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Synthesis of 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline derivatives as potential anticancer agents active on multidrug resistant cell lines

Maria Dzieduszycka,[a] Maria M. Bontemps-Gracz,[a] Barbara Stefańska,[a] Sante Martelli,[b] Agnieszka Piwkowska,[a] Małgorzata Arciemiuk[a] and Edward Borowski[a,*]

[a]*Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, 80-952 Gdańsk, Poland*
[b]*Department of Chemistry, University of Camerino, 62033 Camerino, Italy*

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Abstract—Following our earlier finding that tetracyclic anthraquinone analogs with a fused pyridone ring exhibit cytotoxic activity toward multidrug resistant tumor cells, a series of new potential antitumor agents, 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline derivatives (**3**, **6**–**8**, **10**–**12**, **14**, **15**, and **18**), bearing one or two basic side chains and various substituents at the pyridone ring, have been synthesized. The compounds have been obtained from 1-amino-4-chloroanthraquinone or 1-aminoanthraquinone by cyclization with diethyl malonate and the subsequent reactions of the key intermediates **2**, **4**, and **17**. The compounds exhibited cytotoxic activity toward sensitive human leukemia cell line HL-60 and against its resistant sublines HL-60/VINC (MDR1 type) and HL-60/DX (MRP1 type).

1. Introduction

The appearance of cancer cell populations resistant to multi-based chemotherapy constitutes a major problem to achieve cures in the patients. The multidrug resistance is associated with overexpression of plasma membrane drug efflux pumps such as P-gp (MDR1) and multidrug resistance-associated protein (MRP1).[1,2] One of the strategies to avoid or decrease this effect is the design and synthesis of non-cross-resistant analogs of antitumor agents on a rational basis. The identification of the structural factors of the compounds responsible for their ability to overcome multidrug resistance is essential for this strategy.

We have earlier put forward the hypothesis that the presence of five- or six-membered heterocyclic ring(s) fused with the anthraquinone or acridine moiety is essential for the ability to overcome multidrug resistance by antitumor compounds of these groups.[3,4] The cytotoxic activity toward resistant tumor cell lines has been shown by the following tetracyclic anthracenedione and acridine agents: anthrapyrazoles[5,6] and their aza-analogs,[7] benzoperimidines,[3] anthrapyridazones,[8] anthrapyridones,[9–11] pyrimidoacridines,[12,13] pyrazoloacridines,[14,15] and pyrazolopyrimidoacridines.[14,16]

In the continuous search for optimized compounds, we have synthesized a series of new 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline derivatives (hereafter referred to as anthrapyridones) and studied their ability to overcome multidrug resistance. Our earlier studies with anthrapyridones[9] comprised rather limited number of compounds. The new derivatives presented in this paper should allow better establishment of structural requirements for the ability to overcome multidrug resistance of tumor cells by this novel group of antitumor agents. The structures of the synthesized compounds, shown in Table 1, include compounds with side chain attached to the chromophore at position 6 (**10**–**12**) and to 1-pyridone ring (**6**–**8** and **3**), the analogs of **10**–**12**, namely 2-Cl compounds (**14** and **15**), as well as 1-carboxamide compound **18**. Such selection of compounds enables us to examine the influence of the kind of functional groups attached to the pyridone ring, as well as of the structure of aminoalkylamino side arms at position 6, on the cytotoxicity of anthrapyridones and their ability to overcome multidrug resistance.

Abbreviations: MIT, mitoxantrone; DX, doxorubicin; VINC, vincristine; MDR1, P-gp dependent multidrug resistance; MRP1, multidrug resistance associated protein dependent resistance.

Keywords: Antitumor compounds; Anthracenedione analogs; Anthrapyridones; Cytotoxic activity; Multidrug resistance.

* Corresponding author. Tel.: +48 58 347 2523; fax: +48 58 347 1144; e-mail: borowski@altis.chem.pg.gda.pl

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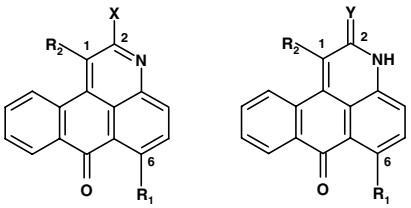
Table 1. Structures and characteristics of anthrapyridone derivatives



