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Original article

Synthesis and cytotoxic activity of substituted 2-phenyl-3-hydroxy-4(1*H*)-quinolinones-7-carboxylic acids and their phenacyl esters

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Abstract

The preparation of 3-hydroxy-2-phenyl-4(1*H*)-quinolinones substituted in position 7 with a carboxyl group is described. The synthesis is based on the reaction of 2-aminoterephthalic acid with substituted α-bromoacetophenones and subsequent cyclization of the resulting bisphenacylesters in polyphosphoric acid. The reaction affords a mixture of substituted 3-hydroxy-2-phenyl-4(1*H*)-quinolinones 7-carboxylic acids as well as their phenacylesters. All quinolinones prepared (acids and phenacylesters) were tested for cytotoxic activity in vitro against five cancer cell lines and the results and a tentative structure–activity relationship are reported.

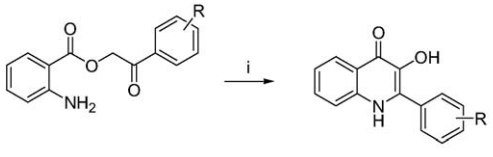
Keywords: Quinolinone; Cytotoxic activity; X-ray structure

1. Introduction

A large number of 4-quinolinone derivatives are known for their biological activity especially in connection with the inhibition of eucaryotic topoisomerase [1–3]. However, the derivatives of 3-hydroxy-4(1*H*)-quinolinone have not been so intensively investigated. Only few papers describing the biological activity of these compounds have been published in spite of the fact that they can be considered as azaanalogues of flavones. In one report, the activity of 3-hydroxy-4(1*H*)-quinolinone as an inhibitor of inosine monophosphate dehydrogenase (IMPDH) is described in [4]. Other studies have focused on the role of these compounds as topoisomerase inhibitors [5,6] and on describing the cytotoxic activity of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone substituted with chlorine atoms in various positions [7].

The synthesis of 3-hydroxy-2-phenyl-4(1*H*)-quinolinone derivatives via thermal cyclization of phenacylesters of anthranilic acid was first described in 1995 by one of us [8] (Scheme 1).

This cyclization is very convenient method affording derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinones (azaanalogues of flavones) as compounds with potential biological acti-



R = halogens, NO_2, NH_2, NH-alkyl

[a] Reagents: i) polyphosphoric acid; 100 °C or heating without solvent at 230 °C.

Scheme 1. General reaction leading to 2-phenyl-3-hydroxy-4(1H)-quinolinones.

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vity. The structure of resulting products was unambiguously proved in previous work by X-ray analyses [7] and NMR experiments [8].

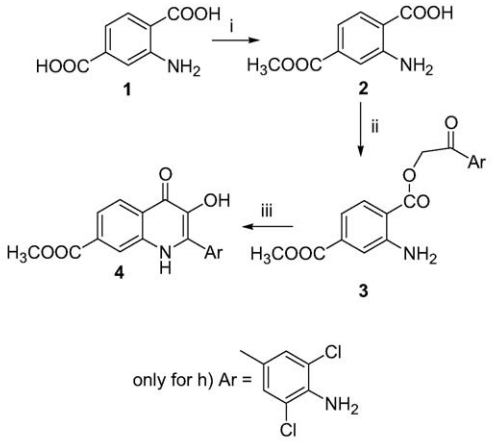
In this communication we have focused on studying the reactivity of 2-aminoterephtalic acid as a model compound able to incorporate the carboxylic group into the quinolinone structure. The carboxyl group presents a number of possibilities to modify the basic skeleton via amides, esters, salts formation, etc., which could cause these compounds to become biologically active or increase their biological activity and/or availability.

2. Chemistry

2-Aminoterephthalic acid **1** was selected as a suitable carboxy-bearing starting material. Its partial esterification to 2-aminoterephtalic acid 4-methylester **2**, already described in Ref. [9], seemed to be the most convenient way to prepare desired 7-carboxyderivatives of 2-aryl-3-hydroxy-4(1*H*)-quinolinones, by cyclization of the appropriate phenacylesters **3** in *N*-methylpyrrolidone or polyphosphoric acid, by a similar method already reported in [8] (Scheme 2). This synthetic route we first tried for preparation of the 3,5-dichloro-4-aminophenyl derivative affording quinolinone **4h** (Scheme 2). This derivative could be subsequently hydrolyzed to the final carboxy derivative.

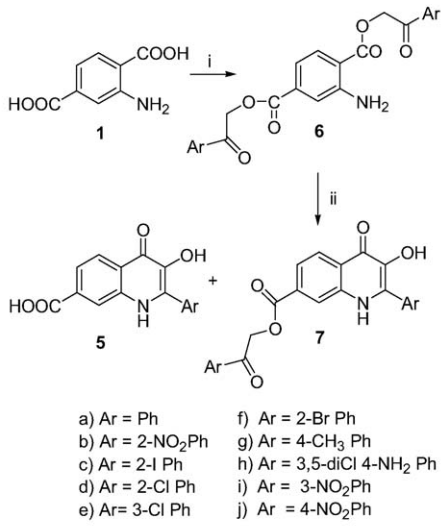
We were interested in the idea of shortening the synthetic procedure by double esterification of 2-aminoterephtalic acid **1** with bromoacetylbenzenes to afford the bis-phenacylesters **6**. We found that cyclization of the compound **6** in polyphosphoric acid was accompanied by partial hydrolysis in position 7, leading to mixtures of compounds **5** and **7**, which were readily separated via the salt formation of **5** (Scheme 3).

Derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinones are only slightly soluble in common organic solvents. The generally poor solubility is probably caused by strong intermolecular



[a] Reagents: (i) MeOH, HCl; (ii) $ArCOCH_2Br$, DMF, rt; (iii) N-methylpyrrolidone, reflux

Scheme 2. Preparation of compound 2-(4-amino-3,5-dichlorophenyl)-3-hydroxy-4(1*H*)-quinolinone **4h**.



[a] Reagents: (i) $ArCOCH_2Br$, DMF, K_2CO_3; (ii) polyphosphoric acid, 120 °C

Scheme 3. Preparation of 7-carboxyquinolinones **5** and their phenacylesters **7**.

bonds as indicated by X-ray diffraction studies of the chloroquinolinone derivatives described in a previous paper [7].

We reasoned that these hydrogen bonding networks in simple substituted 2-phenyl-3-hydroxy-4(1*H*)-quinolinones can be disrupted by compounds capable of interaction with the NH groups. This was confirmed by the X-ray structure of quinolinone **4h**, crystallized in the presence of pyridine (Figs. 1a,1b).

Compounds **5** prepared by above mentioned method contain variously substituted phenyl rings and, most importantly for our purposes, their solubility in common organic solvents is better in comparison to those lacking a carboxylic group. This property greatly facilitates their biological activity testing.

3. Biological experiments: results and discussion

2-Phenyl-3-hydroxy-4(1*H*)-quinolinones possessing a carboxylic group at position 7 (compounds **5a**–**j**, **7a**–**j**) were tested for their cytotoxic activity in vitro. Only selected carboxyquinolone derivatives were active against cell lines under in vitro conditions, suggesting a structure–activity relationship (Tables 1–4).

From the results of the in vitro cytotoxic MTT-tests of compounds **5**, it follows that their cytotoxic activity is relatively weak ($IC_{50} > 100$ μM in five cell lines) except for derivative **5h**, which includes dichloroaminophenyl moiety in position 2 ($IC_{50} = 9.83$–11.98 μM) (Table 1).

Some intriguing results concerning the relationship of solubility and biological activity were obtained in the study of derivatives **7**. When the free carboxylic group of compounds **5** was replaced by a phenacylester group (less soluble derivatives **7**), the activity increased abruptly, as exemplified by compounds **7c**, **7e**, **7f**, **7g**, **7h** which exhibited cytotoxic activity in the range of 0.68–12.03 μM (Table 2).

However, the phenacyl group alone is not responsible for the enhancement of biological activity, because intermediates

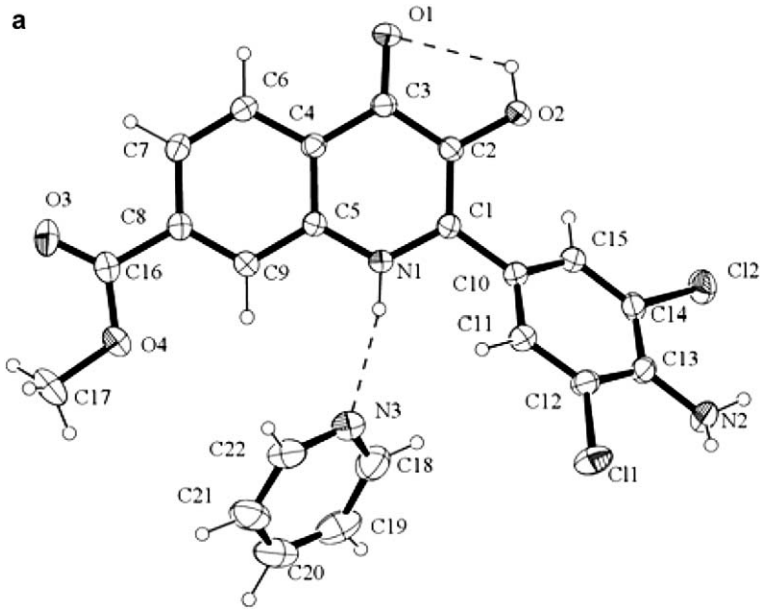


Fig. 1a. ORTEP view of the aggregate between a molecule of compound **4h** and a molecule of pyridine showing the thermal ellipsoids at 40% level of probability.

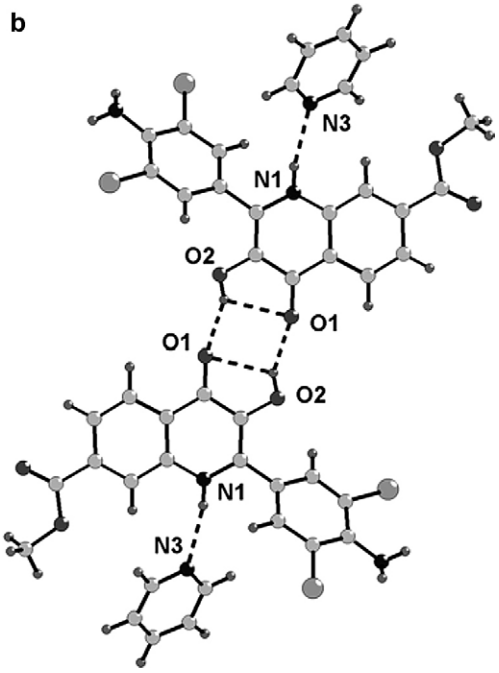


Fig. 1b. Dimer of hydrogen bonded molecules in the crystal packing of compound **4h**·pyridine.

