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Synthesis and Biological Evaluation of 2,7-Dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione Derivatives, a Novel Group of Anticancer Agents Active on a Multidrug Resistant Cell Line

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Abstract—A series of anthrapyridazone derivatives with one or two basic side chains at various positions in the tetracyclic chromophore have been synthesized. The key intermediates in the synthesis are 2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-diones **1**, **12** and **15** monosubstituted at position 2 (**4d**, **16a**–**e**), or 6 (**2a**–**f**) or disubstituted at positions 2 and 6 (**4a**–**c**) or 2 and 8 (**17a**–**e**) with appropriate alkylaminoalkylamines. All analogues showed in vitro cytotoxic activity against murine leukemia (L1210) and human leukemia (K562) cell lines. The compounds were also active against human leukemia multidrug resistant (K562/DX) cell line with resistance index (RI) in the range 1–3 depending on the compound's structure. Two of the most active in vitro compounds **4a** and **11** were tested in vivo against murine P388 leukemia and displayed antileukemic activity comparable with that of Mitoxantrone. DNA-binding assays were performed and DNA affinity data were correlated with the structures of the compounds. The cytoplasmatic membrane affinity values (log k'_{IAM}) have also been determined and the correlation with the resistance indexes discussed. The anthrapyridazones constitute a novel group of antitumor compounds that can overcome multidrug resistance.
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Introduction

The prolonged clinical use of chemotherapeutic agents often causes the appearance of multidrug resistance (MDR) toward numerous antitumor compounds. This effect currently constitutes one of the major problems in clinical chemotherapy and has not yet been successfully solved.[1–3] Many efforts have been directed toward the search for new antitumor anthracenedione derivatives with increased effectiveness against MDR tumor cell lines. This has resulted in the design of anthrapyrazoles[4] and their aza-analogues,[5] benzoperimidines[6] and anthrapyridones,[7] which fulfill this requirement. Recently we postulated that the presence of a heterocyclic ring condensed with the anthracenedione or with the structurally related acridone chromophore determines cytotoxic activity toward the multidrug resistant cell lines.[6] Although the presence of a fused heterocyclic ring seems to be essential for overcoming MDR, the cytotoxic potency depends on the structure of the side chains and other substituents.

In this paper, we describe the synthesis and biological evaluation of a new group of anthracenedione derivatives with a pyridazone ring incorporated into the chromophore, namely 2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-diones (hereafter referred to as anthrapyridazones), to determine the relationships between structure and antitumor activity of derivatives of this novel group. A series of compounds bearing [(alkylamino)-alkyl]amino side chain attached at positions 2, 6 or 8 to the chromophore moiety has been obtained. The presence of a second basic side chain could increase not only the binding to DNA, but also modify the physicochemical properties and solubility of the above derivatives and enhances their cytotoxic activity. Therefore, the 2,6- as well as the 2,8-disubstituted analogues have been synthesized (Fig. 1).

As evidenced earlier, the presence of hydroxyl groups in related ring systems such as anthracenediones,[8] anthrapyrazoles,[9] benzoperimidines[6] and acridine derivatives[10]

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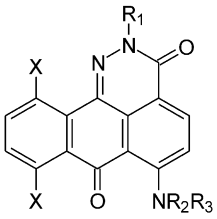
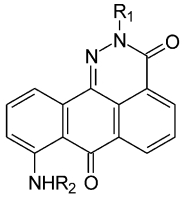


2 a-e	X = H	$R_1 = R_2 = H$	R_3 = alkylaminoalkyl
2 f	X = H	R_1 = H; $R_2 = CH_3$	R_3 = alkylaminoalkyl
4 a-c	X = H	R_1 = alkylaminoalkyl; R_2 =H	R_3 = alkylaminoalkyl
4 d	X = H	R_1 = alkylaminoalkyl	$R_2 = R_3 = H$
11	X = OH	R_1 = alkylaminoalkyl; R_2 =H	R_3 = alkylaminoalkyl
14	X = OH	$R_1 = R_2 = H$	R_3 = alkylaminoalkyl



16 a-e	R_1 = alkylaminoalkyl; R_2 =H
17 a-c	$R_1 = R_2$ = alkylaminoalkyl
17 d-e	R_1 ? R_2 = alkylaminoalkyl
21	$R_1 = CH_3$; R_2 = alkylaminoalkyl

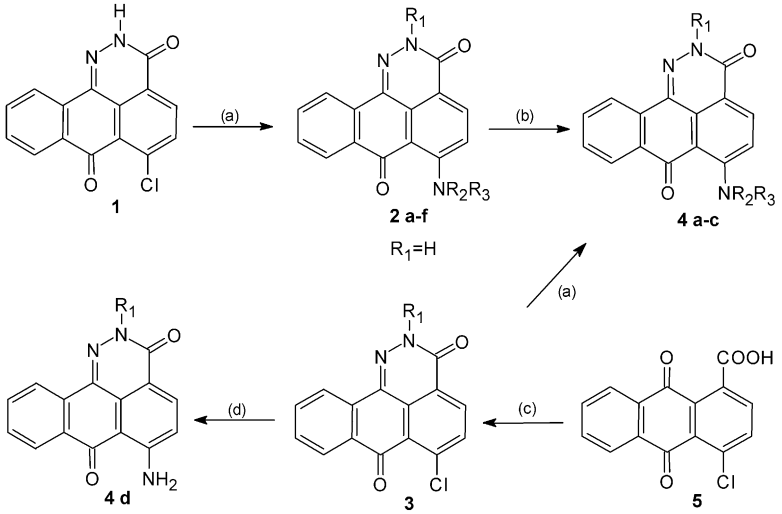
Figure 1. Structure of 2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-diones.

increases antitumor activity in these classes of compounds. For this reason the 2,7-dihydro-3*H*-dibenzo [*de*,*h*]cinnoline-3,7-dione derivatives with hydroxyl groups at positions 8 and 11 have also been prepared.

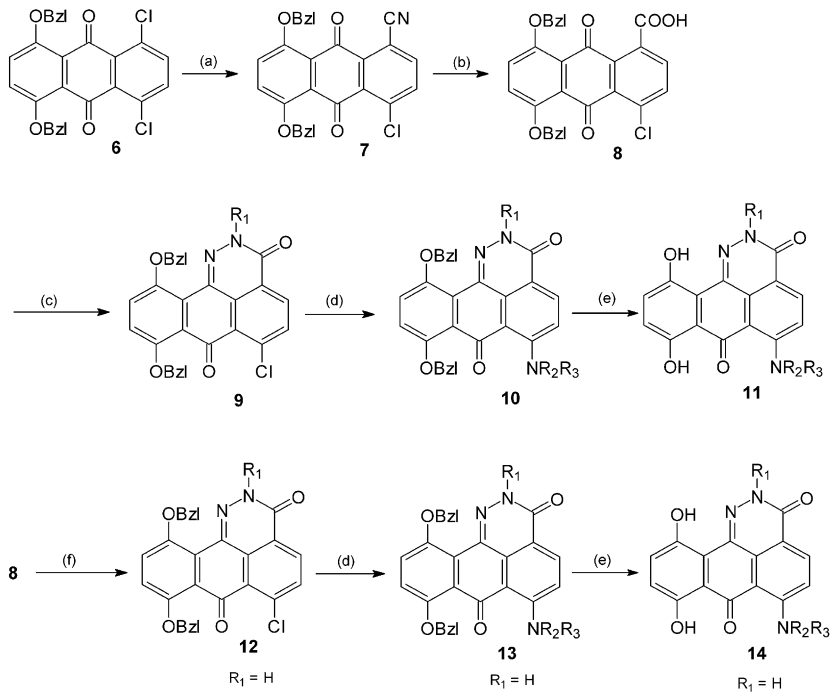
Chemistry

The synthetic pathways leading to anthrapyridazone derivatives are shown in Schemes 1–4. The starting materials 6-chloro- and 8-aminoanthrapyridazone (**1** and **15**), used in the synthesis of **2a–f**, **4a–d**, **16a–e** and **17a–e**, were obtained by previously reported methods[11,12] by treatment of the respective acid chlorides of **5** or **15** with hydrazine. We prepared the 2-methyl-8-aminoanthrapyridazone (**20**) in an analogous way in slightly modified conditions. The reaction of **1** with the appropriate amines occurred very readily and led to the desired 6-[(alkylamino)alkyl]amino-anthrapyridazones (**2a–f**). These derivatives were transformed into, respectively, the symmetrically and unsymmetrically substituted 2-[(alkylamino)alkyl]-6-[(alkylamino)alkyl]amino-anthrapyridazones (**4a–c**) by heating with the appropriate alkylaminoalkyl chloride in the presence of Na_2CO_3. Another possibility to obtain these derivatives is the preparation of the 2-[(alkylamino)alkyl]-6-chloro-anthrapyridazone **3** by refluxing of the acid chloride of **5** with an appropriate alkylaminoalkyl hydrazine followed by heating of **3** with the respective amine. 2-[(Alkylamino) alkyl]-6-amino-anthrapyridazone (**4d**) was obtained in the reaction of **3** with aminoacetaldehyde dimethylacetal and hydrolysis of the product with 10% HCl in tetrahydrofurane (Scheme 1).