Compound	R_1	R_2	X	Y	Mp (°C)	Formula
3·HCl	$NH(CH_2)_2N(CH_3)_2$	$COOC_2H_5$	OH		253–254	$C_{23}H_{24}N_3O_4Cl$
6·2HCl	$NH(CH_2)_2N(CH_3)_2$	$CONH(CH_2)_2N(CH_3)_2$	OH		296	$C_{25}H_{31}N_5O_3Cl_2$
7·2HCl	$NH(CH_2)_2N(C_2H_5)_2$	$CONH(CH_2)_2N(CH_3)_2$	OH		266–268	$C_{27}H_{35}N_5O_3Cl_2$
8·2HCl	$NH(CH_2)_2N$-c-$(CH_2)_5$	$CONH(CH_2)_2N(CH_3)_2$	OH		115–117	$C_{28}H_{35}N_5O_3Cl_2$
10·HCl	$NH(CH_2)_2N(CH_3)_2$	H		O	230–232	$C_{20}H_{20}N_3O_2Cl$
11·HCl	$NH(CH_2)_2N$-c-$(CH_2)_5$	H		O	225–227	$C_{23}H_{24}N_3O_2Cl$
12·HCl	$NH(CH_2)_3N(CH_3)_2$	H		O	220–222	$C_{21}H_{22}N_3O_2Cl$
14·HCl	$NH(CH_2)_2N(CH_3)_2$	H	Cl		260–261	$C_{20}H_{19}N_3OCl_2$
15·HCl	$NH(CH_2)_2N$-c-$(CH_2)_5$	H	Cl		>320	$C_{23}H_{23}N_3OCl_2$
18·HCl	H	$CONH(CH_2)_2N(CH_3)_2$	OH		253–254	$C_{21}H_{20}N_3O_3Cl$