6a–**j** did not show any significant anticancer activity in vitro (Table 3).

4. Conclusion

Synthesized 3-hydroxy-2-phenyl-4(1H)-quinolinone-7-carboxylic acids **5** showed no significant cytotoxic activity against cancer cell lines. The only exception was derivative **5h**, which possesses a dichloroaminophenyl group in position 2. The cytotoxic activity changes significantly on replacing the carboxylic group by a phenacylester group affording the derivatives **7**. The activity of these compounds increases in all substituted derivatives.

Compounds **6d**, **6e** and **7c**–**7f** were less active in daunorubicin or paclitaxel resistant leukemic cell lines CEM-DNR bulk and K562-tax, respectively (Tables 2 and 3), which were shown to overexpress the multidrug resistance proteins Pgp and/or MRP1 [10]. Nonetheless, equivalent effectiveness of compounds **5h** and **7g** was found in both drug resistant and susceptible cell lines (Tables 1 and 3). This indicates that carboxyquinolones with sufficient activity should be equally or even more effective against multidrug resistant tumors and thus they could provide significant therapeutic benefit to chemotherapy-resistant cancer patients.

5. Experimental part

5.1. Chemical synthesis

Melting points were determined on a Boetius stage and are not corrected. ^{1}H NMR spectra were measured in DMSO-d_6 at 300 K on a Bruker Avance 300 spectrometer (300 MHz) with TMS as an internal standard; chemical shifts are reported in ppm, and coupling constants in Hz. Mass spectra were recorded using an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). Elemental analyses were performed using an EA 1108 Elemental Analyzer (Fison Instruments).

*5.1.1. 1-[2-(4-Amino-3,5-dichlorophenyl)-2-oxoethyl]-4-methyl 2-aminoterephthalate (**3h**)*

2-Aminoterephthalic acid 4-methylester **2** [9] (0.47 g; 2.4 mmol) was dissolved in *N*,*N*-dimethylformamide (5 ml) and sodium bicarbonate (0.13 g; 1.2 mmol) was added. The mixture was heated at 90 °C until dissolution was complete. After cooling to room temperature, α-bromo-3,5-dichloro-4-aminoacetophenone (0.67 g; 2.4 mmol) was added. The mix-

Table 1
Cytotoxic activity of 3-hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylic acids **5** on human malignant cell lines of different tissue origin and drug resistance profile

		IC_{50} (μM)[a]				
Compounds	R	A 549	CEM	CEM-DNR bulk	K 562	K 562-tax
5a	R = H	236.2	223.4	171.2	198.9	196.3
5b	R = 2-NO_2	226.1	100.8	153.5	130.0	183.0
5c	R = 2-I	191.8	124.4	134.1	129.0	174.9
5d	R = 2-Cl	201.9	156.1	149.7	161.1	167.5
5e	R = 3-Cl	239.1	224.9	163.4	190.0	197.5
5f	R = 2-Br	192.4	117.0	145.5	146.3	160.6
5g	R = 4-CH_3	190.7	195.2	158.6	172.0	172.0
5h	R = 3,5-diCl; 4-NH_2	9.83	12.3	12.0	11.0	11.7
5i	R = 3-NO_2	250	227.2	166.1	200.1	197.1
5j	R = 4-NO_2	250	235.6	167.2	208.8	192.9

[a] Average values of IC_{50} from three to four independent experiments with S.D. ranging from 10% to 25% of the average values.

Table 2
Cytotoxic activity of 2-oxo-2-phenylethyl 3-hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylates **7** on human malignant cell lines of different tissue origin and drug resistance profile

		IC_{50} (μM)[a]				
Compounds	R	A 549	CEM	CEM-DNR bulk	K 562	K 562-tax
7a	R = H	120.2	147.0	128.0	132.4	141.8
7b	R = 2-NO_2	46.6	25.6	105.2	28.9	40.3
7c	R = 2-I	11.3	5.5	12.0	5.00	8.5
7d	R = 2-Cl	38.2	17.4	36.3	15.7	79.3
7e	R = 3-Cl	4.5	8.0	11.5	5.6	10.7
7f	R = 2-Br	10.4	4.9	12.3	5.2	8.5
7g	R = 4-CH_3	2.8	3.8	4.9	2.9	2.8
7h	R = 3,5-Cl-4-NH_2	0.68	0.76	3.6	1.1	1.2
7i	R = 3-NO2	162.5	102.0	189.8	26.1	43.2
7j	R = 4-NO2	151.7	182.6	174.6	48.6	96.5

[a] Average values of IC_{50} from three to four independent experiments with S.D. ranging from 10% to 25% of the average values.

Table 3
Cytotoxic activity of bis(2-oxo-2-phenylethyl)-2-aminoterephthalates **6** on human malignant cell lines of different tissue origin and drug resistance profile

		IC_{50} (μM) [a]				
Compounds	R	A 549	CEM	CEM-DNR bulk	K 562	K 562-tax
6a	R = H	250	215.2	105.2	206.2	195.4
6b	R = 2-NO2	54.8	224.9	52.9	72.9	110.7
6c	R = 2-I	189.9	159.5	163.4	120.0	184.3
6d	R = 2-Cl	30.6	52.3	82.3	71.8	29.8
6e	R = 3-Cl	18.2	8.9	250	84.8	185.3
6f	R = 2-Br	31.6	66.4	45.4	44.0	81.4
6g	R = 4-CH_3	108.2	68.1	97.0	92.3	198.7
6h	R = 3,5-diCl-4-NH_2	149.7	186.8	55.7	94.1	182.8
6i	R = 3-NO_2	40.3	155.4	95.0	183.2	219.3
6j	R = 4-NO_2	48.9	28.1	96.1	146.2	205.2

