collected at 120(2) K with Mo–Ka radiation using a Rigaku Saturn724 + (2 × 2 bin mode) instrument of the UK EPSRC crystallographic service, based at the University of Southampton. Data collection, data reduction, and unit cell refinement were carried out under the control of the program CrystalClear-SM Expert 2.0 r7 [24]. Correction for absorption was achieved in all cases by a semi-empirical method based upon the variation of equivalent reflections with the program SADABS [25]. The programs ORTEP-3 for Windows [26] and MERCURY [27] were used in the preparation of the figures. SHELXL97 [28] and PLATON [29] were used in the calculation of molecular geometry. The structure was solved by direct methods using SHELXS-97 [28] and fully refined by means of the program SHELXL-97 [28]. Difference map peaks provided positions for the hydrogen atom attached to methine C9. The coordinates, along with isotropic displacement parameters, were fully refined. All other hydrogen atoms were placed in calculated positions. Crystal data and structure refinement details are listed in Table 1. Crystal data of **6e** (colorless crystal) were collected at 120(2) K: 0.40 × 0.34 × 0.16 mm. Formula: $C_{18}H_{16}ClN_3O$, *M* = 325.79; triclinic, *P*-1; *a* = 8.8352(3) Å, *b* = 8.9684(5) Å, *c* = 10.3083(7) Å, α = 83.525(6)°, β = 76.717(5)°, γ = 79.485(6)°, *Z* = 2, *V* = 779.46(7) $Å^3$, independent reflections 3545 [*R*(int) = 0.0188], 2636 observed reflections [$I > 2\sigma(I)$]: parameters refined 213; number of restraints 0; *R*(F) 0.037 (obs data), largest diff peak 0.366 $eÅ^{-3}$. Atomic coordinates, bond lengths, angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, deposition number 1028262.

Cytotoxicity assays

Cytotoxicity against cancer cell lines
Compounds **5a–5i** and **6a–6i** (0.009–5 $\mu g/cm^3$) were tested for cytotoxic activity against three four cancer cell lines: SF-295 (glioblastoma), HCT-116 (colon), OVCAR-8 (ovarium), HL-60 (leukemia) (National Cancer Institute, Bethesda, MD). All cell lines were maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM glutamine, 100 U/cm^3 penicillin, 100 $\mu g/cm^3$ streptomycin at 37 °C with 5 % CO_2. Each compound was dissolved with DMSO to obtain a concentration of 1 mg/cm^3. The final concentration of DMSO in the culture medium was kept constant, below 0.1 % (v/v). Compounds **5a–5i** and **6a–6i** were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.001 % in the highest concentration). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann [11].

Cell membrane disruption

The test was performed in 96-well plates using a 2 % mouse erythrocyte suspension in 0.85 % NaCl containing 10 mM $CaCl_2$ [12]. The compounds **3a–3u** were diluted as mentioned above and were tested at 250 $\mu g/cm^3$. After incubation at room temperature for 30 min and centrifugation, the supernatant was removed and the liberated hemoglobin was measured spectrophotometrically at 540 nm. DMSO was used as a negative control and Triton X-100 (1 %) was used as positive control. After incubation at room temperature for 1 h and centrifugation, the supernatant was removed and the liberated hemoglobin was measured spectrophotometrically at 540 nm. EC_{50} is

the calculated effective concentration that induced lysis on 50 % that of the Triton X-100.

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