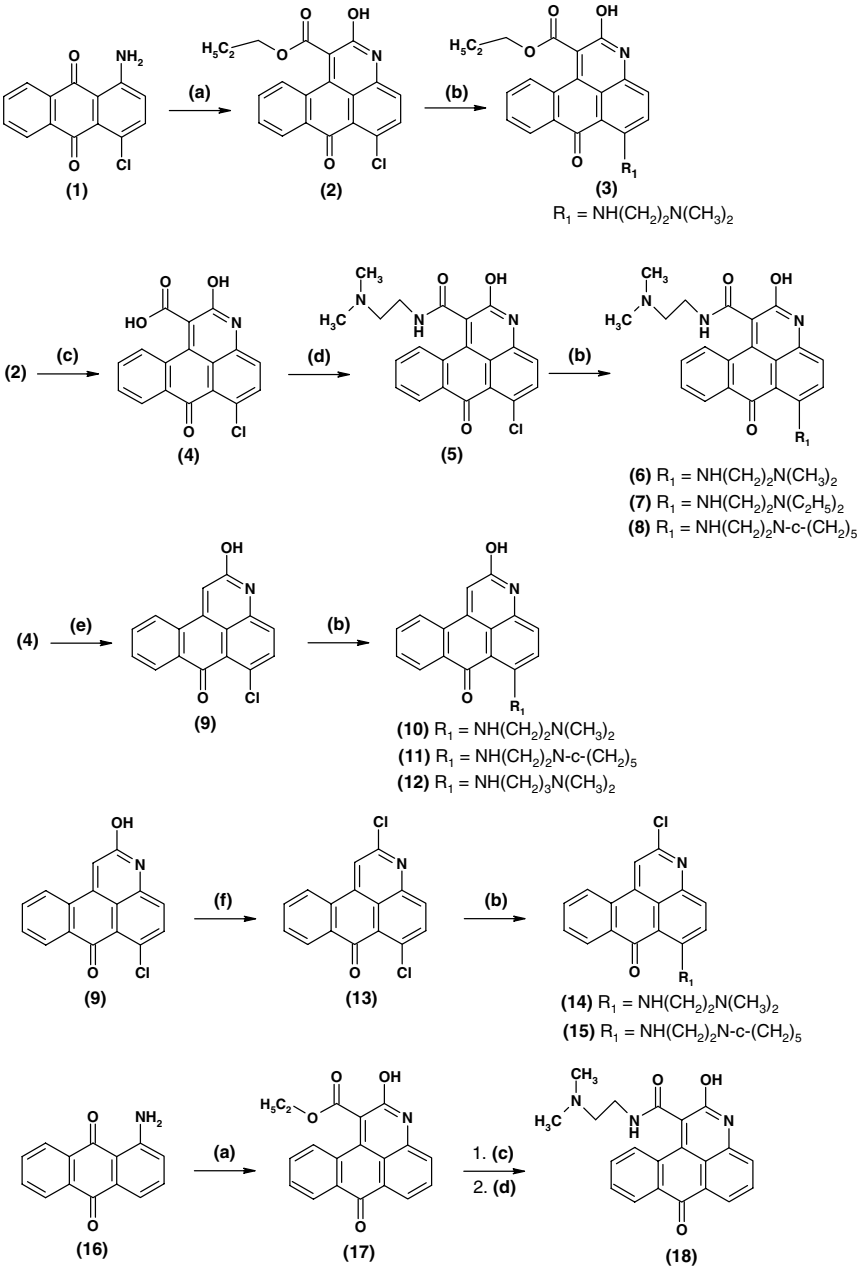
2. Chemistry

The synthetic pathways for derivatives **3**, **6**–**8**, **10**–**12**, **14**, **15**, and **18** are shown in Scheme 1. 1-Amino-4-chloroanthraquinone (**1**), the substrate for the majority of syntheses, was obtained from 1,4-dichloroanthraquinone according to Wormser's method.[17] The cyclization of **1** or commercially available 1-aminoanthraquinone (**16**) to its anthrapyridone derivatives (**2** or **17**, respectively) was performed in the reaction with diethyl malonate and sodium acetate at an elevated temperature. The substitution of the 6-chloro residue in compound **2** by an appropriate amine led to derivative **3** with an 1-ester group and aminoalkylamino chain at position 6. Compounds **6**–**8**, with basic 1-amido chain and various basic 6-aminoalkylamino arms, were prepared by alkaline hydrolysis of **2** to acid **4** followed by the reaction with 1,1′-carbonyldiimidazole (DCI). The intermediate imidazolide was then directly reacted with *N*,*N*-dimethylethylenediamine to give the required amide **5**. The successive displacement of the 6-chloro group by appropriate amines carried out at an elevated temperature led to the desired **6**–**8**. To obtain derivatives unsubstituted in the pyridone ring (**10**–**12**, **14**, and **15**), the decarboxylation of **4** by heating its DMA solution was performed. The resulting **9** on heating with amines readily gave **10**–**12**. The preparation of derivatives **14** and **15**, possessing 2-chloro group instead of 2-hydroxy function at the pyridone ring, was performed by the reaction of **9** with phosphonyl chloride, followed by 6-chloro displacement of resulting 2,6-dichloroanthraquinone (**13**) with amines. All attempts to obtain 2,6-disubstituted aminoalkylamino anthrapyridones failed or, if it were successful, only poor yields of the products were achieved. Compound **18**, with only 1-carboxamide was obtained, started with **16**, in the analogous reaction used for the preparation of the intermediates **2**, **4**, and **5**.

The structures of the compounds were determined by ^1H and ^{13}C NMR spectroscopy, and by determination of the molecular weights. The compounds (Table 1) were proved to occur in two tautomeric forms, as evidenced by ^1H and ^{13}C NMR, and physicochemical property. The compounds **3**, **6**, **7**, and **18** are the 2-hydroxy tautomers, while the compounds **10**–**12** exist in 2-oxo forms. For the biological and physicochemical evaluations the free bases of **3**, **6**–**8**, **10**–**12**, **14**, **15**, and **18** were converted into their hydrochloride or dihydrochloride salts by routine methods.

3. Results and discussion

The cytotoxicity of anthrapyridone derivatives **3**, **6**–**8**, **10**–**12**, **14**, **15**, and **18**, in comparison with reference clinical drugs doxorubicin (**DX**) and mitoxantrone (**MIT**) against sensitive and resistant human leukemia cell lines are presented in Table 2 as IC_{50} values. The examined compounds are different in the respect to their structure concerning various functional groups attached to the pyridone ring as well as different aminoalkylamino arms at position 6 of the chromophore moiety. The highest cytotoxic activity among the new derivatives exhibited compounds **3**, **6**, and **14**. Derivatives **3** and **6** with *N*,*N*-dimethylaminoethylamino chain at position 6 pointed to the effect of cationic 1-carboxamide arm in comparison with the 1-ester function. Compound **3**, possessing an ester group, was about twice more active than **6**. Surprisingly, the introduction of a basic 1-carboxamide moiety to the anthrapyridone chromophore does not change the activity of the compounds (compare the potency of **7** and **8** with that of **10**–**12**). Derivative **18**, holding only an 1-amido chain, is devoid of cytotoxicity. But converting the 2-hydroxy to the 2-chloro functionality restores the cytotoxicity (potency of the compound pair **10** versus **14**). The cytotoxic activity is also affected by the nature of the aminoalkylamino side chain at position 6. Compounds with a *N*,*N*-dimethylaminoethylamino residue (**6**, **10**, **14**) were more potent than those with piperidino moiety (**8**, **11**, **15**). The difference in the potency between the pair **10** versus **12** shows that the optimal distance between the two nitrogen atoms seems to be two methylene units.



Scheme 1. Synthetic route for compounds **3**, **6**–**8**, **10**–**12**, **14**, **15**, and **18**. Reagents and conditions: (a) $CH_2(COOEt)_2$, AcONa, 155–160 °C; (b) amine, Δ; (c) 20% KOH, EtOH, reflux; (d) CDI, DMA, 80 °C/80 min, amine, rt/18 h; (e) DMA, 165–170 °C/2h; (f) $POCl_3$, PCl_5, 100 °C/80 min.

The synthesized anthrapyridones exhibited activity toward the cell lines with induced multidrug cross-resistance of two main types (P-gp and MRP). The resistance indexes (RI) of the examined compounds were low (0.65–6.6). Only compound **3** with a 1-ester group has shown much lower ability to overcome the multidrug resistance (RI values are 20 and 50 against MDR1 and MRP1 cell lines, respectively). No marked influence of the other substituents on this property could be observed.

