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Effect of Modification of 6-[(Aminoalkyl)amino]-7*H*-benzo[*e*]-perimidin-7-ones on Their Cytotoxic Activity Toward Sensitive and Multidrug Resistant Tumor Cell Lines. Synthesis and Biological Evaluation

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Abstract—Benzoperimidines, a novel group of antitumor anthracenedione analogues, are of interest due to their ability to overcome multidrug resistance of tumor cells (Stefańska, B., Dzieduszycka, M., Bontemps-Gracz, M. M., Borowski, E., Martelli, S., Supino, R., Pratesi, G., De Cesare, MA., Zunino, F., Kuśnierczyk, H., Radzikowski, Cz. *J. Med. Chem.* **1999**, 42, 3494). Although the structural factor essential for exhibiting this desirable property is the presence in the molecule of a fused heterocyclic ring, the cytotoxicity against resistant cells is highly influenced by the nature and location of the substituents. A series of novel synthetic derivatives, comprising monohydroxylated benzoperimidines and 2-aminobenzoperimidines, allowed the establishment of an in vitro structure–activity relationship for a panel of leukemia sensitive, as well as P-gp dependent multidrug resistance (MDR) and multidrug resistance associated protein dependent resistance (MRP) resistant cell lines. The membrane affinity for the compounds has also been determined.

Introduction

The prolonged treatment of cancer patients by chemotherapeutic antitumor agents leads to the development of multidrug resistance toward numerous antitumor drugs.[2–4] This effect depends on the overexpression of plasma membrane drug efflux pumps such as P-gp and multidrug resistance associated protein dependent resistance (MRP), and presents one of the major limitations in clinical oncology. Its circumvention is a major challenge in cancer chemotherapy. This serious problem is also exhibited by mitoxantrone (MIT) (**1b**, Fig. 1), a synthetic anthracenedione anticancer agent. Several structural modifications to avoid or decrease the multidrug resistance phenomenon have been done, resulting in some progress in overcoming multidrug resistance.

We have previously postulated that the structural factor essentially favouring the activity toward resistant tumor cell lines is a five- or six-membered heterocyclic ring fused with the anthracenedione moiety.[1] Anthrapyrazoles[5] and their aza-analogues,[6] anthrapyridones,[7,8] anthrapyridazones,[7] and benzoperimidines,[1] all exhibiting activity against resistant cells, fulfill this requirement. Although the presence of a fused heterocyclic ring is essential for overcoming the multidrug resistance by an anthracenedione analogue, appropriate substituents are necessary to achieve optimal results. In this paper, the effect of substituents on benzoperimidines has been studied. The synthesis and biological activity of several benzoperimidines, chromophore-modified anthracenediones with a fused pyrimidine ring, have been previously described by us[1,9] (**2a**, **2b**, Fig. 1). These compounds exhibited in vitro cytotoxic activity against a panel of sensitive and multidrug resistant tumor cell lines with a good resistance index (RI of 1–2), which included solid tumors.

We have performed the synthesis and biological evaluation of two new groups of **2a** and **2b** derivatives: 2-amino-6-[(aminoalkyl)amino]-7*H*-benzo[*e*]-perimidin-7-ones (compounds **3**–**6**, referred to as 2-aminobenzoperimidines) and

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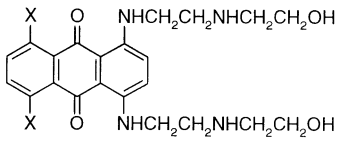
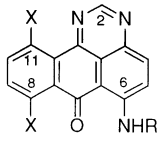
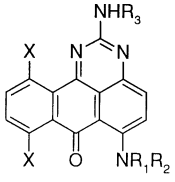
8- or 11-monohydroxy-6-[(aminoalkyl)amino]-7*H*-benzo[*e*]-perimidin-7-ones (**7**, **8**). The structures of the synthesized compounds are shown in Figure 1 and Tables 1 and 2. The 2-aminobenzoperimidine derivatives include compounds with one alkylaminoalkylamino side chain attached at position 2 (Table 1, **5a**, **6b**) and position 6 (Table 1, **3b**–**d**, **4c**–**e**) as well as with two basic chains at position 2 and 6 (Table 1, **5b**, **6c**). The derivatives with an unsubstituted 2-amino group have also been obtained (Table 1, **3a**, **4b**). Additionally, some of the synthesized compounds hold hydroxyl groups at the C-8 and C-11 positions of the ring system, while the others are dehydroxy analogues. Such selection of the derivatives enabled us to examine if, like in the case of related antitumor agents as anthracenediones,[10,11] anthrapyrazoles[12] and acridine derivatives,[13–15] the position of the attached side chains, the presence of the second arm, as well as hydroxyl groups, in the structure of chromophore, are essential for the activity of benzoperimidine analogues and ability to overcome multidrug resistance.