[a] Average values of IC_{50} from three to four independent experiments with S.D. ranging from 10% to 25% of the average values.

ture was stirred overnight and then poured into water (100 ml). The resulting solid was filtered, washed with water and dried in air. Yield 0.78 g (82%), m.p. 187–192 °C (ethanol); ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 3H, CH_3); 5.59 (s, 2H, CH_2); 6.59 (s, 2H, NH_2); 6.86 (s, 2H, NH_2); 7.09 (dd, 1H, $J_1 = 6$ Hz, $J_2 = 2$ Hz, H5); 7.47 (d, 1H, $J = 2$ Hz, H3); 7.88–7.91 (m, 3H, H2′, H6). Elemental analyses are summarized in Table 5.

*5.1.2. 2-(4-Amino-3,5-dichlorophenyl)-3-hydroxy-4-oxo-1,4-dihydroquinoline-7-carboxylic acid methyl ester (**4h**)*

Derivative **3h** (0.5 g; 1.2 mmol) was refluxed in *N*-methylpyrrolidone (1 ml) for 45 min. After cooling ethyl acetate (1 ml) was added. The resulting solid was filtered, washed with water and dried. Yield 0.32 g (70%). Elemental analyses are summarized in Table 5.

Table 4
Yield, melting points and molecular ions from mass spectra of prepared compounds

Compounds	Yield (%)	M.p. (°C)	M.s. (M + 1)	Compounds	Yield (%)	M.p. (°C)	Ms (M + 1)
5a	52	> 360	282.2	**6f**	84	133–135	576.3
5b	62	340–343	327.2	**6g**	80	215–218	446.4
5c	73	> 360	408.2	**6h**	90	253–255	584.2
5d	75	> 360	316.2	**6i**	79	190–192	508.2
5e	50	350–355	316.3	**6j**	82	249–252	508.1
5f	80	336–340	361.1	**7a**	25	294–297	400.2
5g	23	> 360	296.3	**7b**	10	248–251	490.3
5h	55	> 360	366.4	**7c**	35	240–243	652.2
5i	62	> 360	327.2	**7d**	20	268–271	469.2
5j	51	355–360	327.2	**7e**	35	258–262	469.2
6a	90	204–205	418.2	**7f**	23	249–252	558.3
6b	86	178–179	508.1	**7g**	63	256–260	428.4
6c	84	177–179	670.3	**7h**	97	223–226	568.2
6d	80	122–124	486.2	**7i**	21	205–208	490.3
6e	82	184–187	486.2	**7j**	19	296–300	490.3

Table 5
Elemental analyses of target compounds

Compounds	Elemental analyses calcd. (%)/found			Compounds	Elemental analyses calcd. (%)/found		
	C	H	N		C	H	N
3h	51.39/51.63	3.55/3.38	7.05/7.22	**6e**	59.28/58.98	3.52/3.22	2.88/2.64
4h	53.85/53.55	3.19/2.89	7.39/7.46	**6f**	50.11/49.85	2.98/3.11	2.44/2.45
5a	68.33/68.63	4.98/4.97	4.98/4.58	**6g**	70.10/69.90	5.20/5.48	3.14/3.29
5b	58.90/58.55	3.09/2.99	8.59/8.24	**6h**	49.26/48.95	3.52/3.76	2.88/2.97
5c	47.20/46.81	2.48/2.17	3.44/3.52	**6i**	56.81/56.67	3.38/3.32	8.28/8.20
5d	60.87/60.54	3.19/2.89	4.44/4.70	**6j**	56.81/56.69	3.38/3.35	8.28/8.09
5e	60.87/60.49	3.19/2.75	4.44/4.52	**7a**	72.17/71.94	4.29/4.47	3.51/3.68
5f	53.36/53.05	2.80/2.58	3.89/3.53	**7b**	58.90/58.71	3.09/2.72	8.59/8.33
5g	69.15/68.89	4.44/4.04	4.74/4.99	**7c**	44.27/44.14	2.32/2.06	2.15/1.97
5h	52.61/52.43	2.76/2.66	7.67/7.43	**7d**	61.56/61.90	3.23/2.92	2.99/2.69
5i	58.90/58.52	3.09/3.00	8.59/8.39	**7e**	61.56/61.41	3.23/2.95	2.99/2.67
5j	58.90/58.63	3.09/3.27	8.59/8.21	**7f**	51.74/51.54	2.71/2.49	2.51/2.38
6a	69.06/68.74	4.59/4.22	3.36/3.07	**7g**	73.06/72.80	4.95/4.64	3.28/3.09
6b	56.81/56.50	3.38/2.99	8.28/7.93	**7h**	50.82/50.52	2.67/2.35	7.41/7.29
6c	43.08/42.98	2.56/2.49	2.09/1.96	**7i**	58.90/58.57	3.09/3.00	8.59/8.09
6d	59.28/58.99	3.52/3.67	2.88/2.92	**7j**	58.90/58.79	3.09/2.97	8.59/8.33

^{1}H NMR (DMSO-d_6): δ 3.92 (s, 3H, CH_3); 6.05 (s, 2H, NH_2), 7.72 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz, H6), 7.84 (s, 2H, H2′, H6′), 8.22 (d, 1H, J = 8.5 Hz, H5), 8.45 (d, 1H, J = 1.5 Hz, H8).