In conclusion, anthrapyridone derivatives are the group of anthraquinone analog antitumor agents with moderate cytotoxicity. These compounds, however, are of interest because of their ability to overcome multidrug resistance of tumor cell lines. In particular, the cytotoxic activity against MRP cell line should be stressed, because such a property is rather rare among cytostatics.

The presence of an ester group at position 1 of pyridone ring is beneficial for cytotoxic activity but simultaneously causes the loss of activity in respect to resistant cell lines. However, the introduction at this position of aminoalkylamido arm together with an appropriate 6-aminoalkylamino chain resulted in the appearance of activity against sensitive as well as resistant cell lines. On the other hand, lack of a 6-amino side chain affected a drastic decrease of the cytotoxicity but the ability to overcome MDR is retained.

The obtained results corroborate our hypothesis on the essential role of heterocyclic ring fused to anthracenedi-

Table 2. In vitro cytotoxic activity of examined compounds toward sensitive and resistant human cell lines in comparison with reference doxorubicin (**DX**), and mitoxantrone (**MIT**)

Compound	Cell line[a]/IC_{50}[b] (nM) ± SEM[c]				
	HL-60	HL-60/VINC	RI[d]	HL-60/DX	RI[d]
3	146 ± 9	2798 ± 395	19.21	7424 ± 892	50.99
6	311 ± 23	1012 ± 82	3.25	667 ± 275	2.14
7	1141 ± 102	1428 ± 172	1.25	1193 ± 203	1.05
8	1257 ± 86	1258 ± 121	1.00	1296 ± 201	1.03
10	1159 ± 74	1551 ± 124	1.34	2092 ± 129	1.81
11	1543 ± 120	1541 ± 128	1.00	1784 ± 140	1.16
12	1463 ± 295	3301 ± 262	2.26	3515 ± 8	2.40
14	327 ± 61	415 ± 35	1.27	2178 ± 363	6.66
15	1312 ± 126	853 ± 23	0.65	2113 ± 84	1.61
18	10,759 ± 79	10,235 ± 510	0.95	13,987 ± 31	1.30
DX	18.6 ± 0.7	443.9 ± 28.1	23.87	4163 ± 310	223.82
MIT	1.0 ± 0.1	33.6 ± 1.8	33.60	925.1 ± 20.2	925.10

[a] HL-60—human promyelocytic leukemia, and vincristine resistant (MDR1 type) subline HL-60/VINC, and doxorubicin resistant (MRP1 type) subline HL-60/DX.
[b] IC_{50}—the concentration of compound causing 50% inhibition of cell growth after 72 h continuous exposure.
[c] SEM—standard error of the mean.
[d] RI—resistance index; the ratio of IC_{50} value for resistant cell line to IC_{50} value for sensitive cell line.

one compounds in the ability to overcome multidrug resistance of tumor cells.

4. Experimental

4.1. Chemistry

Melting points were determined with a Boeticus PHMK05 apparatus and are uncorrected. Combustion analyses are within +0.4% of the theoretical values and were carried out on a Carlo Erba CHNS-O-EA1108 instrument for C, H, and N. NMR spectra were taken on a Varian 300 MHz or 500 MHz spectrometer using tetramethylsilane as an internal standard. The following NMR abbreviations were used: ex (exchangeable with D_2O), d ex (exchangeable with D_2O, but with difficulty). Mass spectra were recorded on a Quadrupolic Mass Spectrophotometer Trio-3 (FAB technique). Thin-layer chromatography (TLC) was carried out on silica gel (Kieselgel 60 plates, Merck), column chromatography was performed on silica gel (Kieselgel Merck, −200 mesh), if do not marked otherwise, and on Sephadex LH-20 (Pharmacia).

4.1.1. Ethyl 6-chloro-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylate (2). A mixture of 250 mg (2 mmol) of **1**, 250 mg of sodium acetate, and 2.5 mL of diethyl malonate was stirred at 155–160 °C for about 3.5 h. To facilitate the stirring, a small amount of diethyl malonate was added. The course of the reaction was followed by TLC in $CHCl_3$/MeOH (20:1). To the reaction mixture a portions of ethyl ether and hexane were added and the resulted yellow-green precipitate was filtered and then washed with hexane and water. Yield 90%. An analytical sample was dissolved in warm $CHCl_3$ and flash-chromatographed (using $CHCl_3$/MeOH, 20:1 and 10:1 as eluent). Mp 291–294 °C.

^{1}H NMR (DMSO-d_6) δ 1.3 (t, 3H, J = 7.1 Hz); 4.4 (q, 2H, J = 7.1 Hz); 7.64 (d, 1H, J = 8.8 Hz); 7.8–7.93 (m, 3H); 7.96 (dd, 1H, J = 6.6, J = 1.5 Hz); 8.3 (dd, 1H, J = 1.9 Hz); 12.7 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %); 355 [$(M+1)^+$, 100].