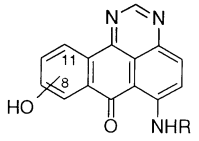
1a X = H
1b X = OH



2a X = H
2b X = OH



3,5 X = H
4,6 X = OH



7 8-OH
8 11-OH

Figure 1. The structures of AMET, MIT, benzoperimidines and synthesized compounds.

The monohydroxybenzoperimidine derivatives (hydroxyl groups at position 8 or 11) have been synthesized (Fig. 1, **7** and **8**, respectively), following procedures used for anthrapyrazoles,[16] where the presence of one hydroxyl group instead of two, increased the cytotoxic activity of the derivatives.

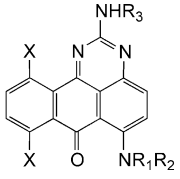
In vitro cytotoxic activity of the obtained compounds, together with reference compounds was examined against sensitive murine L1210 and human K562 and HL-60 leukemia cell lines and resistant P-gp dependent multidrug resistance (MDR) (K562/DX and HL-60/VINC) and MRP (HL-60/DX) sublines. The membrane affinity of compounds (log k'_{IAM})[17,18] has been also determined and related to their structural characteristics.

Chemistry

In the synthesis of derivatives **3**–**6** (Fig. 1), the starting compounds were 1,4-dichloro-9,10-anthracenedione (**9**)[19] or its 5,8-bis(phenylmethoxy) analogue (**10**)[20] (see Fig. 2).

The cyclization of these compounds to 2-amino benzoperimidines (**3a** or **4a**, respectively) with the use of guanidine, in the presence of copper, in DMF solution at an elevated temperature was performed by following Hollin's method.[21] Surprisingly, under these reaction conditions, not only cyclization to 2-aminopyrimidine ring occurred, but also the substitution of the chlorine atom at position 6 of the substrates by the $-N(CH_3)_2$ residue (which comes from DMF) took place. The course of the reaction was followed by ^1H NMR and ^{13}C NMR

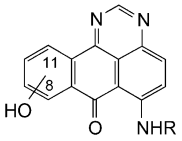
Table 1. Structure and characteristic of compounds **3**–**6**



Compound	X	R_1	R_2	R_3	Mp (°C)	% Yield	Formula
3a	H	CH_3	CH_3	H	208–210	40	$C_{17}H_{14}N_4O$
3b	H	H	$(CH_2)_2N(CH_3)_2$	H	218–220	86	$C_{19}H_{19}N_5O$
3c × HCl	H	H	$(CH_2)_3NH_2$	H	276–277	90	$C_{18}H_{18}N_5OCl \times 1.1\ H_2O$
3d	H	H	$(CH_2)_2N(CH_2)_5$	H	178–179	70	$C_{22}H_{23}N_5O$
5a	H	CH_3	CH_3	$(CH_2)_2N(CH_3)_2$	110–112	25	$C_{21}H_{23}N_5O$
5b × 2HCl	H	H	$(CH_2)_2N(CH_3)_2$	$(CH_2)_2N(CH_3)_2$	213–115 (dec)	65	$C_{23}H_{30}N_6OCl_2 \times 2.1\ H_2O$
4a	$OCH_2C_6H_5$	CH_3	CH_3	H	189–190	26	$C_{31}H_{26}N_4O_3$
4b	OH	CH_3	CH_3	H	>330	95	$C_{17}H_{14}N_4O_3$
4c	OH	H	$(CH_2)_2N(CH_3)_2$	H	282–285	65	$C_{19}H_{19}N_5O_3$
4d	OH	H	$(CH_2)_2N(CH_2)_5$	H	270–273 (subl)	70	$C_{22}H_{23}N_5O_3$
4e	OH	H	$(CH_2)_2N(CH_2)_4O$	H	260–263	94	$C_{21}H_{21}N_5O_4$
6a	$OCH_2C_6H_5$	CH_3	CH_3	$(CH_2)_2N(CH_3)_2$	170–172	22	$C_{35}H_{35}N_5O_3$
6b	OH	CH_3	CH_3	$(CH_2)_2N(CH_3)_2$	200–202 (dec)	95	$C_{21}H_{23}N_5O_3$
6c	OH	H	$(CH_2)_2N(CH_3)_2$	$(CH_2)_2N(CH_3)_2$	242–244	80	$C_{23}H_{28}N_6O_3$

spectroscopy and by determination of the molecular weight of resulting compounds. In the reaction of **9** with guanidine the main product **3a,** as well as small amounts of the 6 substituted derivative **11** were isolated and identified. The subsequent reaction of **3a** or **4b** with the appropriate aminoalkylamines occurred very readily and led to the desired **3b–d** or **4c–e** (**4b** was obtained by debenzylation of **4a** with trifluoroacetic acid). In contrast, substitution of chlorine in **11** by amines required more drastic reaction conditions. The acetylation of **3a** to **12** gave us a derivative suitable for spectral analysis.