5.2. General procedure for preparation of 2-aminoterephthalic acid bis-[2-phenyl-2-oxo-ethyl] esters (6)

2-Aminoterephthalic acid **1** (0.362 g, 2.9 mmol) was dissolved in dimethylformamide (10 ml) and potassium carbonate (0.4 g, 2.9 mmol) was added. The reaction mixture was heated to 90 °C and stirred for 1 hour. Then the solution was cooled to 20 °C and phenacyl bromide (3.5 mmol) was added. After stirring for 60 min the solution was poured into an aqueous solution of sodium bicarbonate (10%; 50 ml) mixed with ice. The precipitated solid was collected by filtration, washed with water and dried. The sample used for analysis was crystallized from acetone.

Yields, melting points and mass spectra data are presented in Table 4. Elemental analyses are summarized in Table 5.

^{1}H NMR (DMSO-d_6) of bis-(2-oxo-2-phenylethyl) 2-aminoterephthalates (**6**).

5.2.1. 2-Aminoterephthalic acid bis-[2-phenyl-2-oxo-ethyl] ester (6a)

δ 5.75 (s, 2H, CH_2); 5.80 (s, 2H, CH_2); 6.92 (s, 2H, NH_2); 7.18 (d, 1H, J_1 = 9 Hz, H5); 7.57 (s, 1H, H3); 7.59 (t, 4H J = 4 Hz, 4H, H_{phenyl}); 7.72 (t, 2H J = 8 Hz, H_{phenyl}); 7.97 (d, 1H J = 8 Hz, H6); 8.03 (d, 4H J = 8 Hz, H_{phenyl}).

5.2.2. 2-Aminoterephthalic acid bis-[2-(2-nitrophenyl)-2-oxo-ethyl] ester (6b)

δ 5.43 (s, 2H, CH_2); 5.47 (s, 2H, CH_2); 6.91 (s, 2H, NH_2); 6.98 (dd, 1H, J_1 = 8 Hz, H_{phenyl}; 7.46 (d, 1H, J = 2 Hz, H3); 7.73 (d, 1H, J = 8 Hz, H_{phenyl}); 7.81–7.86 (m, 3H, H_{phenyl}); 7.92–7.97 (m, 2H, H6 and H_{phenyl}); 8.18–8.22 (m, 3H, H_{phenyl}).

*5.2.3. 2-Aminoterephthalic acid bis-[2-(2-iodophenyl)-2-oxo-ethyl] ester (**6c**)*

δ 5.51 (s, 2H, CH_2); 5.53 (s, 2H, CH_2); 6.92 (s, 2H, NH_2); 7.14 (d, 1H, $J_1 = 9$ Hz, H5); 7.34 (t, 2H, $J = 7$ Hz, H_{phenyl}); 7.53 (s, 1H, H3); 7.55–7.60 (m, 2H, H_{phenyl}); 7.81 (d, 2H, $J = 8$ Hz, H_{phenyl}); 7.92 (m, $J = 9$ Hz, 1H, H6); 8.05 (d, 2H, $J = 8$ Hz, H_{phenyl}).

*5.2.4. 2-Aminoterephthalic acid bis-[2-(2-chlorophenyl)-2-oxo-ethyl] ester (**6d**)*

δ 5.50 (s, 2H, CH_2); 5.55 (s, 2H, CH_2); 6.92 (s, 2H, NH_2); 7.12 (d, 1H, $J_1 = 9$ Hz, $J_2 = 1$ Hz, H5); 7.49–7.54 (m, 2H, H_{phenyl}); 7.53 (s, 1H, H3); 7.61 (d, 4H, $J = 6$ Hz, H_{phenyl}); 7.84 (d, $J = 8$ Hz, 2H, H_{phenyl}); 7.90 (d, 1H, $J = 9$ Hz, H6).

*5.2.5. 2-Aminoterephthalic acid bis-[2-(3-chlorophenyl)-2-oxo-ethyl] ester (**6e**)*

δ 5.74 (s, 2H, CH_2); 5.77 (s, 2H, CH_2); 7.17 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 2$ Hz, H5); 7.56 (d, 1H, $J = 2$ Hz, H3); 7.60 (t, 2H, $J = 7$ Hz, H_{phenyl}); 7.77–7.81 (m, 2H, H_{phenyl}); 7.95–8.00 (m, 3H, H6 and H_{phenyl}); 8.05 (s, 2H, H_{phenyl}).

*5.2.6. 2-Aminoterephthalic acid bis-[2-(2-bromophenyl)-2-oxo-ethyl] ester (**6f**)*

δ 5.50 (s, 2H, CH_2); 5.53 (s, 2H, CH_2); 6.92 (s, 2H, NH_2); 7.12 (d, 1H, $J_1 = 10$ Hz, H5); 7.54 (s, 1H, H3); 7.48–7.58 (m, 4H, H_{phenyl}); 7.76–7.82 (m, 4H, H_{phenyl}); 7.90 (d, 1H, $J = 10$ Hz, H6).

*5.2.7. 2-Aminoterephthalic acid bis-[2-(4-methylphenyl)-2-oxo-ethyl] ester (**6g**)*

δ 2.41 (s, 3H, CH_3); 5.70 (s, 2H, CH_2); 5.72 (s, 2H, CH_2); 6.91 (s, 2H, NH_2); 7.18 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 2$ Hz, H5); 7.40 (d, 4H, $J = 8$ Hz, H_{phenyl}); 7.57 (d, 1H, $J = 2$ Hz, H3); 7.92 (d, 4H, $J = 8$ Hz, H_{phenyl}); 7.97 (d, 1H, $J = 9$ Hz, H6).