4.1.2. Ethyl-6[[2-(dimethylamino)ethyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylate (3). A sample of **2** with *N*,*N*-dimethylethylenediamine was stirred under reflux in a nitrogen atmosphere for 2 h. The reaction mixture was diluted with $CHCl_3$ and washed with diluted HCl to removed excess amine. The organic layer was flash-chromatographed (eluent $CHCl_3$/MeOH, 10:1 and 5:1) to give **3** as a dark pink solid (yield 86%). Mp (as hydrochloride) 253–254 °C.

^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7.1 Hz); 2.4 (s, 6H); 2.7 (t, 2H, J = 6.3 Hz); 3.35–3.45 (m, 2H); 4.6 (q, 2H, J = 7.1 Hz); 7.1 (d, 1H, J = 8.2 Hz); 7.6–7.7 (m, 2H); 7.75 (d, 1H, J = 7.3 Hz), 8.2 (d, 1H, J = 8.3 Hz); 8.6 (d, 1H, J = 7.75 Hz); 10.8 (t, 1H, d ex), 12.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 406 [$(M+1)^+$, 100].

Found: C, 68.0; H, 5.71; N, 10.31. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36.

4.1.3. 6-Chloro-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylic acid (4). A sample of 354 mg (1 mmol) of **2** was dissolved in 22 mL of 20% KOH solution and 18.5 mL of EtOH. The solution was refluxed for 3.5 h, then cooled and poured into cold 5% HCl solution. The resulted solid was separated and dried at 90 °C to give 320 mg of **4**, as a yellow powder (yield 98%). Mp > 320 °C.

MS *m*/*z* (relative intensity, %): 326 [$(M)^+$, 80]; 282 [$(M-44)^+$].

4.1.4. 6-Chloro-1-[(2-dimethylamino)ethyl]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxamide (5). A suspension of 650 mg (2 mmol) of acid **4** in 10 mL of DMA was stirred at 80 °C for 80 min. Next 500 mg

(3 mmol) of 1,1′-carbonyldiimidazole was added and the stirring was continued at 40–50 °C for 3 h. After then the reaction mixture was treated with 1 mL of *N*,*N*-dimethylethylenediamine and the solution was left at room temperature for 18 h. The course of the reaction was monitored by TLC in $CHCl_3$/MeOH (10:1). The solution was diluted with $CHCl_3$, washed with 10% Na_2CO_3 solution, water, and dried over Na_2SO_4. The solvents were removed and the residue was flash-chromatographed (eluent $CHCl_3$/MeOH, 20:1), to afford **5** (79%) as a yellow solid, fluorescing at 366 nm. Mp 218–220 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.28 (s, 6H); 2.4 (t, 2H, J = 5.3 Hz); 3.0 (q, 2H, J = 5.2 Hz); 6.9 (t, 1H, J = 4.9 Hz, ex); 7.48–7.56 (m, 3H); 7.7 (t, 1H, J = 7.8 Hz); 7.83 (d, 1H, J = 8.2 Hz); 7.85 (d, 1H, J = 7.8 Hz), 12.0 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 396 [(M)]$^+$, 100].

4.1.5. 1-[2-(Dimethylamino)ethyl]-6-[[(2-dimethylamino)ethyl]-amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxamide (6). A sample of **5** with *N*,*N*-dimethylethylenediamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was stirred at 130 °C in a nitrogen atmosphere for 6 h. The course of the reaction was monitored by TLC in $CHCl_3$/MeOH (5:1). The reaction mixture was diluted with $CHCl_3$ and carefully washed with diluted HCl to remove excess of amines. The organic layer was dried over Na_2SO_4, evaporated and the residue was flash-chromatographed (eluent $CHCl_3$/MeOH, 10:1 and 5:1). The desired chromatography fractions were collected and **6** was converted into its dihydrochloride salt (orange-red powder; yield 20%). The solid was purified by column chromatography (Sephadex LH-20) eluting with MeOH. Mp (as dihydrochloride) 296 °C (dec).

^{1}H NMR ($CDCl_3$) δ 2.25 (s, 6H); 2.4 (s, 6H); 2.45 (t, 2H, J = 5.8 Hz); 2.7 (t, 2H, J = 5.8 Hz); 2.9 (q, 2H, J = 5.2 Hz); 3.4 (m, 2H); 6.85 (t, 1H, J = 5.8 Hz, ex); 6.95 (d, 1H, J = 8.5 Hz); 7.5 (t, 1H, J = 7.2 Hz); 7.6 (d, 1H, J = 8.6 Hz); 7.75 (t, 1H, J = 7.4 Hz); 8.32 (d, 1H J = 7.9 Hz); 8.5 (d, 1H, J = 7.9); 10.45 (t, 1H, J = 4.9 Hz, ex); 12.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 447 [(M)$^+$, 100].

Found: C, 67.00; H, 6.51; N, 15.60. Calcd for $C_{25}H_{29}N_5O_3$: C, 67.10; H, 6.53; N, 15.65.