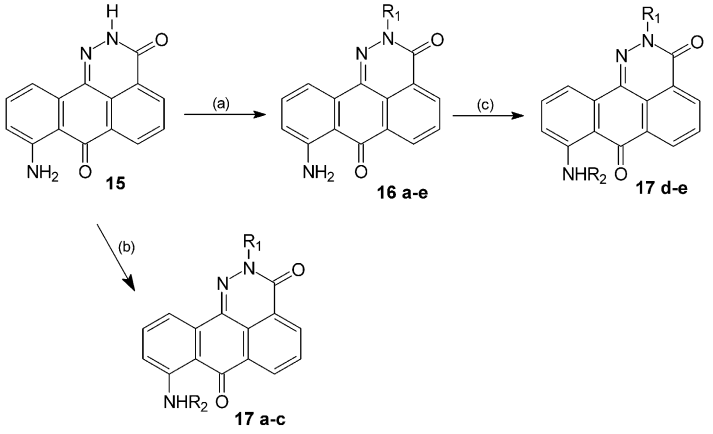
The synthetic route to the 8,11-dihydroxyanthrapyridazone derivatives is illustrated in Scheme 2.



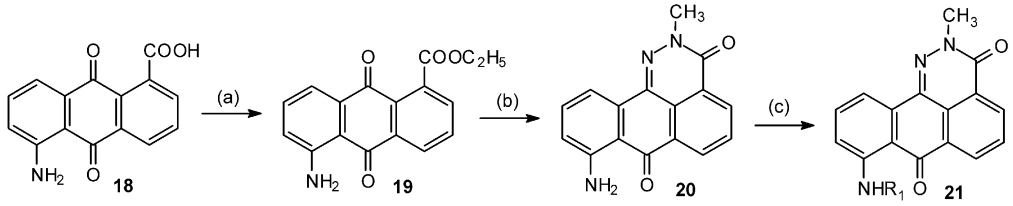
Scheme 1. Synthetic route for compounds **2–4**. Reagents: (a) NHR_2R_3, pyridine; (b) R_1Cl, Na_2CO_3, DMA; (c) 1. benzene, PCl_5, 2. R_1NHNH_2; (d) 1. $H_2NCH_2CH(OCH_3)_2$, 2. 10% HCl, THF.



Scheme 2. Synthetic route for compounds **11**–**14**. Reagents: (a) 1. CuCN, DMA, 2. 3N HNO_3; (b) 15% NaOH, MeOH, DMA; (c) 1. benzene, PCl_5, 2. R_1NHNH_2; (d) NHR_2R_3, DMA; (e) TFA; (f) 1. benzene, PCl_5, 2. NH_2NH_2.



Scheme 3. Synthetic route for compounds **16** and **17**. Reagents: (a) R_1Cl, Na_2CO_3, DMA; (b) $R_1Cl{=}R_2Cl$, NaH, DMA; (c) R_2Cl, NaH, DMA.



Scheme 4. Synthetic route for compound **21**. Reagents: (a) EtOH saturated with HCl; (b) CH_3NHNH_2, pyridine; (c) R_1Cl, NaH, DMA.

Treatment of **6** with cupric cyanide in DMA resulted in the desired monosubstituted derivative **7**. The basic hydrolysis of nitrile group in a 15% solution of NaOH led to 5,8-di(benzyloxy)-4-chloro-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylic acid (**8**). The reaction of the acid chloride of **8** with hydrazine or 2-(*N*,*N*-dimethylamino)ethylhydrazine followed by heating **9** or **12** with 2-(dimethylamino)ethylamine in DMA gave compounds substituted with one or two basic side chains, respectively (**13**, **10**). The removal of the protecting benzyl

groups in the presence of trifluoroacetic acid yielded the required 8,11-dihydroxyanthrapyridazones **11** and **14**.

Compounds **16a–e** were prepared starting from **15** using similar conditions as described above for the synthesis of **4a–c** (Scheme 3).

Derivatives **17a–c** were obtained in the reaction of **15** with sodium hydride in DMA followed by the treatment of obtained sodium salts with an appropriate (alkylamino)alkyl chloride or, if the side chains at positions 2 and 8 should be different, under similar conditions, using **16** as starting material (**17d–e**, Scheme 3).

For structure–activity relationship, the anthrapyridazone derivative **21** with one side chain at position 8 has been synthesized. Compound **19** was obtained in the usual way by heating **18** in hydrogen chloride saturated ethanol. After that **19** refluxed with methylhydrazine led to **20**, which was transformed into **21** in an analogous way as described for **17d–e** (Scheme 4).

The structures, melting points, yields and formulas of compounds **2a–f**, **4a–d**, **11**, **14**, **16a–e**, **17a–e** and **21** are presented in Table 1.

For the biological and physicochemical evaluations, all the obtained compounds were converted into their hydrochloride or dihydrochloride salts by routine methods.

Results and Discussion

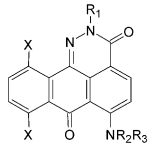
Cytotoxic activity

The anthrapyridazone derivatives were evaluated for their cytotoxic potency in vitro using the sensitive leukemia cell lines: murine L1210 and human K562 as well as human leukemia multidrug resistant subline K562/DX.

Mitoxantrone (MIT) and Doxorubicin (DX) were used as reference compounds. The cytotoxicity data are presented in Table 2 as IC_{50} values.

Most of the studied anthrapyridazones demonstrated high cytotoxic activity against L1210 leukemia. The 2,6-disubstituted derivatives (**4a–e**) are more active than their 6-monosubstituted analogues (**2a–e**). Generally derivatives with side chains at positions 2 and 8 are less

Table 1. Substituents, melting points, yields, and formulas of anthrapyridazone derivatives



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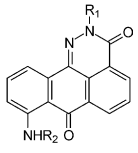

B

Compd	Fm	X	R_1	R_2	R_3	Yield[a]	Mp (°C)	Molecular formula[b]
2a	A	H	H	H	$CH_2CH_2N(CH_3)_2$	85	274–276	$C_{19}H_{18}N_4O_2$
2b	A	H	H	H	$CH_2CH_2NHCH_2CH_2OH$	50	243–245	$C_{19}H_{18}N_4O_3$
2c	A	H	H	H	CH_2CH_2–*c*-$N(CH_2)_5$	65	254–256	$C_{22}H_{22}N_4O_2$
2d	A	H	H	H	CH_2CH_2–*c*-$N(CH_2)_4NH$	75	260–261	$C_{21}H_{21}N_5O_2$
2e	A	H	H	H	CH_2CH_2–*c*-$N(CH_2)_4O$	75	281–283	$C_{21}H_{20}N_4O_3$
2f	A	H	H	CH_3	$CH_2CH_2N(CH_3)_2$	70	188–190	$C_{20}H_{20}N_4O_2$
4a	A	H	$CH_2CH_2N(CH_3)_2$	H	$CH_2CH_2N(CH_3)_2$	54[c]	179–181	$C_{23}H_{27}N_5O_2$
4b	A	H	$CH_2CH_2N(CH_3)_2$	H	CH_2CH_2–*c*-$N(CH_2)_5$	52[d]	134–136	$C_{26}H_{31}N_5O_2$
4c	A	H	CH_2CH_2–*c*-$N(CH_2)_5$	H	CH_2CH_2–*c*-$N(CH_2)_5$	56[c]	181–182	$C_{29}H_{35}N_5O_2$
4d	A	H	$CH_2CH_2N(CH_3)_2$	H	H	27	225–227	$C_{19}H_{18}N_4O_2$
11	A	OH	$CH_2CH_2N(CH_3)_2$	H	$CH_2CH_2N(CH_3)_2$	69[c]	196–198	$C_{23}H_{27}N_5O_4$
14	A	OH	H	H	$CH_2CH_2N(CH_3)_2$	42	>300	$C_{19}H_{18}N_4O_4$
16a	B		$CH_2CH_2N(CH_3)_2$	H		54	230–232	$C_{19}H_{18}N_4O_2$
16b	B		$CH_2CH_2N(C_2H_5)_2$	H		60	233–235	$C_{21}H_{22}N_4O_2$
16c	B		$CH_2CH_2CH_2N(CH_3)_2$	H		50	231–233	$C_{20}H_{20}N_4O_2$
16d	B		CH_2CH_2–*c*-$N(CH_2)_5$	H		45	238–240	$C_{22}H_{22}N_4O_2$
16e	B		CH_2CH_2–*c*-$N(CH_2)_4$	H		48	243–245	$C_{21}H_{20}N_4O_2$
17a	B		$CH_2CH_2N(CH_3)_2$	$CH_2CH_2N(CH_3)_2$		35	134–135	$C_{23}H_{27}N_5O_2$
17b	B		$CH_2CH_2N(C_2H_5)_2$	$CH_2CH_2N(C_2H_5)_2$		43	>290	$C_{27}H_{37}Cl_2N_5O_2$
17c	B		$CH_2CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$		38	>290	$C_{25}H_{33}Cl_2N_5O_2$
17d	B		$CH_2CH_2N(CH_3)_2$	CH_2CH_2–*c*-$N(CH_2)_5$		25	136–138	$C_{26}H_{31}N_5O_2$
17e	B		CH_2CH_2–*c*-$N(CH_2)_4$	$CH_2CH_2N(CH_3)_2$		20	>290	$C_{25}H_{31}Cl_2N_5O_2$
21	B		CH_3	$CH_2CH_2N(CH_3)_2$		55	280–281	$C_{20}H_{20}N_4O_2$