*5.2.8. 2-Aminoterephthalic acid bis-[2-(4-amino-3,5-dichlorophenyl)-2-oxo-ethyl] ester (**6h**)*

δ 5.61 (s, 2H, CH_2); 5.64 (s, 2H, CH_2); 6.56 (s, 4H, NH_2); 6.87 (s, 2H, NH_2); 7.53 (d, 1H, $J = 2$ Hz, H3); 7.14 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 2$ Hz, H5); 7.93 (d, 1H, $J = 10$ Hz, H6); 7.89 (s, 4H, H_{phenyl}).

*5.2.9. 2-Aminoterephthalic acid bis-[2-(3-nitrophenyl)-2-oxo-ethyl] ester (**6i**)*

δ 5.83 (s, 2H, CH_2); 5.87 (s, 2H, CH_2); 6.93 (s, 2H, NH_2); 7.18 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 2$ Hz, H5); 7.58 (d, 1H, $J = 2$ Hz, H3); 7.91 (t, 2H, H_{phenyl}); 7.98 (d, 1H, $J = 10$ Hz, H6); 8.45 (d, 2H, $J = 8$ Hz, H_{phenyl}); 8.54 (d, 2H, $J = 8$ Hz, H_{phenyl}); 8.72 (s, 2H, H_{phenyl}).

*5.2.10. 2-Aminoterephthalic acid bis-[2-(4-nitrophenyl)-2-oxo-ethyl] ester (**6j**)*

δ 5.80 (s, 2H, CH_2); 5.83 (s, 2H, CH_2); 6.93 (s, 2H, NH_2); 7.18 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 1$ Hz, H5); 7.57 (d, 1H, $J = 1$ Hz, H3); 7.98 (d, 1H, $J = 9$ Hz, H6); 8.25 (d, 4H, $J = 8$ Hz, H_{phenyl}); 8.40 (d, 4H, $J = 8$ Hz, H_{phenyl}).

*5.3. General procedures for preparation of 3-hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylic acids (**5**) and 2-oxo-2-phenylethyl 3-hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylates (**7**)*

Anthranilate **6** (0.5 mmol) was added to polyphosphoric acid (5 g) at 120 °C and the reaction mixture was stirred for 4 hours at this temperature. Then the reaction mixture was diluted with cold water (50 ml) and cooled to room temperature. The pH of the solution was adjusted to between 9 and 10 by the addition of aqueous 10% sodium hydroxide solution and the precipitated solid was collected by filtration, washed with 50 ml of water, dried and recrystallized from acetone. The filtrate was acidified with 10% sulfuric acid, the precipitated solid was collected, washed with water and dried.

Yields, melting points and mass spectral data are presented in Table 4. Elemental analyses are summarized in Table 5.

^{1}H NMR (DMSO-d_6) of 3-hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylic acid (**5**).

*5.3.1. 3-Hydroxy-4-oxo-2-phenyl-1,4-dihydroquinoline-7-carboxylic acid (**5a**)*

δ 7.53–7.60 (m, 3H, $H_{2\text{-phenyl}}$); 7.74 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 1$ Hz , H6); 7.83 (dd, 2H, $J_1 = 10$ Hz, $J_2 = 2$ Hz, $H_{2\text{-phenyl}}$); 8.23 (d, 1H, $J = 10$ Hz, H5); 8.41 (d, 1H, $J = 1$ Hz, H8); 11.80 (s, 1H, COOH).

*5.3.2. 3-Hydroxy-4-oxo-2-(2-nitrophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5b**)*

δ 7.70 (d, 1H, $J_1 = 9$ Hz, H6); 7.79–7.84 (m, 2H, $H_{2\text{-phenyl}}$); 7.95 (t, 1H, $J = 2$ Hz, $H_{2\text{-phenyl}}$); 8.20 (d, 1H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 8.23 (d, 1H, $J = 9$ Hz, H5); 8.27 (s, 1H, H8); 12.21 (s, 1H, COOH).

*5.3.3. 3-Hydroxy-4-oxo-2-(2-iodophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5c**)*

δ 7.29 (dt, 1H, $J_1 = 10$ Hz, $J_2 = 2$ Hz , $H_{2\text{-phenyl}}$); 7.51–7.61 (m, 2H, $H_{2\text{-phenyl}}$); 7.75 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 2$ Hz, H6); 8.05 (d, 1H, $J = 10$ Hz, $H_{2\text{-phenyl}}$); 8.25 (d, 1H, $J = 1$ Hz, H8); 8.27 (d, 1H, $J = 9$ Hz, H5); 11.97 (s, 1H, COOH).

*5.3.4. 3-Hydroxy-4-oxo-2-(2-chlorophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5d**)*

δ 7.53–7.69 (m, 4H, $H_{2\text{-phenyl}}$); 7.75 (d, 1H, $J = 8$ Hz, H6); 8.25 (s, 1H, H8); 8.27 (d, 1H, $J = 8$ Hz, H5); 12.06 (s, 1H, COOH).

*5.3.5. 3-Hydroxy-4-oxo-2-(3-chlorophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5e**)*

δ 7.60–7.62 (m, 2H, $H_{2\text{-phenyl}}$); 7.75 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 1$ Hz, H6); 7.83 (t, 1H, $J = 3$ Hz, $H_{2\text{-phenyl}}$); 7.90 (s, 1H, $H_{2\text{-phenyl}}$); 8.23 (d, 1H, $J = 9$ Hz, H5); 8.40 (d, 1H, $J = 1$ Hz, H8); 11.84 (s, 1H, COOH).