Table 2. Structure and characteristic of compounds **7–8**



Compound	X	R	Mp (°C)	% Yield	Formula
7a × HCl	8-OH	$(CH_2)_2N(CH_3)_2$	175–177 (dec)	30	$C_{19}H_{19}N_4O_2Cl \times 0.5\ H_2O$
7b	8-OH	$(CH_2)_2N(CH_2)_4NH$	155–157	50	$C_{21}H_{21}N_5O_2$
7c	8-OH	$(CH_2)_2N(CH_2)_5$	135–136	35	$C_{22}H_{22}N_4O_2$
8a	11-OH	$(CH_2)_2N(CH_3)_2$	275–280 (dec)	43	$C_{19}H_{18}N_4O_2$
8b	11-OH	$(CH_2)_2N(CH_2)_4NH$	205–207	50	$C_{21}H_{21}N_5O_2$
8c	11-OH	$(CH_2)_2N(CH_2)_5$	137–139	55	$C_{22}H_{22}N_4O_2$

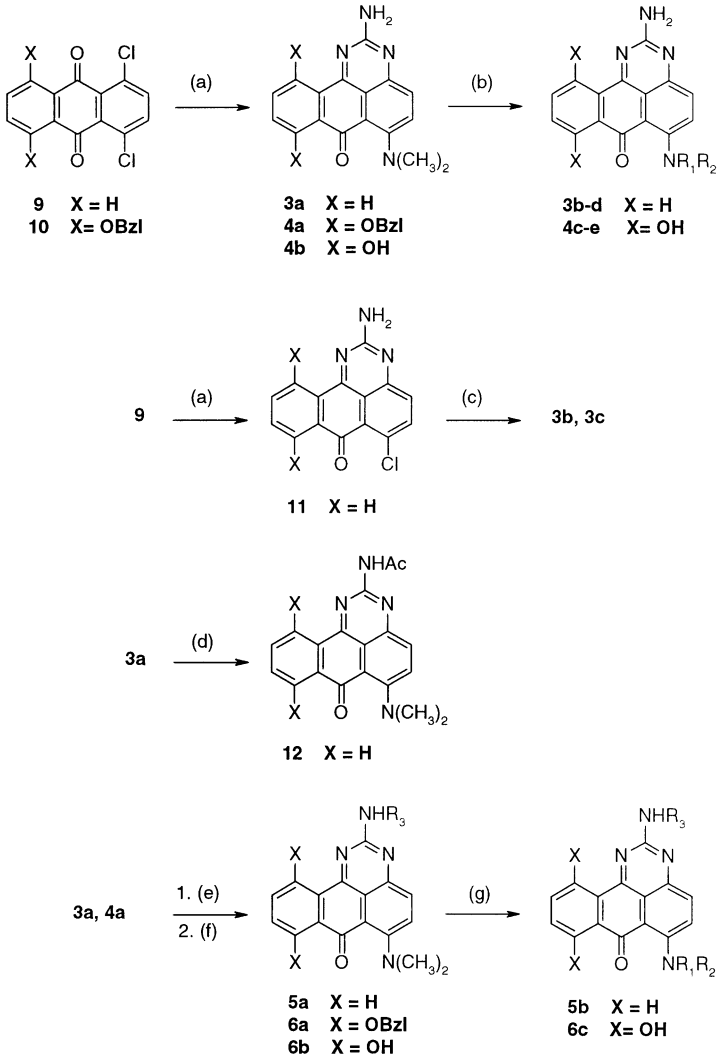


Figure 2. Synthetic route for compounds **3–6**. Reagents: (a) for **3a**: guanidine dicarbonate or hydrochloride, Cu, NaOH, DMF, 125–130 °C/4 h; for **4a**: the same reagents, 145 °C/80 h; (e) $NaNH_2$, toluene, 125 °C/2 h; (f) for **5a**: aminoethyl chloride, toluene, 112–115 °C/2 h; for **6a**: the same reagents, 118–120 °C/40–120 min; for **6b**: **6a**, TFA, room temp/18 h; (g) amine, 120 °C/60 min.

According to the literature, the alkylation of the 2-amino group of pyrimidine derivatives frequently failed and, if it was successful, only poor yield of the product were achieved.[22] The 2-*N*-alkylamino derivatives **5a** and **6a** were obtained by reaction of **3a** or **4a** with sodium amide in toluene followed by the treatment of the obtained sodium salts of **3a** or **4a** with alkylaminoalkyl chloride. Debenzylation of **6a** with trifluoroacetic acid gave **6b**. The subsequent treatment of **5a** or **6b** with aminoalkylamines afforded **5b** or **6c**. Structures, melting points, yields and formulas of compounds **3**–**6** are reported in Table 1.

The 8- or 11-monohydroxy-6-aminoalkylamino substituted benzoperimidines (**7a**–**c** and **8a**–**c**, Fig. 1) were obtained starting from 1,4-dichloro-5-(phenylmethoxy)-9,10-anthracenedione (**13**)[23] (Fig. 3).