[a]The yields are not optimized.
[b]The structures of compounds presented in Table 1 were confirmed by their spectral data (^{1}H NMR, IR, UV–vis, MS-FAB) and by elemental analyses (C, H, N).
[c]Method A.
[d]Method B.

Table 2. In vitro cytotoxic activity against L1210, K562 and K562/DX tumor cell lines, DNA binding and membrane affinity of target compounds **2a–f**, **4a–d**, **11**, **14**, **16a–e**, **17a–e**, **21**, and of reference compounds Mitoxantrone (MIT) and Doxorubicin (DX)

Compd	Cell line[a]/IC_{50} (nM)±SEM			RI[b]	K_{app}[c]$\times10^{-7}$ CT-DNA	log k'_{IAM}[d]
	L1210	K562	K562/DX			
2a	43.4±1.4	446.0±60.2	1217.6±208.3	2.73	0.43 (2.93)	1.649
2b	91.6±3.6	338.6±29.3	7604.9±1394.0	22.5	0.32 (3.89)	1.793
2c	532.3±31.1	1536.3±243.3	2009.0±306.5	1.31	0.55 (2.30)	2.226
2d	165.9±26.6	ND	ND			1.415
2e	1781.6±123.5	2101.6±825.3	4944.9±852.8	2.35		2.008
2f	191.1±11.0	792.5±125.2	2389.0±317.5	3.01		1.308
4a	2.1±0.1	14.0±1.4	31.6±8.2	2.26	2.28 (0.55)	1.754
4b	31.9±1.7	138.1±18.6	138.5±9.8	1.00	0.88 (1.49)	2.077
4c	75.1±1.2	286.9±11.2	605.9±33.8	2.11	1.12 (1.15)	2.622
4d	66.0±2.8	185.2±7.4	331.6±67.5	1.79	0.46 (2.76)	1.688
11	0.43±0.06	3.53±0.51	13.61±2.51	3.86	3.32 (0.39)	1.982
14	19.0±4.7	ND	ND			4.575
16a	30.3±3.5	133.8±16.2	263.0±34.7	1.96		1.733
16b	124.7±15.0	330.0±36.8	582.8±140.9	1.77		
16c	300.8±35.5	963.7±220.0	1750.5±65.8	1.82		1.521
16d	239.7±14.3	573.4±26.5	1125.5±55.3	1.96		
16e	38.5±2.1	148.4±6.8	379.2±86.0	2.56		
17a	137.8±9.2	591.3±48.4	716.4±39.1	1.21	4.58 (0.28)	2.235
17b	122.5±31.5	672.4±100.7	631.8±75.8	0.94		1.745
17c	254.4±47.3	996.7±114.6	2655.0±52.3	2.66		
17d	44.1±2.7	335.6±45.1	687.2±115.3	2.04		
17e	40.6±7.1	267.2±49.8	716.6±164.1	2.68		2.230
21	242.1±17.9	701.3±47.5	1417.6±226.6	2.02		
MIT	1.4±0.1	21.0±4.0	554.0±49.5	26.4	19 (0.053)[e]	1.841
DX	19.4±1.3	42.1±3.3	7031.5±542.0	167.0		1.434

ND, not determined.
[a]L1210, murine lymphocytic leukemia; K562, human myelogenous leukemia and Doxorubicin resistant (MDR type) subline K562/DX.
[b]RI, resistance index: the ratio of IC_{50} value for resistant cell line to IC_{50} value for sensitive cell line.
[c]$K_{app}=1.26/C_{50}\times10^7$, in which 1.26 is the concentration (μM) of ethidium–DNA complex, C_{50} is drug concentration (μM) to effect 50% drop in fluorescence of bound ethidium and 10^7 is the value of K_{app} assumed for ethidium in the complex. The C_{50} values are in parentheses.
[d]Logarithm of HPLC capacity factor determined in an immobilized artificial membrane column with acetonitrile buffer, pH 7.0.
[e]According to ref 13.

active than their 2,6-disubstituted analogues. However, anthrapyridazone derivatives with two side arms at positions 2 and 8 (**17a–e**) exhibit comparable or lower activity than their analogues with one side chain at position 2 (**16a–e**). A conclusion could be drawn that not only the presence of the second side chain but also its respective position in the chromophore of anthrapyridazones is important for cytotoxic activity. A similar structure–activity relationship was found in the anthrapyrazole and anthracenedione systems.[8,9] The alkylaminoalkylamino side chains at positions 2 and 6 are indispensable for the high cytotoxicity of anthrapyridazone derivatives. As expected,[6] for the activity was a optimal substitution of the ring with a 2-[(dimethylamino)ethyl]amino side arm regardless of its position. Derivatives with a piperidyl-, morpholinyl-, or piperazyl-terminal residue in the aminoethyl side arm are about tenfold less active than derivatives with a 2-[(dimethylamino)ethyl]amino side chain, independently, if they are situated at position 2 or 6. The cytotoxic activity of monosubstituted 2-(alkylamino)alkylanthrapyridazones is similar to that of 6-[(alkylamino)alkyl]amino-8-aminoderivatives.

Compound **4a** with two dimethylaminoethylamino side chains at positions 2 and 6 is the most active, while the 6-monosubstituted analogue, as well as the 2-substituted, 6-amino or 6-chloro ones exhibit tenfold less activity than **4a**.

The introduction of hydroxyl groups at positions 8 and 11 of compounds **2a** and **4a** increased the cytotoxic activity of these derivatives, similarly as was found for other anthracenedione and acridine derivatives. It should be noted that the compound **11** is more active against sensitive cell lines than Mitoxantrone.

The anthrapyridazones also exhibit significant cytotoxic activity against human leukemia sensitive cell line K562 and the multidrug resistant subline K562/DX. A good resistance index (RI) in the range 1–3 depending on the compound's structure was evidenced for the evaluated compounds regardless of their cytotoxic potency. A much higher RI was exhibited by the derivative **2b** with the hydroxyethylaminoethylamino side arm at position 6.

We assumed that the possible hydrogen bond between the carbonyl at position 7 and the hydrogen of the amino group at position 6 of **2a** could mimic an additional fifth ring, which may be important for MDR activity as has been shown for the pyrimidoacridines.[13] To examine this effect, we synthesized compound **2f** (the *N*-methyl derivative of **2a**) in which the formation of the hydrogen bond is not possible. However, **2f** is 2-fold less active than **2a** and has a similar resistance index.

The best effectiveness in overcoming the multidrug resistance was exhibited by derivatives bearing a

2-[(dimethylamino)ethyl]amino side chain, which correlates well with data obtained for the benzoperimidines, anthrapyridones, aza-anthrapyrazoles and pyrimidoacridines.