4.1.6. 1-[2-(Dimethylamino)ethyl]-6[[(2-diethylamino)ethyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxamide (7). The reaction of **5** with *N*,*N*-diethylethylenediamine was carried out as described for the preparation of **6**; time of the reaction being 6 h. The reaction mixture was worked up and purified by flash-chromatography (eluent $CHCl_3$/MeOH, 10:1 and 5:1) to give **7**, as a red solid. Yield 35%. Mp (as dihydrochloride) 266–268 °C.

^{1}H NMR ($CDCl_3$) δ 1.12 (t, 6H, J = 7.3 Hz); 2.52 (s, 3H); 2.54 (s, 3H); 2.54–2.57 (m, 2H); 2.7 (q, 4H, J = 7.3 Hz); 2.9 (t, 2H, J = 7.3 Hz); 3.05 (m, 2H); 3.5 (t, 2H, J = 6.9 Hz); 6.85 (t, 1H, J = 6.0 Hz, ex); 6.9 (d, 1H, J = 8.8 Hz); 7.52 (t, 1H, J = 7.3 Hz); 7.6 (d, 1H, J = 8.8 Hz); 7.7 (t, 1H, J = 7.42 Hz); 8.3 (d, 1H, J = 7.9 Hz); 8.5 (d, 1H, J = 77.81 Hz); 10.45 (t, 1H, J = 4.88 Hz, d ex); 12.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 476 [(M+1)$^+$ 100].

Found: C, 67.9; H, 6.97; N, 14.70. Calcd for $C_{27}H_{33}N_5O_3$: C, 68.19; H, 6.99; N, 14.73.

4.1.7. 1-[(2-Dimethylamino)ethyl]-6-[[(2-piperidin-1-yl)ethyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxamide (8). Compound **8** was obtained in the reaction of **5** with 1-(2-aminoethyl)piperidine by a procedure similar to that described for **6**, as an orange-red solid. Yield 40%. Mp (as dihydrochloride) 115–117 °C.

^{1}H NMR ($CDCl_3$) δ 1.5 (m, 2H); 1.7 (m, 4H); 2.25 (s, 6H); 2.45 (t, 2H, J = 5.85 Hz); 2.6 (m, 4H); 2.8 (t, 2H, J = 6.3 Hz); 3.1 (d, 2H, J = 5.4 Hz); 3.6 (m, 2H); 6.75 (t, 1H, J = 4.8 Hz, ex); 6.95 (d, 1H, J = 8.8 Hz); 7.52–7.58 (m, 2H); 7.7 (t, 1H, J = 7.3 Hz); 8.3 (d, 1H, J = 8.3 Hz), 8.5 (d, 1H, J = 7.8 Hz); 10.4 (m, 1H, ex); 11.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 488 [(M+1)$^+$, 100].

Found: C, 68.99; H, 6.81; N, 14.32. Calcd for $C_{28}H_{33}N_5O_3$: C, 68.97; H, 6.82; N, 14.36.

4.1.8. 6-Chloro-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (9). A sample of 200 mg of **4** in 7 mL DMA was stirred at 165–170 °C for 2h. The progress of the reaction was followed by TLC in $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$ and washed with 10% Na_2CO_3 solution and H_2O. The organic layer was dried over Na_2SO_4, the solvents were evaporated, and the residue was flash-chromatographed (eluent $CHCl_3$, then $CHCl_3$/MeOH, 20:1). Compound **9** was obtained as a yellow-brown solid (yield 40%), with fluorescence at 366 nm. Mp 164–166 °C (dec).

^{1}H NMR (DMSO-d_6) δ 7.6 (d, 1H, J = 8.8 Hz); 7.75 (s, 1H); 7.78–7.85 (d, 1H, J = 8.8 Hz overlapping with m, 2H); 8.22 (d, 1H, J = 6.8 Hz); 8.5 (d, 1H, J = 8.3 Hz); 12.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 282 [(M)$^+$, 100].

4.1.9. 6-[[(2-Dimethyloamino)ethyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (10). A sample of **9** with *N*,*N*-dimethylethylenediamine was stirred at 100 °C in a nitrogen atmosphere for 80 min. The course of the reaction was monitored by TLC in $CHCl_3$/MeOH (5:1). The reaction mixture was worked up by the procedure described for the preparation of **6**. Further purification by flash-chromatography (eluent $CHCl_3$/MeOH, 5:1 and 2:1) gave **10** as a pink solid. Yield 25%. Mp (as hydrochloride) 230–232 °C (subl).

^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H); 2.8 (m, 2H); 3.5 (m, 2H); 7.7 (m, 5H); 8.2 (m, 1H); 8.5 (m, 1H); 10.6 (br s,

1H, ex); 10.75 (br s, 1H, ex); ($CDCl_3$ + TFA) δ 3.2 (s, 6H); 3.7 (m, 2H); 4.15 (m, 2H); 7.6 (d, 1H, J = 9.8 Hz); 7.8–7.85 (m, 1H); 7.9–8.0 (m, 2H); 8.0 (d, 1H, J = 9.3 Hz); 8.1 (s, 1H); 8.4 (m, 1H); 9.4 (br s, 1H).