All our attempts according to Showalter's method[24] to obtain 1-amino-4-hydroxy-5-(phenylmethoxy)-9,10-anthracenedione, the starting material for the cyclization step, leading to the benzoperimidine ring were unsuccessful. Instead, we utilized 1,4-diamino-5-(phenylmethoxy)-9,10-anthracenedione (**14**), which in the reaction with formamide yielded benzoperimidines **15** and **16**. Compound **14** was synthesized by modification of Gabriel's method in the reaction of **13** with potassium phthalimide, followed by hydrazinolysis of phthalylaminoanthracenedione.[25] Then **14** was treated with formamide in phenol to afford mixture of **15** and **16** (1.4:1, respectively). The ratio of the regioisomers was calculated on the basis of ^{1}H NMR spectra of **15** and **16**, as well as of their debenzyl derivatives (**17** and **18**, Fig. 3). The signals at 5.39, 9.4 and 14.2 ppm were assigned to the $-CH_2-$ benzyl group, proton of perimidine ring and hydroxyl group of **15** or **17**, while the signals at 5.4, 9.3 and 13.8 ppm to the appropriate protons of **16** or **18**, respectively. Because of the similar mobility of **15** and **16** on flash silica gel chromatography in eluents used, their separation required repeated tedious operations. The removal of benzyl groups by trifluoroacetic acid afforded **17** and **18**, which were reacted with appropriate amines to provide the desired derivatives **7a**–**b** and **8a**–**c**. It was noticed that the transamination of an amino group of 11-hydroxy isomer by aminoalkylamines occurred more easily then a similar reaction of the 8-hydroxy isomer. Structures, melting points, yields and molecular formulas of **7** and **8** are presented in Table 2.

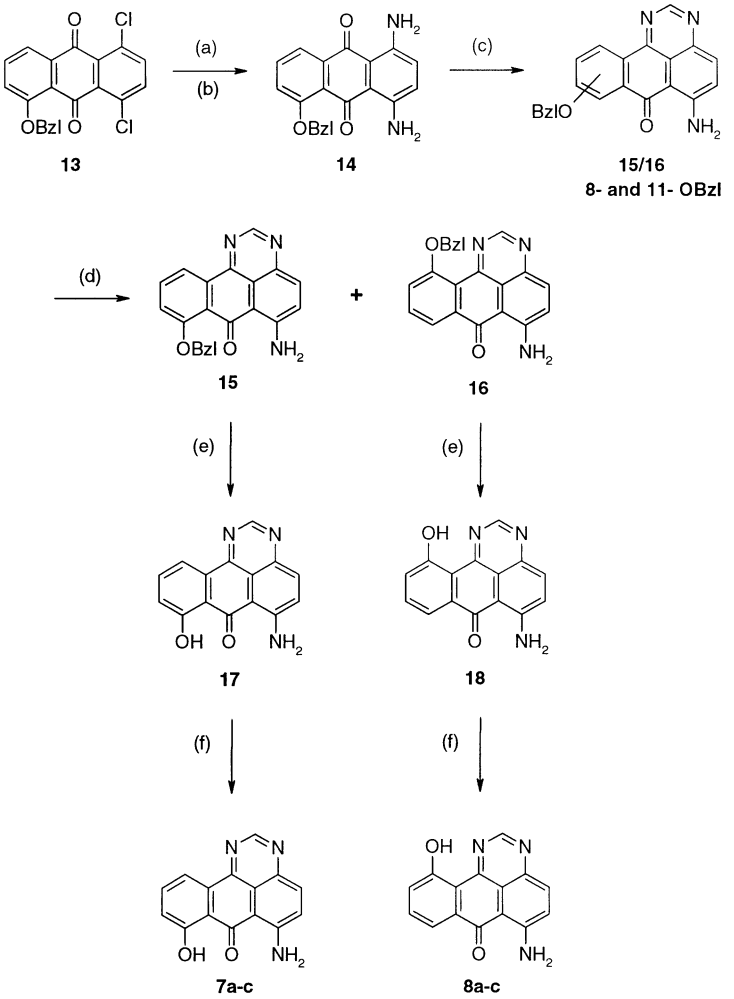


Figure 3. Synthetic route for compounds **7**–**8**. Reagents: (a) $(C_6H_4)_2NK$, Cu_2I_2, DMA, reflux/20 h; (b) N_2H_4, K_2CO_3, pyridine, 100 °C/10 min; (c) $HCONH_2$/PhOH, 160 °C/95 min; (d) flash chromatography; (e) TFA, room temp/18 h; (f) amine, Δ.

For the biological and physicochemical evaluations, the free bases of **3**–**8** were converted into their hydrochloride or dihydrochloride salts by routine methods.