Among the synthesized anthrapyridazones, the most active against the MDR cell line is the derivative with a dimethylaminoethylamino arm at positions 2 and 6 (**4a**), as well as its 8,11-dihydroxy analogue **11**. Compounds **4a–c** with two basic side chains at positions 2 and 6 were much more active than compounds bearing only one chain at position 6. However, a second side chain at position 8 is rather unfavorable.

The above data demonstrate that both the presence of a condensed heterocyclic ring and the structure, number and position in the ring system of the side chains are of importance for the overcoming of multidrug resistance by anthrapyridazone derivatives.

DNA binding

Competitive displacement (C_{50}) in fluorometric assays with DNA-bound ethidium was applied to determine 'apparent' equilibrium constants (K_{app}) for drug binding, as the C_{50} value is approximately inversely proportional to the binding constant.[14] In the present work, fluorescence displacement assays were performed at pH 7 to enable comparison with biological conditions. The K_{app} values for the representative anthrapyridazones (Table 2) indicate that: (a) the disubstituted derivatives **4a**, **4c**, **11** and **17b** are stronger DNA-binding ligands than ethidium, with the exception of **4b**, (b) the presence of 8,11-hydroxy groups in the chromophore moiety markedly increased the K_{app} value (**11**), (c) the side chain at position 8 (**17a**) seems to be more important for binding than that in position 6 (**4a**), which does not correlate with the cytotoxicity data.

Membrane affinity

We have reported earlier that the ability of the anthracenedione analogues to overcome multidrug resistance is dependent on the velocity of their uptake, exceeding the ABC proteins mediated efflux. This is ensured by the presence in the molecule of a fused heterocyclic ring; however, various substituents quantitatively influence this effect.[6,15]

The essential factor governing the diffusion of a drug to the cells is its cytoplasmatic membrane affinity. The determinations have been performed on an HPLC-reverse-phase chromatographic IAM (column immobilized artificial membrane) constituting chromatographic surfaces prepared by covalently immobilized cell membrane phospholipids to solid surfaces at monolayer densities. IAM surfaces mimic the fluid cell membrane.[16,17] The retention time for a given compound, expressed as log k'_{IAM} values, comprises not only lipophilicity characteristics but also other interactions with membrane like hydrogen bond formation, electrostatic interactions, and so on. Therefore we prefer to interpret the log k'_{IAM} values as characterizing the membrane affinity rather then as commonly used lipophilicity characteristics.

Most studied anthrapyridazone derivatives maintain cytotoxic activity against human leukemia multidrug resistant subline K562/DX with P-gp efflux pump (MDR), and exhibit low RI values regardless of their cytotoxic potency. There is a qualitative correlation tendency between resistance indexes of examined compounds and their log k'_{IAM} values (Table 2). One should not expect a quantitative correlation, as the ability to overcome multidrug resistance is a rather complex phenomenon, influenced by the changes in the structure of the examined compounds. Besides the velocity of drug uptake, other factors like its affinity to the drug exporting protein (P-gp) and intracellular distribution characteristics also play a defined role. In the latter case, the nuclear DNA binding characteristics and the ability to be entrapped in other organelles, like lysosomes, influence the final response of the cell to the drug action.[18] However, the data obtained for anthrapyridazones point to the uptake characteristics, expressed by logk'_{IAM} values, as essential ones. The logk'_{IAM} values for anthrapyridazones with low RI are located in the range optimal for good uptake. Values near or below 1 would slow down the drug diffusion into cells, while values near 3 or more would cause the retention of the compounds in cytoplasmatic membrane. The obtained data are similar to the earlier ones found for the benzoperimidine derivatives.[19] The exception is compound **2b** with a very high RI. However, due to the presence of the hydroxyl group in the side chain, the compound is expected to acquire a specific conformation that might influence its properties. We have earlier evidenced that anthraquinone derivatives with this type of side chain form a very stable hydrogen bond network essentially influencing the conformation of the molecule.[20] Other authors also observed that aza-anthrapyrazole[5] derivatives as well as pyrimidoacridines[13] with the same type of side chains exhibit high resistant indexes.

Antileukemic activity

On the basis of results of the cytotoxicity studies, compounds **4a** and **11** were selected for preliminary evaluation of their antitumor activity. Compounds **4a** and **11** were examined with intraperitoneal (ip) administration on P388 murine leukemia. The data, in comparison with reference Mitoxantrone, are shown in Table 3.

Both compounds **4a** and **11** exhibit significant antileukemic efficacy (T/C% >200). The in vivo activity of **4a** with maximum T/C% $=240$ (at the dose 0.8 mg/kg) is comparable with that of Mitoxantrone (T/C% $=211$, at the same dose). It should be noticed that the antileukemic activity of **11**, which is the 8,11-dihydroxy analogue of **4a**, is lower than that for **4a** (T/C% $=200$ at 0.8 mg/kg and T/C% $=222$ at a 2-fold higher dose). These data are rather surprising because as noted above the introduction of hydroxyl groups into the chromophore of different antitumor compounds of anthracenedione and acridine groups increases their antitumor activity. On the other hand, **11** is less toxic than its dehydroxy

Table 3. Antitumor activity of selected compounds **4a** and **11** against P388 murine leukemia in comparison with Mitoxantrone

Compd	Total dose (mg/kg)[a]	% T/C[b]
4a	0.1	108
	0.2	165
	0.4	130
	0.8	240
	1.6	216
	2.4	tox
11	0.1	130
	0.2	162
	0.4	170
	0.8	200 (1/6 LTS)[c]
	1.6	219
	2.4	222
MIT	0.1	163
	0.2	165
	0.4	179
	0.8	211

[a]Single dose ip administration.
[b]The average value of two separate experiments, %T/C, the ratio of medium survival time of treated to control mice, expressed as a percentage. %T/C $\geq$125 are considered to be a significant activity. Long-term survivors were not included in the calculation.
[c]LTS, number of long-term survivors (cures)/total number of mice.

analogue **4a**.

In conclusion

Anthrapyridazones constitute a novel group of anthracenedione analogues able to overcome the multidrug resistance of tumor cells. The presence of respective heterocyclic ring, here the pyridazone one, fused with the anthracenedione chromophore determines cytotoxic activity toward the multidrug resistant cell lines. The positive effect of this structural factor in overcoming the multidrug resistance of tumor cells is quantitatively influenced, regarding the cytotoxic potency, by the type and location of substituents.

Experimental

Chemistry

Melting points, determined with a Boeticus PHMK05 apparatus, are uncorrected. Analyses are within $\pm$0.4% of the theoretical values and were carried out on a Carlo Erba CHNS-O-EA1108 instrument for C, H, N. A Beckman 3600 spectrophotometer was used for UV spectral determination. IR spectra were recorded on a UR 10 Zeiss spectrometer in KBr pellets; ^{1}H NMR spectra were taken on a Varian 300-MHz or 500-MHz spectrometer using tetramethylsilane as an internal standard. The following NMR abbreviations are 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 Kieselgel 60 plates (Merck), column chromatography on Kieselgel Merck (70–230 mesh) and on Sephadex LH-20 (Pharmacia).

Typical procedure for the preparation of hydrochloride salts of 2a–f, 4a–d, 11, 14, 16a–e, 17a–e, 21. To a stirred solution of the compound (as free base) in $CHCl_3$ or $CHCl_3$/MeOH a slightly molar excess of hydrogen chloride in absolute ethanol was added dropwise at 5 °C. The hydrochloride salt was precipitated by adding dry diethyl ether and collected by filtration. The solid was purified by column chromatography (Sephadex LH-20) eluting with MeOH and crystallized from MeOH/Et_2O.

General procedure for the synthesis of compounds 2a–f

6-[[2-(Dimethylamino)ethyl]amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (2a). A sample of 400 mg (1.4 mmol) of 6-chloro-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (**1**)[12] with 2 mL of 2-(dimethylamino)ethylamine in pyridine was stirred at 100 °C under a nitrogen atmosphere for 7 h. The progress of the reaction was followed by TLC in the solvent system $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$, washed several times with dilute HCl to remove an excess of amines and then washed with water. The organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column flash chromatography (silica gel) eluting with $CHCl_3$/MeOH mixture (10:1, 5:1). The chromatographic fractions were concentrated to afford **2a** (300 mg, 85%) as a yellow powder. Mp 274–276 °C. ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 6H, 2$\times$$CH_3$), 2.64 (t, 2H, J = 5.9 Hz), 3.6 (q, 2H, J = 5.9 Hz), 7.2 (d, 1H, J = 9.3 Hz), 7.58–7.76 (m, 2H), 8.4–8.58 (m, 3H), 10.8 (t, 1H, NH), 13.2 (br s, 1H, NH, ex). Found: C, 67.87; H, 5.56; N, 16.42; calcd for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76.