*5.3.6. 3-Hydroxy-4-oxo-2-(2-bromophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5f**)*

δ 7.49–7.61 (m, 3H, $H_{2\text{-phenyl}}$); 7.75 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 1$ Hz, H6); 7.83 (d, 1H, $J = 11$ Hz, $H_{2\text{-phenyl}}$); 8.24 (d, 1H, $J = 1$ Hz, H8); 8.27 (d, 1H, $J = 10$ Hz, H5); 12.03 (s, 1H, COOH).

*5.3.7. 3-Hydroxy-4-oxo-2-(4-methylphenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5g**)*

δ 2.42 (s, 6H, CH_3); 7.38 (d, 2H, $J = 12$ Hz, $H_{2\text{-phenyl}}$); 7.73 (d, 1H, $J = 9$ Hz, H6); 7.75 (d, 2H, $J = 10$ Hz, $H_{2\text{-phenyl}}$); 8.22 (d, 1H, $J = 9$ Hz, H5); 8.41 (s, 1H, H8); 11.71 (s, 1H, COOH).

*5.3.8. 3-Hydroxy-4-oxo-2-(4-amino-3,5-dichlorophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5h**)*

δ 6.01 (s, 2H, NH_2), 7.70 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 1.5$ Hz, H6), 7.81 (s, 2H, $H_{2\text{-phenyl}}$), 8.17 (d, 1H, $J = 8.5$ Hz, H5), 8.40 (d, 1H, $J_1 = 1.5$ Hz, H8).

*5.3.9. 3-Hydroxy-4-oxo-2-(3-nitrophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5i**)*

δ 7.76 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 2$ Hz , H6); 7.88 (t, 1H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 8.25 (d, 1H, $J = 9$ Hz, H5); 8.31 (d, 1H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 8.37 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 2$ Hz, $H_{2\text{-phenyl}}$); 8.42 (d, 1H, $J = 2$ Hz, H8); 8.72 (t, 1H, $J = 1$ Hz, $H_{2\text{-phenyl}}$); 12.00 (s, 1H, COOH).

*5.3.10. 3-Hydroxy-4-oxo-2-(4-nitrophenyl)-1,4-dihydroquinoline-7-carboxylic acid (**5j**)*

δ 7.76 (d, 1H, $J = 9$ Hz, H6); 8.13 (d, 2H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 8.25 (d, 1H, $J = 9$ Hz, H5); 8.40 (s, 1H, H8); 8.42 (d, 2H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 11.93 (s, 1H, COOH).

^{1}H NMR (DMSO-d_6) of 3-hydroxy-4-oxo-2-phenyl-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-phenyl-ethyl ester (**7**).

*5.3.10.1. 3-Hydroxy-4-oxo-2-phenyl-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-phenyl-ethyl ester (**7a**)*. δ 5.84 (s, 2H, CH_2); 7.53–7.63 (m, 5H, $H_{2\text{-phenyl}}$); 7.73 (t, 1H, $J = 7$ Hz, H_{phenyl}); 7.84 (m, 3H, H5 and H_{phenyl}); 8.04 (d, 2H, $J = 7$ Hz, H_{phenyl}); 8.31 (d, 1H, $J = 9$ Hz, H6); 8.55 (s, 1H, H8).

*5.3.10.2. 3-Hydroxy-4-oxo-2-(2-nitrophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(2-nitrophenyl)-ethyl ester (**7b**)*. δ 5.53 (s, 2H, CH_2); 7.67 (d, 1H, $J = 8$ Hz, H5); 7.76–7.95 (m, 6H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 8.12–8.26 (m, 3H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 8.31 (s, 1H, H8).

*5.3.10.3. 3-Hydroxy-4-oxo-2-(2-iodophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(2-iodophenyl)-ethyl ester (**7c**)*. δ 5.61 (s, 2H, CH_2); 7.28–7.35 (m, 2H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 7.54–7.59 (m, 3H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 7.83 (m, 2H, H5 and H_{phenyl}); 8.05 (d, 2H, $J = 8$ Hz, $H_{2\text{-phenyl}}$); 8.33 (d, 1H, $J = 8$ Hz, H6); 8.37 (s, 1H, H8).

*5.3.10.4. 3-Hydroxy-4-oxo-2-(2-chlorophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(2-chlorophenyl)-ethyl ester (**7d**)*. δ 5.61 (s, 2H, CH_2); 7.51–7.69 (m, 7H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 7.79 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 1$ Hz , H_{phenyl}); 7.86 (d, 1H, $J = 8$ Hz, H5); 8.32 (d, 1H, $J = 8$ Hz, H6); 8.34 (s, 1H, H8).

*5.3.10.5. 3-Hydroxy-4-oxo-2-(3-chlorophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(3-chlorophenyl)-ethyl ester (**7e**)*. δ 5.85 (s, 2H, CH_2); 7.63 (m, 3H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 7.79–7.83 (m, 2H, H5 and $H_{2\text{-phenyl}}$); 7.92 (s, 1H, H_{phenyl}); 7.99–8.06 (m, 3H, H_{phenyl}); 8.31 (d, 1H, $J = 8$ Hz, H6); 8.53 (s, 1H, H8).