Found: C, 72.00; H, 5.75; N, 12.59. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60.

4.1.10. 6-[[(2-Piperidin-1-yl)ethyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (11). The reaction of **9** with 1-(2-aminoethyl)piperidine was carried out at 80–90 °C for 30 min. The reaction mixture was worked up by the procedure described for the preparation of **6** and purified by flash-chromatography (eluent $CHCl_3$/MeOH, 10:1 then 5:1) to afford **11** as a red solid. Yield 32%. Mp (as hydrochloride) 225–227 °C (subl).

^{1}H NMR (DMSO-d_6) δ 1.68–1.72 (m, 2H); 1.72–1.84 (m, 4H); 2.9 (t, 2H, J = 8.2 Hz); 3.3 (t, 2H, J = 6.9 Hz); 3.5 (m, 2H); 4.0 (t, 2H, J = 6.9 Hz); 7.5 (d, 1H, J = 9.3 Hz); 7.65 (s, 1H); 7.7 (d, 1H, J = 9.3 Hz); 7.72–7.78 (m, 1H); 7.82 (q, 1H, J = 7.3 Hz); 8.35 (d, 1H, J = 5.3 Hz); 8.51 (d, 1H, J = 8.3 Hz); 10.4 (br s, 1H, ex); 10.5 (br s, 1H, ex).

MS m/z (relative intensity, %): 374 [$(M+1)^+$, 100].

Found: C, 74.00; H, 6.19; N, 11.24. Calcd for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25.

4.1.11. 6-[[(3-Dimethylamino)propyl]amino]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (12). Compound **12** was prepared in the reaction of **9** with 3-(dimethylamino)propylamine by the procedure similar to that described for **10**, as a pink-orange solid. Yield 26%. Mp (as hydrochloride) 220–222 °C (subl).

^{1}H NMR ($CDCl_3$) δ 2.04 (q, 2H, J = 6.8 Hz); 2.4 (s, 6H); 3.0 (t, 2H, J = 7.15 Hz); 3.68 (q, 2H, J = 6.3 Hz), 7.5 (d, 1H, J = 9.1 Hz); 7.63 (s, 1H); 7.68 (d, 1H, J = 9.2 Hz); 7.69–7.75 (m, 1H), 7.8 (q, 1H, J = 7.2 Hz); 8.3 (d, 1H, J = 5.15 Hz), 8.49 (d, 1H, J = 8.2 Hz); 10.6 (br s, 1H, ex); 10.7 (br s, 1H, ex).

Found: C, 72.30; H, 6.10; N, 12.05. Calcd for $C_{21}H_{21}N_3O_2$: C, 72.37; H, 6.08; N, 12.06.

4.1.12. 2,6-Dichloro-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (13). A sample of 140 mg (0.5 mmol) of **9**, 2 mL of $POCl_3$ and a slightly amount of PCl_5 was stirred at 100 °C for 80 min. The course of the reaction was followed by TLC in $CHCl_3$/MeOH (10:1); the loss of substrate fluorescence indicating the completion of the reaction. The unreacted $POCl_3$ was removed under reduced pressure and to the residue ice was added. The mixture was diluted with $CHCl_3$ and washed with 2 N NaOH solution and H_2O. The organic layer was dried over Na_2SO_4, evaporated, and the residue was flash-chromatographed (eluent $CHCl_3$/MeOH, 50:1 then 20:1) to afford **13** as a yellow-brown solid. Yield 60%. Mp 240–241 °C.

MS m/z (relative intensity, %): 301 [$(M)^+$, 100].

4.1.13. 2-Chloro-6-[[(2-dimethylamino)ethyl]amino]-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (14). A sample of **13** with *N*,*N*-dimethylethylenediamine was stirred at 100 °C in a nitrogen atmosphere for 20 min. The course of the reaction was monitored by TLC in $CHCl_3$/MeOH (5:1). The reaction mixture was worked up as described for **6**. The flash-chromatography (eluent $CHCl_3$/MeOH, 20:1 then 5:1) gave **14** as a yellow solid strongly fluorescing at 366 nm. Yield 60%. Mp (as hydrochloride) 260–261 °C.

^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H), 2.75 (t, 2H, J = 5.8 Hz); 3.5 (m, 2H); 7.78–7.82 (m, 3H); 8.1 (d, 1H, J = 9.6 Hz); 8.4 (d, 1H, J = 7.8 Hz); 8.5 (s, 1H); 8.7 (d, 1H, J = 7.8 Hz); 11.45 (t, 1H, J = 5.9 Hz, d ex).

Found: C, 68.15; H, 5.12; N, 11.8. Calcd for $C_{20}H_{18}N_3OCl$: C, 68.28; H, 5.16; N, 11.94.

4.1.14. 2-Chloro-6-[[(2-piperidin-1-yl)ethyl]amino]-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (15). Compound **15** was prepared in the reaction of **13** with 1-(2-aminoethyl)piperidine by a procedure similar to that described for **14**. Compound **15** is a yellow solid with a strong fluorescence at 366 nm. Yield 76%. Mp > 320 °C (dec).