6-Chloro-2-[2-(dimethylamino)ethyl]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (3). A sample of 500 mg (1.75 mmol) of 4-chloro-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylic acid (**5**)[11] and 500 mg of PCl_5 was suspended in 6 mL benzene at room temperature to obtain an intimate mixture. Additionally, 16 mL of benzene was added and then dropwise 500 mg (4.85 mmol) of 2-(*N*,*N*-dimethylamino)ethylhydrazine during 10 min. After stirring for 1 h, the resulting suspension was diluted with $CHCl_3$. The mixture was extracted with a 5% water solution of Na_2CO_3 (2$\times$50 mL) and with water (2$\times$50 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was chromatographed on silica gel column (eluent $CHCl_3$/MeOH from 20:1 to 5:1) to yield **3** (312 mg, 51%) as a yellow solid. Mp 163–164 °C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H, 2$\times$$CH_3$), 2.9 (t, 2H, J = 6.6 Hz), 4.5 (t, 2H, J = 6.6 Hz), 7.58–7.78 (m, 2H), 7.9 (d, 1H, J = 8.5 Hz), 8.34 (dd, 1H, J = 1.4 Hz, J = 7.8 Hz), 8.46 (dd, 1H, J = 1.3 Hz, J = 9.0 Hz).

General procedure for the synthesis of compounds 4a–c (Method A)

2-[2-(Dimethylamino)ethyl]-6-[[2-(dimethylamino)ethyl]amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (4a). A mixture of 50 mg (0.14 mmol) of **3** and 1 mL of *N*,*N*-dimethylethylenediamine in 2 mL of DMA was stirred for 30 min at 60 °C. The reaction mixture was

partitioned between $CHCl_3$ with Et_2O and water. The organic layer was worked up to give a residue which was flash chromatographed on silica gel column eluted with $CHCl_3$/MeOH (from 20:1 to 5:1) to afford 31.5 mg (54%) of pure **4a**. Mp 179–181 °C. ^{1}H NMR (as dihydrochloride, DMSO-d_6) δ 2.84 (s, 6H, 2×CH_3), 2.89 (s, 6H, 2×CH_3), 3.39 (t, 2H, J = 6.3 Hz), 3.65 (t, 2H, J = 5.7 Hz), 4.0 (q, 2H, J = 6.3), 4.68 (t, 2H, J = 5.6), 7.68 (d, 1H, J = 9.8 Hz), 7.76 (t, 1H, J = 7.6 Hz), 7.87 (t, 1H, J = 7.6 Hz), 8.31–8.53 (m, 2H), 10.06 (br s, 1H, NH^+), 10.62 (br s, 1H, NH^+), 10.78 (t, 1H, NH). Found: C, 68.10; H, 6.80; N, 17.01; calcd for $C_{23}H_{27}N_5O_2$: C, 68.13; H, 6.71; N, 17.27.

Method B

2-[2-(Dimethylamino)ethyl]-6-[[2-(piperidin-1-yl)ethyl]-amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (4b). A sample of 185 mg (0.5 mmol) of **2c**, 50 mg (0.5 mmol) of Na_2CO_3 and 0.15 mL (1.5 mmol) of 2-(dimethylamino)ethyl chloride in 5 mL of DMA was stirred for 1 h at 80 °C. The course of reaction was monitored by TLC in the solvent system $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$, washed twice with water and the organic layer dried over Na_2SO_4. After evaporation under reduced pressure, the residue was chromatographed on silica gel column eluting with $CHCl_3$/MeOH (10:1) to afford **4b**. Yield 115 mg (52%). Mp 134–136 °C. ^{1}H NMR ($CDCl_3$) δ 1.6–1.7 (m, 6H), 2.4 (s, 6H, 2×CH_3), 2.56 (t, 4H, J = 4.8 Hz), 2.8 (t, 2H, J = 6.6 Hz), 2.87 (t, 2H, J = 6.6 Hz), 3.64 (q, 2H, J = 4.8 Hz), 4.48 (t, 2H, J = 6.6 Hz), 7.24 (d, 1H, J = 10.3 Hz), 7.58–7.76 (m, 2H), 8.38–8.48 (m, 2H), 8.56 (d, 1H, J = 7.7 Hz), 11.0 (t, 1H, NH). Found: C, 70.10; H, 7.28; N, 15.54; calcd for $C_{26}H_{31}N_5O_2$: C, 70.09; H, 7.01; N, 15.72.

6-Amino-2-[2-(dimethylamino)ethyl]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (4d). Step (i). A suspension of 70 mg (0.2 mmol) of **3** and aminoacetaldehyde dimethylacetal (0.5 mL) in 2 mL of DMA was heated at 110 °C with stirring, until all starting material was consumed. After cooling, the reaction mixture was diluted with $CHCl_3$ and extracted with water. The organic layer was dried over Na_2SO_4. Removal of the solvent in vacuo gave a residue, which was chromatographed eluting with $CHCl_3$. **Step (ii)**. The aminoacetal, obtained by the above procedure, was treated with 10% HCl. The reaction mixture was stirred at 30–40 °C for 30 min and then the suspension was poured into ice water and neutralized with $NaHCO_3$. The precipitate was collected by filtration, washed with THF, then with water and dried in vacuo. The solid was purified by crystallization from DMA to give **4d** (18 mg, 27%). Mp 225–227 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H, 2×CH_3), 2.9 (t, 2H, J = 6.6 Hz), 4.5 (t, 2H, J = 6.6 Hz), 7.0 (d, 1H, J = 9.0 Hz), 7.58–7.78 (m, 2H), 8.24–8.32 (m, 2H), 8.5 (d, 1H, J = 8.0 Hz), 9.8 (br s, 2H, NH_2). MS m/z (relative intensity, %): 334 ($[M]^+$, 100); 335 ($[M+1]^+$, 41). Found: C, 67.95; H, 5.65; N, 16.48; calcd for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76.

5,8-Di(benzyloxy)-4-chloro-9,10-dioxo-9,10-dihydro-1-anthracenecarbonitrile (7). A sample of 500 mg (1.02 mmol) of 1,4-di(benzyloxy)-5,8-dichloro-9,10-dihydro-9,10-anthracenedione (**6**)[9] and 108 mg (1.22 mmol) CuCN in 5 mL of DMA was stirred and heated at 125 °C for 4 h. The course of the reaction was followed by TLC in the solvent system toluene/acetone (30:1). The reaction mixture was allowed to cool to room temperature and poured into cold water. The brown solid was collected by filtration and washed well with water. The material was suspended in 3 N HNO_3 and stirred at 70° C for 4 h. The resulting suspension was filtered, and the precipitate was washed several times with cold water. The deep-orange product was air dried overnight and then flash chromatographed eluting with benzene and benzene/acetone (50:1). The chromatographic fractions containing the product were concentrated giving 300 mg (61%) of product (**7**) as an orange powder. Mp 199–201 °C. ^{1}H NMR ($CDCl_3$) δ 5.34 (s, 2H), 5.35 (s, 2H), 7.36–7.60 (m, 12H), 7.95 (d, 1H, J = 8.2 Hz), 8.02 (d, 1H, J = 8.2 Hz).

5,8-Di(benzyloxy)-4-chloro-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylic acid (8). To a stirred solution of 300 mg (0.62 mmol) of **7** in 8 mL MeOH and 2 mL of DMA was added dropwise 1 mL of 20% NaOH. The mixture was refluxed for 4 h and then allowed to cool to room temperature. To the reaction mixture dilute HCl was added giving an orange suspension. The solid was collected by filtration, washed with cold water, dried and then purified by column chromatography using as eluent $CHCl_3$/MeOH (20:1 to 5:1) to afford 258 mg (82.7%) of pure acid **8** as a yellow powder. Mp 212–214 °C. ^{1}H NMR (DMSO-d_6) δ 5.34 (s, 2H), 5.36 (s, 2H), 7.4–7.6 (m, 12H), 8.0 (d, 1H, J = 8.5 Hz), 8.4 (d, 1H, J = 8.5 Hz), 13.3 (s, 1H, ex).