*5.3.10.6. 3-Hydroxy-4-oxo-2-(2-bromophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(2-bromophenyl)-ethyl ester (**7f**)*. δ 5.60 (s, 2H, CH_2); 7.49–7.61 (m, 5H, H_{phenyl} and $H_{2\text{-phenyl}}$); 7.77–7.86 (m, 4H, H5 and $H_{2\text{-phenyl}}$ and H_{phenyl}); 8.33 (d, 1H, $J = 10$ Hz, H6); 8.34 (s, 1H, H8).

*5.3.10.7. 3-Hydroxy-4-oxo-2-(4-methylphenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(4-methylphenyl)-ethyl ester (**7g**)*. δ 2.41 (s, 6H, CH_3); 5.79 (s, 2H, CH_2); 7.37–7.41 (m, 4H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 7.76–7.81 (m, 3H, H5 and $H_{2\text{-phenyl}}$); 7.93 (d, 2H, $J = 6$ Hz, H_{phenyl}); 8.29 (d, 1H, $J = 9$ Hz, H6); 8.55 (s, 1H, H8).

*5.3.10.8. 3-Hydroxy-4-oxo-2-(4-amino-3,5-dichlorophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(4-amino-3,5-dichlorophenyl)-ethyl ester (**7h**)*. δ 5.71 (s, 2H, CH_2); 6.02 (s, 2H, NH_2); 6.59 (s, 2H, NH_2); 7.77 (d, 2H, $J = 8$ Hz, H5); 7.88 (s, 2H, H_{phenyl}); 7.90 (s, 2H, $H_{2\text{-phenyl}}$); 8.25 (d, 1H, $J = 8$ Hz, H6); 8.52 (s, 1H, H8).

*5.3.10.9. 3-Hydroxy-4-oxo-2-(3-nitrophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(3-nitrophenyl)-ethyl ester (**7i**)*. δ 5.94 (s, 2H, CH_2); 7.79–7.96 (m, 3H, H5 and H_{phenyl}); 8.28–8.55 (m, 6H, H8, H6 and $H_{2\text{-phenyl}}$); 8.73 (s, 1H, H_{phenyl}); 8.81 (s, 1H, $H_{2\text{-phenyl}}$).

*5.3.10.10. 3-Hydroxy-4-oxo-2-(4-nitrophenyl)-1,4-dihydro-quinoline-7-carboxylic acid 2-oxo-2-(4-nitrophenyl)-ethyl ester (**7j**)*. δ 5.88 (s, 2H, CH_2); 7.75 (d, 1H, $J = 11$ Hz, H5); 8.17 (d, 1H, $J = 11$ Hz, H6); 8.24–8.42 (m, 8H, $H_{2\text{-phenyl}}$ and H_{phenyl}); 8.51 (s, 1H, H8).

5.4. Biological activity

5.4.1. Cell lines

All cells were purchased from the American Tissue Culture Collection (ATCC), unless otherwise indicated. The daunorubicin resistant subline of CEM cells (CEM-DNR bulk) and paclitaxel resistant subline K562-tax were selected in our laboratory by the cultivation of maternal cell lines in increasing concentrations of daunorubicine or paclitaxel, respectively [10]. The cells were maintained in Nunc/Corning 80 cm^2 plas-

tic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 $g \cdot l^{-1}$ glucose, 2 mM glutamine, 100 $U \cdot ml^{-1}$ penicillin, 100 μg ml^{-1} streptomycin, 10% fetal calf serum, and $NaHCO_3$).

5.4.2. Cytotoxic MTT assay [11]

Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500–30,000 cells per well based on cell growth characteristics). Cells were added by pipette (80 μl) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Fourfold dilutions, in 20-μl aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μl) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period the formazan produced was dissolved by the addition of 100 μl per well of 10% aq SDS (pH 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumor cell inhibitory concentration (IC) was calculated using the following equation: $IC = (OD_{\text{drug-exposed well}}/\text{mean } OD_{\text{control wells}}) \times 100\%$. The IC_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose–response curves.

*5.5. X-ray structure determination of **4h***

X-ray diffraction data for compound **4h** were collected on a Nonius Kappa CCD diffractometer, at room temperature ($T = 295$ K), with graphite monochromated Mo Kα radiation ($\lambda = 0.7107$ Å). The structure was solved by direct methods (SIR97) [12] and refined (SHELXL-97) [13] by full-matrix least-squares with anisotropic non-H and hydrogens isotropically.

Crystal data: $C_{17}H_{12}Cl_2N_2O_4 \cdot C_5H_5N$; triclinic, space group *P-1*, $a = 7.6468(2)$, $b = 10.7236(2)$, $c = 13.1177(3)$ Å, $\alpha = 103.961(1)$, $\beta = 94.337(1)$, $\gamma = 96.292(1)°$, $V = 1031.78(4)$ $Å^3$, $Z = 2$, $Dc = 1.475$ g cm^{-3}. Intensity data collected with $\theta \leq 30°$; 5979 independent reflections measured; 4225 reflections observed [$I > 2\sigma(I)$]. Final $R = 0.043$ (observed reflections) and $Rw = 0.125$ (all reflections). The crystal contains molecules of pyridine linked to compound **4h** by means of a N1–H…N3 hydrogen bond [N1…N3 = 2.988(2) Å]. An ORTEP [14] view of molecular aggregate **4h** pyridine is given in Fig. 1a. Furthermore, the molecules of **4h** form an intramolecular hydrogen bond O2–H…O1 [O2…O1 = 2.730(2) Å] and dimers linked by intermolecular hydrogen bonds O2–H…O1 (–*x*, 1 – *y*, –*z*) [O2…O1 = 2.718(2) Å] (Fig. 1b).

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