^{1}H NMR (DMSO-d_6) δ 1.6–1.9 (m, 6H); 3.0 (m, 2H); 3.5 (t, 4H, J = 11 Hz); 4.2 (q, 2H, J = 6.8 Hz); 7.8–7.9 (m, 3H); 8.2 (d, 1H, J = 9.7 Hz); 8.5 (d, 1H, J = 7.8 Hz); 8.7 (s, 1H); 8.8 (d, 1H, J = 7.8); 11.5 (t, 1H, J = 5.9 Hz, d ex).

Found: C, 70.45; H, 5.58; N, 10.73. Calcd for $C_{23}H_{22}N_3OCl$: C, 70.49; H, 5.66; N, 10.72.

MS m/z (relative intensity, %): 392 [$(M)^+$, 100].

4.1.15. Ethyl 2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylate (17). Compound **17** was prepared in the reaction of 1-aminoanthraquinone (**16**) with sodium acetate and diethyl malonate by a procedure similar to that described for **2**. Yield 91%. Mp 310 °C (dec).

^{1}H NMR (DMSO-d_6) δ 1.3 (t, 3H, J = 7.1 Hz); 4.5 (q, 2H, J = 7.1 Hz); 7.72 (d, 1H, J = 7.1 Hz); 7.83 (t, 1H, J = 7.3 Hz); 7.86 (d, 1H, J = 8.0 Hz); 7.92 (t, 1H, J = 2.9 Hz); 8.02 (d, 1H, J = 8.3 Hz); 8.12 (d, 1H, J = 8.0 Hz); 8.4 (d, 1H, J = 6.0 Hz); 12.7 (br s, 1H, ex).

^{13}C NMR (DMSO-d_6) δ 181.34; 166.90; 159.32; 137.57; 134.16; 132.87; 131.75; 131.63; 131.73; 128.39; 128.24; 126.56; 126.32; 122.49; 121.33; 115.62; 62.15; 13.69.

MS m/z (relative intensity, %): 320 [$(M+1)^+$, 100].

4.1.16. 1-[2-(Dimethylamino)ethyl]-2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxamide (18). A sample of **17** was converted into acid by the same manner as described for **4**. The obtained 2-hydroxy-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylic acid in DMA solution was then treated with 1,1′-carbonyldiimidazole and the intermediate imidazolide was reacted with *N*,*N*-dimethylethylenediamine. The procedure of the reaction

was similar to that described for **5**. Yield 80%. Mp (as hydrochloride) 253–254 °C.

^{1}H NMR ($CDCl_3$) δ 2.3 (s, 6H); 2.45 (t, 2H, J = 6.0 Hz); 3.55 (q, 2H, J = 7.6 Hz); 6.45 (t, 1H, ex); 7.3–7.7 (m, 3H); 7.8 (t, 1H, J = 7.2 Hz); 8.18 (d, 1H, J = 7.5 Hz); 8.34 (dd, 1H, J = 7.1, 1.6 Hz); 8.5 (dd, 1H, J = 6.7, 1.36 Hz); 11.8 (br s, 1H, ex).

MS *m*/*z* (relative intensity, %): 362 [$(M+1)^+$, 100].

Found: C, 69.74; H, 5.26; N, 11.62. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63.

5. Biological evaluation

5.1. Cell lines

Human promyelocytic leukemia sensitive cell line HL-60 and resistant sublines: vincristine-resistant HL-60/VINC and doxorubicin-resistant HL-60/DX (Kansas State University, Manhattan, KS, USA) were grown in RPMI 1640 medium supplemented with 10% FBS penicillin G (100,000 units/L), streptomycin (100 mg/L). Reselection of the resistant cell lines was performed once a month by exposure to 200 nM doxorubicin and 1 μM vincristine for HL-60/DX and HL-60/VINC, respectively. Cell lines were grown in a controlled (air—5% CO_2) humidified atmosphere at 37 °C and were transplanted three times a week. For the experiments the cells in logarithmic growth were suspended in the growth medium to give a final required density. The resistant cell lines were maintained without the reselection drugs at least one week before the experiments.

5.2. In vitro cytotoxic evaluation

Cells of required density were seeded and different concentrations of the drugs were added. The experiments were carried out in a controlled (air—5% CO_2) humidified atmosphere at 37 °C. The exposure time was 72 h for all cell lines. The cytotoxic activity (IC_{50} values) of the compounds was defined as their in vitro concentrations causing 50% inhibition of cell growth after continuous exposure to the drug, as measured by cell counting with Z2 Cell Analyzer (Beckman Coulter) or by the protein content of the cells as described previously.[18] Results are given as means of at least three independent experiments ± standard error of the mean (SEM). The resistance index (RI) was defined as the ratio of IC_{50} value for resistant cell line to IC_{50} value for sensitive one.

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