8,11-Di(benzyloxy)-6-chloro-2-[2-(dimethylamino)ethyl]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (9). A sample of 200 mg (0.4 mmol) of 5,8-di(benzyloxy)-4-chloro-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylic acid and 200 mg of PCl_5 was stirred in 5 mL of benzene at room temperature to obtain an intimate mixture. Additional 5 mL of benzene was added and then dropwise 500 mg (4.85 mmol) of 2-(*N*,*N*-dimethylamino)ethylhydrazine during 5 min. The resulting suspension was kept in these conditions for additional 1 h. After this the mixture was partitioned between $CHCl_3$ and aqueous 5% Na_2CO_3. The organic layer was worked up to give a residue, which was flash chromatographed (eluent $CHCl_3$/MeOH 20:1) to yield pure **9** (114 mg, 50%). Mp 157–160 °C. ^{1}H NMR ($CDCl_3$) δ 2.19 (s, 6H, 2×CH_3), 2.61 (t, 2H, J = 6.6 Hz), 4.31 (t, 2H, J = 6.6 Hz), 5.2 (s, 2H), 5.32 (s, 2H), 7.1 (d, 1H, J = 9.2 Hz), 7.29–7.61 (m, 11H), 7.87 (d, 1H, J = 8.6 Hz), 8.52 (d, 1H, J = 8.6 Hz).

8,11-Di(benzyloxy)-2-[2-(dimethylamino)ethyl]-6-[[2-(dimethylamino)ethyl]amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]-cinnoline-3,7-dione (10). A sample of **9** (100 mg, 0.17 mmol) with 2 mL of 2-(dimethylamino)ethylamine in 2 mL of DMA was stirred at 60 °C for 1 h. The progress of the reaction was followed by TLC in the solvent system $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$ and Et_2O and then washed several times with water. The organic layer was dried over

Na_2SO_4, evaporated in vacuo and the residue was purified by column flash chromatography (silica gel) eluting with $CHCl_3$/MeOH (10:1, then 5:1). The chromatographic fractions were concentrated to afford **10** (300 mg, 84.6%) as a yellow powder. Mp 186—188 °C. ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6H, 2×CH_3), 2.41 (s, 6H, 2×CH_3), 2.66 (t, 2H, J = 6.7 Hz), 2.74 (t, 2H, J = 6.7 Hz), 3.56 (q, 2H, J = 5.1 Hz), 4.36 (t, 2H, J = 6.7 Hz), 5.23 (s, 2H), 5.28 (s, 2H), 7.1 (d, 1H, J = 9.2 Hz), 7.2 (d, 1H, J = 9.2 Hz), 7.27–7.64 (m, 10H), 7.68 (d, 1H, J = 6.7 Hz), 8.44 (d, 1H, J = 9.3 Hz).

2-[2-(Dimethylamino)ethyl]-6-[[2-(dimethylamino)ethyl]amino]-8,11-dihydroxy-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (11). A sample of **10** (100 mg, 0.16 mmol) was left in 1 mL of trifluoroacetic acid at room temperature for 18 h. The TFA was removed under reduced pressure by coevaporation with benzene. The residue was suspended in $CHCl_3$ and the solution was carefully washed with $NaHCO_3$ water solution and then with water. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and the product was solidified under Et_2O to yield **11** (48.8 mg, 69%) as red powder. Mp 196–198 °C. IR (KBr, major peaks cm^{-1}): 1209, 1468, 1570, 1631, 1656. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H, 2×CH_3), 2.41 (s, 6H, 2×CH_3), 2.75 (t, 2H, J = 6.2 Hz), 2.86 (t, 2H, J = 6.3 Hz), 3.54 (q, 2H, J = 6.2 Hz), 4.48 (t, 2H, J = 6.3 Hz), 7.1 (d, 1H, J = 9.0 Hz), 7.19–7.41 (m, 2H), 8.43 (d, 1H, J = 9.3 Hz), 10.57 (s, 1H, NH), 11.48 (s, 1H, ex), 13.21 (s, 1H, ex). Found: C, 63.01; H, 6.50; N, 15.84; calcd for $C_{23}H_{27}N_5O_4$: C, 63.14; H, 6.22; N, 16.01.

8,11-Di(benzyloxy)-6-chloro-2,7-dihydro-3*H*-dibenzo[*de*,*h*]-cinnoline-3,7-dione (12). A sample of 200 mg (0.4 mmol) of 5,8-di(benzyloxy)-4-chloro-9,10-dioxo-9,10-dihydro-1-anthracene-carboxylic acid (**8**) and 200 mg of PCl_5 was stirred in 2 mL of benzene at room temperature to obtain an intimate mixture. Then, the mixture was diluted with 5 mL of benzene and 0.5 mL of hydrazine hydrate 98% was added dropwise during 5 min. The resulting suspension was kept in these conditions for additional 1 h. The isolation and purification procedures of the reaction mixture to obtain the product **12** were the same as described for compound **9**. Yield 176 mg (89%). Mp 277–280° C. ^{1}H NMR (DMSO-d_6) δ 5.24 (s, 2H), 5.27 (s, 2H), 7.3–7.6 (m, 12H), 8.0 (d, 1H, J = 8.5 Hz), 8.4 (d, 1H, J = 8.5 Hz), 13.5 (s, 1H, NH, ex).

8,11-Di(benzyloxy)-6-[[2-(dimethylamino)ethyl]amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (13). To a stirred suspension of **12** (100 mg, 0.2 mmol) in 2 mL of DMA, 1 mL of *N*,*N*-dimethylethylenediamine was added. The mixture was stirred for 1 h at 60 °C, diluted with $CHCl_3$ and Et_2O and extracted with water. The organic layer was dried over Na_2SO_4, and concentrated to dryness. The residue was purified by flash chromatography (silica gel) eluting with $CHCl_3$/MeOH (10:1). The fractions containing the product were pooled and concentrated to dryness. The product **13** (65 mg, 59%) was obtained as a yellow powder. Mp 224–226° C. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6H, 2×CH_3), 2.75 (t, 2H, J = 6.5 Hz), 3.56 (q, 2H, J = 6.5 Hz), 5.21 (s, 2H), 5.29 (s, 2H), 7.2 (d, 1H, J = 9.2 Hz), 7.27–7.70 (m, 12H), 8.42 (d, 1H, J = 9.2 Hz), 10.42 (s, 1H, NH ex), 11.14 (s, 1H, NH).

6-[[(Dimethylamino)ethyl]amino]-8,11-dihydroxy-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (14). A solution of 50 mg (0.1 mmol) of **13** in 1 mL of trifluoroacetic acid was left at room temperature for 12 h. The excess of TFA was removed under reduced pressure by coevaporation with benzene to afford a salt of 6-[2-(dimethylamino)ethyl]amino-8,11-dihydroxy-2,7-dihydro-3*H*-dibenzo-[*de*,*h*]cinnoline-3,7-dione. The residue was dissolved in aqueous 5% Na_2CO_3 and extracted several times with chloroform. The organic layer was washed with water, then collected and dried over Na_2SO_4. The solvent was evaporated and the crude product was purified using silica gel column chromatography (eluent $CHCl_3$/MeOH 10:1, 5:1) to give **14** as a dark red solid (14.2 mg, 42%). Mp > 300 °C. ^{1}H NMR (as hydrochloride, DMSO-d_6) δ 2.85 (s, 6H, 2×CH_3), 3.68 (t, 2H, J = 7.1 Hz), 4.06 (q, 2H, J = 7.1 Hz), 7.17–7.32 (m, 2H), 7.61 (d, 1H, J = 9.5 Hz), 8.30 (d, 1H, J = 9.5 Hz), 9.85 (br s, 1H, NH), 10.40 (br s, 1H, NH ex), 11.49 (s, 1H, NH^+), 13.25 (s, 1H, ex), 13.59 (s, 1H, ex). MS m/z (relative intensity, %): 365 ($[M-1]^+$, 100); 366 ($[M]^+$, 50). Found: C, 62.03; H, 5.10; N, 15.04; calcd for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29.