Results and Discussion

Benzoperimidines, the novel group of anthracenediones analogues, are of interest because of their ability to overcome multidrug resistance of tumor cells.[1,9] Of importance is the determination of the effect of benzoperimidines modification on susceptibility to these compounds of tumor cell lines representing the two major types of multidrug resistance. They are based on the existence of two types of xenobiotics exporting pumps. One of these is P-glycoprotein directly effluxing the drugs as its substrates (MDR type resistance).[26] Another type of exporting pump is multidrug resistance associated protein (MRP type resistance), exporting anionic conjugates of neutral or basic drugs with glutathione or with glucuronic acid formed upon the action of appropriate transferases, or cotransporting the drugs with mentioned metabolites.[27] The present paper is devoted to fulfillment of these goals.

It should be noted that the influence of substituents is often quite different for different types of analogues. To establish the structure–activity relationship in this novel group of anthracenedione analogues, the series of benzoperimidine derivatives, including monohydroxybenzoperimidines (**7**–**8**) and the novel subgroup of 2-aminobenzoperimidines (**3**–**6**) have been synthesized. Membrane affinity of the compounds, essential for the uptake characteristics, and their in vitro effectiveness against a panel of leukemia cell lines have been studied. The latter determinations comprised sensitive leukemia cell lines: murine L1210 and human K562 and HL-60, as well as human multidrug resistant leukemia cell sublines: K562/doxorubicin (DX) and HL-60/vincristine (VINC) (resistance of P-gp dependent, MDR) and HL-60/DX (multidrug resistance associated protein dependent, MRP). The results are presented in Table 3.

The membrane affinity characteristic of the examined compounds has been based on a new method for the characterization of abilities to diffuse the anthracenedione compounds. The affinity of a drug to cytoplasmatic membrane is an essential factor governing its diffusion to the cells. The determinations have been done on HPLC chromatographic column IAM (an 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. The retention time for a given compound, expressed as log k'_{IAM} values, comprises not only lypophilicity characteristic but also other interactions with membrane like hydrogen bond formation, electrostatic

Table 3. In vitro cytotoxic activity of examined compounds in a panel of sensitive and resistant leukemia cell lines and membrane affinity of tested compounds

Compound	Cell line[a]/IC_{50} (nM) ± SEM									log k'_{IAM}[c]
	L1210	K562	K562/DX	RI[b]	HL-60	HL-60/VINC	RI[b]	HL-60/DX	RI[b]	
3a	19,075±2907	32,662±2436	33,729±2279	1.03	26,951±765	24,975±887	0.93	25,348±222	0.94	ND
3b	359±53	2112±467	4033±595	1.91	761±46	1067±54	1.40	2244±368	2.95	1.894
3c	333±36	1611±138	3477±563	2.16	942±234	1494±267	1.59	1301±28	1.38	1.166
3d	1020±111	3615±516	2251±218	0.62	1306±82	1143±65	0.88	2167±197	1.66	2.157
4b	382±25	4395±43	4062±832	0.92	216±49	907±36	4.20	1620±135	7.50	ND
4c	2.2±0.1	8.0±1.1	13.8±2.3	1.73	4.7±0.4	6.9±0.1	1.47	777.0±107.3	165	2.231
4d	12.7±1.0	131±22	140±23	1.07	40±7	321±65	8.03	2102±580	52.6	2.817
4e	71.7±4.0	330±20	469±116	1.42	104±26	712±127	6.85	4122±1189	39.6	2.469
5a	1664±111	7710±641	2828±218	0.37	1619±187	1544±192	0.95	1679±196	1.04	1.442
5b	21.7±2.6	94±17	144±6	1.53	51±14	199±50	3.90	8048±401	158	1.765
6b	34.3±3.5	256±34	730±203	2.85	42±20	178±11	4.24	1830±222	43.6	1.883
6c	0.87±0.09	7.0±0.3	15.1±0.5	2.16	2.2±0.1	3.7±0.2	1.68	752.9±77.6	342	2.343
7a	23.1±3.6	151±27	237±55	1.57	42±7	142±29	3.38	741±33	17.6	2.080
7b	911±24	7617±224	8808±758	1.16	2556±114	4257±242	1.67	12,068±1560	4.72	2.500
7c	568±90	488±30	574±138	1.18	211±40	710±179	3.36	2201±325	10.4	2.537
8a	6.5±0.9	46.3±3.5	72.5±15.1	1.57	23.8±5.9	50.8±9.9	2.13	434.8±30.4	18.3	2.170
8b	841±95	3974±749	7540±1080	1.90	2249±125	3182±304	1.41	10,258±2407	4.56	2.460
8c	67.7±4.7	340±119	392±21	1.15	176±11	565±16	3.21	1070±92	6.08	2.700
2a	203±22[d]	1700±340[d]	1756±316[d]	1.03	634±162	619±39	0.98	1371±239	2.16	1.808
2b	1.3±0.3[d]	5.6±0.2[d]	10.4±1.1[d]	1.86	5.2±1.0	14.2±3.0	2.73	736±163	142	2.464
AMET	146±47[d]	181±18	12,392±886	68.5	36.5±2.7	5256±2704	144	6096±109	167	1.255
MIT	1.4±0.1	21.0±4.0	554.0±49.5	26.4	2.2±0.3	56.5±4.2	25.7	1179±203	536	1.841
DX	19.4±1.3	42.1±3.3	7031.5±542.0	167	20.0±3.0	647±60	32.4	3928±262.9	196	1.434

ND, not determined.

[a]L1210, murine lymphocytic leukemia; K562, human myelogenous leukemia and doxorubicin resistant (MDR type) subline K562/DX; HL-60, human promyelocytic leukemia, and vincristine resistant (MDR type) subline HL-60/VINC, and doxorubicin resistant (MRP type) subline HL-60/DX.

[b]RI, resistance index; the ratio of IC_{50} value for resistant cell line to IC_{50} value for sensitive cell line.

[c]Logarithm of HPLC capacity factor determined in an immobilized artificial membrane column with acetonitrile-buffer pH 7.0 eluent.

[d]According to ref 1.

interactions and so on. Therefore we prefer to interpret the log k'_{IAM} values as characterizing the membrane affinity rather then as commonly used lypophilicity characteristics. The effect of both factors: lypophilicity and other interactions, on the membrane affinity, expressed by the log k'_{IAM} values, is well illustrated taking into account various examined compounds (for structures, see Tables 1 and 2). For instance, **3d**, **4d**, **7c**, **8c** bearing lypophilic piperidyl moiety exhibit high log k'_{IAM}. On the other hand, the presence in the molecule of hydrophilic groups, able to interact with the membrane by other mechanisms, also show elevated log k'_{IAM}. The latter case could be exemplified by phenolic derivatives and compounds bearing hydrophilic side chains (e.g., **5b**, **6c**, and **7b**). The great impact of hydrophilic phenolic groups on log k'_{IAM} values can be also illustrated by comparing date for MIT and ametantrone (AMET).

The results obtained in the examination of structure–activity relationships of benzoperimidines allowed us to draw the following conclusions. The nature and location of substituents essentially influence cytotoxic activity of the derivatives, ability to overcome multidrug resistance and to differentiate between tumor cell lines with the two above mentioned types of drug exporting pumps (MDR and MRP). In regard to sensitive cell lines, the presence of two phenolic groups at positions 8 and 11 essentially increases the cytotoxicity. The lack of these groups causes the dramatic decrease of activity. The presence of one phenolic group, the best at position 11, gives an intermediate effect. In regard to this, benzoperimidines behave differently than compounds of the anthrapyrazole group, where the presence of one phenolic group is more beneficial than two.[16] The alkylaminoalkylamino side chain at position 6 is indispensable for the high cytotoxicity and the optimal substituent is dimethylaminoethylamino residue. The introduction of amino group at position 2 does not change essentially the activity of the compound. The substitution at this group of dimethylaminoethyl chain is very beneficial for the cytotoxicity of the compound providing the simultaneous presence of the second dimethylaminoethylamino side chain at position 6 (**5b**). Moreover, the simultaneous presence of two hydroxyl groups at positions 8 and 11 provides compound with very high cytotoxic activity (**6c**). We have noticed the differential effect of substituents on cytotoxicity against multidrug resistance cell lines exhibiting two types of resistance (MDR, MRP). All benzoperimidine derivatives exhibit good or very good resistance indexes (RI) against tumor cell lines with P-gp dependent efflux pump (MDR), regardless of their cytotoxic potency. No marked influence of substituents on this property could be observed. We assume that this is due to the log k'_{IAM} values of examined compounds (above 1) optimal for their ability to diffuse to the cells. Much lower log k'_{IAM} values would slow down the drug diffusion into cells and high values (log k'_{IAM} 3–4) would cause the retainment of the compounds in cytoplasmatic membrane (unpublished data). We postulated that fast diffusion of drugs into the cells with simultaneous functioning of drug exporting pump according to the saturation kinetics, allows for the retainment in the cells of therapeutic concentration of a drug.[28]

Tumor cell lines with multidrug resistance of MRP type behave quite differently. The data indicate that membrane affinity (log k'_{IAM}) and consequently the ability to diffuse of the compounds is not a decisive factor in overcoming MDR-type multidrug resistance. Two structural factors of benzoperimidines which positively influence the membrane affinity, dramatically decrease the activity toward MRP cells. One of these factors is the presence of phenolic groups. One phenolic group exerts a negative effect and two such groups increase it. The same effect of phenolic groups on RI of the compounds is also observed for MIT and AMET. Another factor also negatively affecting the activity is the presence of alkylaminoalkylamino side chains at positions 6 and 2. The presence of both chains in both these positions essentially decreases the cytotoxic activity toward MRP cell line. The highest RI index exhibits compound with two phenolic groups and two side chains (**6c**). It could be postulated that a possible explanation of this behaviour is, that the subject compounds are poor substrates for transferases participating in the formation of anionic drug conjugates (the substrates of MRP pumps) or are not suitable for their cotransport with glutathione or glucuronate.