General procedure for the synthesis of compounds 16a–e

2-[2-(Dimethylamino)ethyl]-8-amino-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (16a). A sample of 130 mg (0.5 mmol) of **15**,[12] 50 mg (0.5 mmol) of Na_2CO_3 and 0.15 mL (1.5 mmol) of 2-(dimethylamino)ethyl chloride in 5 mL of DMA was stirred for 1 h at 60–70 °C. The course of reaction was monitored by TLC in the solvent system $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$, washed twice with water and the organic layer dried over Na_2SO_4. After evaporation under reduced pressure, the residue was chromatographed on silica gel column eluting with $CHCl_3$/MeOH (10:1) to afford **16a** as a red powder. Yield 110 mg (54%). Mp. 230–232 °C. IR (KBr, major peaks cm^{-1}): 1395, 1590, 1630, and 1650. ^{1}H NMR (as hydrochloride, DMSO-d_6) δ 2.8 (s, 6H, 2×CH_3); 3.62 (t, 2H, J = 5.5 Hz); 4.64 (t, 2H, J = 5.5 Hz); 6.99 (d, 1H, J = 7.4 Hz); 7.46 (t, 1H, J = 7.7 Hz); 7.59 (d, 1H, J = 7.1 Hz); 8.11 (t, 1H, J = 7.7 Hz); 8.48 (t, 1H, J = 6.9 Hz); 8.65 (d, 1H, J = 7.4 Hz); 10.22 (br s, 1H, NH ex). MS m/z (relative intensity, %): 333 ($[M-1]^+$, 100); 334 ($[M]^+$, 70). Found: C, 67.89; H, 5.70; N, 16.54; calcd for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76.

General procedure for the synthesis of compounds 17a–c

2-[2-(Dimethylamino)ethyl]-8-[[2-(dimethylamino)ethyl]-amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (17a). A suspension of NaH (60% in mineral oil, 30 mg) in 3 mL DMA and 100 mg (0.4 mmol) of **15** was stirred at room temperature for 15 min. After that 0.15 mL (1.5 mmol) of 2-(dimethylamino)ethyl chloride in 1 mL of benzene was added dropwise and the reaction mixture heated for 1.5 h at 70 °C. The mixture was cooled, diluted with $CHCl_3$, washed several times with

water, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified on column chromatography (deactivated silica gel) using the solvent system $CHCl_3$/MeOH/25% NH_4OH (5:1:0.1). The purple product was purified as described for **2a–f**. Yield 65 mg (35%). Mp 134–135 °C dec. IR (KBr, major peaks cm^{-1}): 1390, 1480, 1590, 1620, 1650. ^1H NMR ($CDCl_3$) δ 2.35 (s, 6H, 2×CH_3); 2.45 (s, 6H, 2×CH_3); 2.75 (t, 2H, J = 3.2 Hz); 2.95 (t, 2H, J = 6.6 Hz); 3.45 (q, 2H, J = 3.1 Hz); 4.5 (t, 2H, J = 6.6 Hz); 6.83 (d, 1H, J = 7.5 Hz); 7.54 (t, 1H, J = 7.6 Hz); 7.75 (d, 1H, J = 7.1 Hz); 7.94 (t, 1H, J = 7.8 Hz); 8.53 (d, 1H, J = 6.8 Hz); 8.62 (d, 1H, J = 7.4 Hz); 9.95 (t, 1H, NH d ex). MS m/z (relative intensity, %): 403 ($[M-2]^+$, 100); 405 ($[M]^+$, 30); 406 ($[M+1]^+$, 70). Found: C, 67.89; H, 6.98; N, 17.10; calcd for $C_{23}H_{27}N_5O_2$: C, 68.13; H, 6.71; N, 17.27.

General procedure for the synthesis of compounds 17d–e

2-[2-(Dimethylamino)ethyl]-8-[[2-(piperidin-1-yl)ethyl]-amino]-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (17d). A suspension of NaH (60% in mineral oil, 30 mg) in 2.5 mL DMA and 130 mg (0.4 mmol) of **16a** was stirred at room temperature for 15 min. After that 0.2 mL (1.5 mmol) of 1-(2-chloroethyl)piperidine in 1 mL of benzene was added dropwise and the reaction mixture heated for 1 h at 70 °C. The product was isolated and purified similarly as described for **17a**. Yield 38 mg (25%). Mp 136–138 °C. ^1H NMR (as dihydrochloride, DMSO-d_6) δ 1.4–1.8 (m, 6H, 2×CH_3); 2.8 (s, 6H, 2×CH_3); 2.85 (t, 4H, J = 7.3 Hz); 3.33 (t, 2H, J = 6.5 Hz); 3.58 (t, 2H, J = 6.4 Hz); 4.05 (q, 2H, J = 6.4 Hz); 4.65 (t, 2H, J = 6.4 Hz); 6.97 (d, 1H, J = 7.35 Hz); 7.45 (t, 1H, J = 7.6 Hz); 7.6 (d, 1H, J = 7.0 Hz); 8.10 (t, 1H, J = 7.7 Hz); 8.46 (t, 1H, J = 6.9 Hz); 8.50 (d, 1H, J = 7.4 Hz); 9.8 (t, 1H, NH d ex). Found: C, 69.83; H, 6.74; N, 15.45; calcd for $C_{26}H_{31}N_5O_2$: C, 70.09; H, 7.01; N, 15.72.

Ethyl 5-amino-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylate (19). 5-Amino-9,10-dioxo-9,10-dihydro-1-anthracenecarboxylic acid (**18**) was refluxed for 10 h in absolute ethanol saturated with hydrogen chloride. The crude product was crystallized from $CHCl_3/Et_2O$ to afford **19** as a purple solid. Mp 158–160 °C. ^1H NMR ($CDCl_3$) δ 1.4 (t, 3H, J = 7.12 Hz); 4.5 (q, 2H, J = 7.13 Hz); 6.0–6.2 (br s, ex); 7.0 (d, 1H, J = 8.4 Hz); 7.46 (t, 1H, J = 6.5 Hz); 7.6–7.72 (m, 2H); 7.8 (t, 1H, J = 7.6 Hz); 8.38 (dd, 1H, J = 7.6 Hz, J = 1.4 Hz).

8-Amino-2-methyl-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (20). A suspension of 300 mg (1 mmol) of **19** and 0.53 mL (10 mmol) of methylhydrazine was stirred at room temperature for 18 h. The progress of the reaction was monitored by TLC in the solvent system $CHCl_3$/MeOH (50:1). To the reaction mixture some amount of water was added and the collected precipitate was washed with dilute HCl and water. The solid was purified by crystallization from DMF to give **20** (160 mg, 52%). Mp 287–289 °C. ^1H NMR (DMSO-d_6) δ 3.8 (s, 3H, 1×CH_3); 6.85 (t, 1H, J = 4.1 Hz); 7.4 (m, 1H); 7.5 (t, 1H, J = 4.2 Hz); 8.0 (t, 1H, J = 4.0 Hz); 8.38–8.58 (m, 2H), 13.2 (s, 2H, NH_2 ex).

8-[[2-(Dimethylamino)ethyl]amino]-2-methyl-2,7-dihydro-3*H*-dibenzo[*de*,*h*]cinnoline-3,7-dione (21). A suspension of 110 mg (0.4 mmol) of **20** and NaH (60% in mineral oil, 30 mg) in 3 mL DMA was stirred at 60 °C for 5 min. Then 2-(dimethylamino)ethyl chloride in benzene solution (four times molar excess) was dropped in and the reaction mixture was stirred at 60° C for 1 h. After cooling the reaction mixture was diluted with $CHCl_3$ and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under diminished pressure. The residue was chromatographed on a silica gel column (eluent $CHCl_3$/MeOH 5:1) to afford **21** as a red powder (yield 55%). Mp 280–281 °C. ^1H NMR ($CDCl_3$) δ 2.4 (s, 6H, 2×CH_3); 2.7 (t, 2H, J = 3.2 Hz); 3.4 (q, 2H, J = 3.0 Hz); 4.0 (s, 3H); 6.8 (d, 1H, J = 7.4 Hz); 7.5 (t, 1H, J = 7.7 Hz); 7.7 (d, 1H, J = 7.4 Hz); 7.9 (t, 1H, J = 7.8 Hz); 8.5 (d, 1H, J = 6.7 Hz); 8.6 (d, 1H, J = 7.4 Hz); 9.9 (t, 1H, NH d ex). Found: C, 68.57; H, 5.89; N, 15.83; calcd for $C_{20}H_{20}N_4O_2$: C, 68.95; H, 5.79; N, 16.08.