Experimental

Chemistry

Melting points, determined with a Boeticus PHMK05 apparatus, are uncorrected. Thin layer chromatography (TLC) was carried out on Kieselgel 60 plates (Merck), column chromatography on Kieselgel Merck (minus 200 mesh). NMR spectra were taken on a Varian 300-MHz spectrometer using tetramethylsilane as an internal standard. MS spectra were recorded on a Quadrupolic Mass Spectrophotometer Trio-3 (FAB technique). The elemental analyses were performed on a Carlo Erba CHNS-O-EA1108 instrument for C, H, N.

2-Amino-6-(dimethylamino)-7*H*-benzo[*e*]perimidin-7-one (3a). A sample of 550 mg (2 mmol) of **9**,[19] 400 mg of guanidine hydrochloride, 180 mg of copper and 240 mg of NaOH in DMF (14 mL) was stirred at 125–130 °C for 4 h. The course of the reaction was followed by TLC in toluene, and in $CHCl_3$/MeOH (20:1). After cooling, the reaction mixture was filtered. The filtrate was diluted with $CHCl_3$ and Et_2O, washed several times with water and dried over Na_2SO_4. The solvents were evaporated and the residue was purified on flash column (eluent $CHCl_3$/MeOH 100:1) to afford **3a** (40%) as a purple powder and **11** (5%) as a yellow-brown powder. ^{1}H NMR of **3a** (DMSO-d_6) 3.4 (s, 6H, 2×CH_3), 6.6 (s, 2H, NH_2, ex), 7.68 (d, 1H, ar, J = 9.5 Hz), 7.8 (m, 2H, ar), 7.83 (d, 1H, ar, J = 9.7 Hz), 8.3 (dd, 1H, ar, J = 10, 1.22 Hz), 8.7 (dd, 1H, ar, J = 10, 0.73 Hz). ^{13}C NMR (DMSO-d_6) δ 177.13; 159.58; 152.58; 150.83; 147.44; 135.5; 133.19; 132.42; 131.14; 127.47; 126.55; 124.04; 115.03; 107.26; 40.0. MS m/z (relative intensity, %): 291

([M + 1]$^+$, 100). Found: C, 70.1; H, 4.75; N, 19.1; calcd for $C_{17}H_{14}N_4O$: C, 70.40; H, 4.87; N, 19.32.

11: mp 284–286 °C, MS *m*/*z* (relative intensity, %): 282 ([M]$^+$, 100).

2-Acetamido-6-(dimethylamino)-7*H*-benzo[*e*]perimidin-7-one (12). A sample of **3a** was left with Ac_2O at room temperature for 18 h. The reaction mixture was worked up to give a residue, which was flash-chromatographed (eluent $CHCl_3$/MeOH 20:1). ^{1}H NMR ($CDCl_3$) δ 2.7 (s, 3H, CH_3), 3.4 (s, 6H, 2×CH_3), 7.7–7.8 (m, 3H, ar), 7.9 (d, 1H, ar, *J* = 9.8 Hz), 8.25 (br s, 1H, NH), 8.4–8.5 (m, 1H, ar), 8.7–8.8 (m, 1H, ar).

2-Amino-6-[(2-dimethylamino)ethyl]amino]-7*H*-benzo[*e*]-perimidin-7-one (3b). A sample of **3a** with 2-(dimethylamino)ethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was stirred under reflux in a nitrogen atmosphere for 30 min. The reaction mixture was diluted with $CHCl_3$ and washed with dilute HCl to remove excess of amine. The organic layer was flash-chromatographed (eluent $CHCl_3$/MeOH 5:1) to give **3b** as a dark pink powder (yield 86%), with a fluorescence at 366 nm. Mp 218–220 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H, 2×CH_3), 2.8 (t, 2H, *J* = 6.5 Hz), 3.65 (q, 2H, *J* = 6.5 Hz), 4.8 (br s, 2H, NH_2, ex), 7.5 (d, 1H, ar, *J* = 9.6 Hz), 7.9–8.0 (m, 3H, ar), 8.4 (dd, 1H, ar, *J* = 6.3, 1.95 Hz), 8.7 (d, 1H, ar, *J* = 7.8 Hz), 11.1 (br, s, 1H, NH, ex). MS *m*/*z* (relative intensity, %): 333 ([M]$^+$, 100). Found: C, 68.20; H, 5.70; N, 19.87; calcd for $C_{19}H_{19}N_5O$: C, 68.40; H, 5.74; N, 21.01.

2-Amino-6-[(3-aminopropyl)amino]-7*H*-benzo[*e*]perimidin-7-one (3c). Compound **3c** was obtained in the reaction of **3a** with 1,3-diaminopropane by a procedure similar to that described for **3b**, as a dark pink solid yield 90%. ^{1}H NMR ($CDCl_3$) δ 2.04 (quintet, 2H, *J* = 6.8 Hz), 2.95 (t, 2H, *J* = 7.2 Hz), 3.7 (q, 2H, *J* = 6.4 Hz), 4.9 (br s, 2H, NH_2, ex), 7.5 (d, 1H, ar, *J* = 9.5 Hz), 7.9–8.0 (m, 3H, ar), 8.2 (br s, 2H, NH_2, ex), 8.45 (dd, 1H, ar, *J* = 6.3, 1.95 Hz), 8.68 (d, 1H, ar, *J* = 7.8 Hz), 11.1 (br s, 1H, NH, ex). MS *m*/*z* (relative intensity, %): 319 ([M]$^+$, 100).