Membrane affinity measurements

HPLC column containing as stationary phase 1-myristoyl-2-[13-carbonylimidazolide-tridecanoyl]sn-3-glycerophospholine(lecithin-imidazolide) bonded to silica-propylamine with the unreacted propylamine moieties end-capped with C10 and C3 alkyl chains (IAM.PC.DD2) was purchased from Regis Technologies Inc. (Morton Grove, IL, USA). The IAM.PC.DD2 column was 3 cm×4.6 mm; particle diameter 12 μm; pore diameter 300 Å. For all studies the injection volume was ca. 10 μL of a solute aqueous solution. Acetonitrile/0.1 M Sörensen buffer (K_2HPO_4/KH_2PO_4) pH 7.2 eluent was used in proportions 50:50, 40:60, 35:65, 30:70 and 25:75% (v/v). The flow rate was 1 mL/min and solute detection was at 495 nm. The chromatographic system consisted of a Model L-6200 A pump, Model L-4250 UV–vis detector and a Model D-2500 chromatointegrator (all from Merck-Hitachi, Vienna, Austria). Capacity factors, k'_{IAM}, were calculated assuming that the dead volume of the column was the signal given by 50 μg/mL citric acid solution. A standard, commercially available statistical package for regression analysis was employed on a personal computer.

Fluorescence binding studies

The C_{50} values for ethidium displacement from CT-DNA were determined using aqueous buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.0) containing 1.26 μM ethidium bromide and 1 μM CT-DNA respectively.

All measurements were made in 10-mm quartz cuvettes at 20 °C using a Perkin–Elmer LS5 instrument (excitation at 546 nm; emission at 595 nm) following a serial addition of aliquots of a stock drug solution (~1 mM in H_2O). The C_{50} values are defined as the drug concentrations that reduce the fluorescence of the DNA-bound ethidium by 50% and are reported as the mean from three determinations. Apparent equilibrium binding constants were calculated from the C_{50} values (in

μM) using: $K_{app} = (1.26/C_{50}) \times K_{ethidium}$, with a value of $K_{ethidium} = 10^7\ M^{-1}$ for ethidium bromide.[13,14]

Biological tests

Cell lines. Murine L1210 lymphocytic leukemia cells were grown in RPMI 1640 medium supplemented with 5% FBS (fetal bovine serum), penicillin G (100,000 units/L), and streptomycin (100 mg/L). Human myelogenous leukemia sensitive cell line K562 and Doxorubicin resistant subline K562/DX (ICIG, Villejuif, France) were grown in RPMI 1640 medium supplemented with 10% FBS, penicillin G (100,000 units/L), streptomycin (100 mg/L), and 2 mM L-glutamine. K562/DX cells were exposed to Doxorubicin (500 nM) for 1 week each month. Cells were not exposed to the drug during the week prior to experimental protocol. 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 the final required density.

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 times were 48 h for L1210 cells and 72 h for other 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 Zb_I Coulter Counter (Coulter Electronics, Ltd., UK). Results are given as the mean of at least three independent experiments ± standard error of the mean (SEM). The resistance index was defined as the ratio of IC_{50} value for resistant cell line to IC_{50} value for sensitive cell line.

Antitumor activity evaluation. Murine leukemia P388 was maintained by ip passages of tumor cells in DBA/2 mice. Studies were conducted according to standard protocols of the US National Cancer Institute.[21] For experimental purposes, 10^6 cells were injected ip to CD2F1 (Balb/c×DBA/2) mice on day 0. Twenty-four h after tumor implantation, solutions of compounds in physiological saline were administrated ip in a single dose. The treated group consisted of six and control group of 14 animals. The medium survival time (MST) of the treated (T) and control (C) groups was determined, and the percent of T/C was calculated by using the following formula: %T/C = [(MST treated)/(MST control)]×100.

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References and Notes

1. Bradley, G.; Juranca, P. F.; Ling, V. *Biochim. Biophys. Acta* **1988**, *948*, 87.
2. Dicato, M.; Duhem, C.; Pauly, M.; Ries, F. *Cytokines Cell. Mol. Ther*. **1997**, *3*, 91.
3. Selassie, C. D.; Hansch, C.; Khwaja, T. *J. Med. Chem.* **1990**, *33*, 1914.
4. Showalter, H. D. W.; Weber, L. W.; Leopold, W. R.; Fry, D. W.; Klohs, W. D.; Jackson, R. C.; Design, Tumor, Biology and Biochemical Pharmacology of Anthrapyrazoles. In *Anthracycline and Anthracenodione-based Anticancer Agents*; Lown, J. W., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1988; p 201.
5. Krapcho, A. P.; Menta, E.; Oliva, A.; Di Domenico, R.; Fiocchi, L.; Maresch, M. E.; Gallagher, C. E.; Hacker, M. P.; Beggiolin, G.; Giuliani, F. C.; Pezzoni, G.; Spinelli, S. *J. Med. Chem*. **1998**, *41*, 5429.
6. Stefańska, B.; Dzieduszycka, M.; Bontemps-Gracz, M. M.; Borowski, E.; Martelli, S.; Supino, R.; Pratesi, G.; De Cesare, MA.; Zunino, F.; Kuśnierczyk, H.; Radzikowski, C. *J. Med. Chem*. **1999**, *42*, 3494.
7. (a) Tarasiuk, J.; Stefańska, B.; Płodzich, I.; Tkaczyk-Gobis, K.; Bontemps-Gracz, M. M.; Garnier-Suillerot, A.; Borowski E. *Abstract Book*, 7th International Symposium on Molecular Aspects of Chemotherapy; Sept. 8–11, 1999, Gdańsk, Poland; Abstract 20. (b) Bontemps-Gracz, M. M.; Stefańska, B.; Dzieduszycka, M.; Borowski, E.; Antonini, I.; Martelli, S.; Supino, R.; Zunino, F. *Abstract Book*, Multidyscyplinarna Konferencja Nauki o Leku (Multidisciplinary Conference of Medicinal Science), March 3–5, 1999, Muszyna, Poland; Abstract P-80. (c) Tarasiuk, J.; Stefańska, B.; Płodzich, I.; Tkaczyk-Gdois, K.; Seksek, O.; Martelli, S.; Garnier-Suillerot, A.; Borowski, E. *Brit J. Pharmacol*. **2002**, *135*, 1513.
8. Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024.
9. Showalter, H. D. H.; Johnson, J. L.; Hoftiezer, J. M.; Turner, W. R.; Werbel, L. M.; Leopold, W. R.; Shillis, J. L.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1987**, *30*, 121.
10. (a) Chołody, W. M.; Martelli, S.; Konopa, J. *J. Med. Chem*. **1992**, *30*, 493. (b) Kato, N.; Sugaya, T.; Mimura, T.; Ikuta, M.; Kato, S.; Kuge, Y.; Tomioka, S.; Kasai, M. *Synthesis* **1997**, 625.
11. (a) Ullmann, F.; van der Schalk, W. *Liebigs Ann*. **1912**, *388*, 199. (b) Ullmann, F.; Minaew, W. *Liebigs Ann*. **1912**, *388*, 217.
12. Dokunikhin, N. S.; Fain, V. A. I. *Zh. Obshch. Khim. RU* **1964**, *34*, 2372.
13. Antonini, I.; Polucci, P.; Kelland, L. R.; Menta, E.; Pescalli, N.; Martelli, S. *J. Med. Chem*. **1999**, *42*, 2535.
14. Morgan, A. R.; Lee, J. S.; Pulleyblank, D. E.; Murray, N. L.; Evans, D. H. *Nucleic Acids Res*. **1979**, *7*, 547.
15. Tkaczyk-Gobis, K.; Tarasiuk, J.; Stefańska, B.; Haber, P.; Garnier-Suillerot, A.; Borowski, E. *Abstract Book*, 6th International Symposium on Molecular Aspects of Chemotherapy; July 9–12, 1997, Gdańsk, Poland; Abstract 28.
16. Kaliszan, R.; Nasal, A.; Buciński, A. *Eur. J. Med. Chem.* **1994**, *29*, 163.
17. Ong, S.; Liu, H.; Pidgeon, Ch. *J. Chromatogr. A* **1996**, *728*, 113.

18. Tkaczyk-Gobis, K.; Tarasiuk, J.; Seksek, O.; Stefańska, B.; Borowski, E.; Garnier-Suillerot, A. *Eur. J. Pharmacol.* **2001**, *413*, 131.
19. Dzieduszycka, M.; Martelli, S.; Arciemiuk, M.; Bontemps-Gracz, M. M.; Kupiec, A.; Borowski, E. *Bioorg. Med. Chem.* **2002**, *10*, 1025.
20. Mazerski, J.; Martelli, S.; Borowski, E. *Polish J. Chem.* **1997**, *71*, 338.
21. (a) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. *J. Cancer Chemother. Rep.* **1972**, *3*, 1. (b) In Vivo Cancer Models; NIH Publication No. 84–2635, 1984; p 27.