2-Amino-6-[2-(1-piperydyl)ethyl]amino]-7*H*-benzo[*e*]perimidin-7-one (3d). The reaction of **3a** with 1-(2-aminoethyl)piperidine was carried out as described for **3b** for 2.5 h. The reaction mixture was worked up and purified by flash-chromatography (eluent $CHCl_3$/MeOH 20:1) to give **3d** (yield 70%), as pink powder with a fluorescence at 366 nm. Mp 178–179 °C. ^{1}H NMR ($CDCl_3$) δ 1.6–1.7 (m, 6H), 2.5 (t, 4H, *J* = 4.9 Hz), 2.75 (t, 2H, *J* = 7.0 Hz), 3.6 (q, 2H, *J* = 6.6 Hz), 5.2 (s, 2H, NH_2, ex), 7.5 (d, 1H, ar, *J* = 9.68 Hz), 7.7–8.0 (m, 3H, ar), 8.56 (m, 1H, ar), 8.7 (m 1H, ar), 11.2 (br s, 1H, NH, ex). MS *m*/*z* (relative intensity, %): 373 ([M]$^+$, 100).

2-[(2-Dimethylamino)ethyl]amino]-6-(dimethylamino)-7*H*-benzo[*e*]perimidin-7-one (5a). A sample of **3a** in dry toluene was stirred under reflux with sodium amide for 2 h. After cooling the product was isolated by filtration followed by washing with Et_2O. The subsequent reaction with 2-(dimethylamino)ethyl chloride was performed in toluene at 125 °C for 30–60 min. The reaction mixture was worked up to give a residue, which was flash-chromatographed (eluent $CHCl_3$/MeOH 5:1 then 2:1) to afford **5a** (yield 25%) as a fluorescing at 366 nm purple powder. Mp 110–112 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H, 2×CH_3), 2.7 (t, 2H, *J* = 6.1 Hz), 3.2 (s, 6H, 2×CH_3), 3.75 (q, 2H, *J* = 5.8 Hz), 5. 7 (t, 1H, NH, *J* = 5.3 Hz, ex), 7.4–7.8 (m, 4H, ar), 8.4–8.5 (m, 2H, ar), 8.8–8.9 (m, 2H, ar). MS *m*/*z* (relative intensity, %): 362 ([M]$^+$, 100). Found: C, 69.41; H, 6.32; N, 18.8; calcd for $C_{21}H_{23}N_5O$: C, 69.59; H, 6.40; N, 19.31.

2,6-Di[2-(dimethylamino)ethyl]amino]-7*H*-benzo[*e*]perimidin-7-one (5b). A sample of **5a** in 2-(dimethylamino)ethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was stirred under nitrogen atmosphere at 120 °C for 2 h. The reaction mixture was worked up to give a residue, which was flash-chromatographed (eluent $CHCl_3$/MeOH 5:1 then $CHCl_3$/MeOH/NH_4OH 5:1:0.1) to afford **5b** (yield 65%) as a fluorescing at 366 nm purple powder. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H, 2×CH_3), 2.39 (s, 6H, 2×CH_3), 2.6–2.8 (two overlapping t, 4H), 3.6–3.8 (two overlapping q, 4H), 5.7 (t, 1H, NH, *J* = 5.0 Hz, ex), 7.45 (d, 1H, *J* = 9.67 Hz, ar), 7.75–7.95 (m, 3H, ar), 8.6 (m, 1H, ar), 9.0 (m, 1H, ar), 11.2 (t, 1H, NH, *J* = 4.2 Hz, ex). MS *m*/*z* (relative intensity, %): 404 ([M]$^+$, 100). Found: C, 53.48; H, 6.64; N, 15.41; calcd for $C_{23}H_{30}N_6OCl_2 \times 2.1\ H_2O$: C, 53.60; H, 6.65; N, 16.32.

2-Amino-6-(dimethylamino)-8,11-bis(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (4a). Compound **4a** was obtained from **10**[20] by a procedure similar to that described for **3a**. The reaction was carried out for 80 min at 145 °C. ^{1}H NMR ($CDCl_3$) δ 3.1 (s, 6H, 2×CH_3), 4.9 (br s, 2H, NH_2, ex), 5.25 (s, 2H), 5.72 (s, 2H), 7.2–7.7 (m, 14H, ar). MS *m*/*z* (relative intensity, %): 503 ([M + 1]$^+$, 100). Found: C, 73.82; H, 5.18; N, 11.01; calcd for $C_{31}H_{26}N_4O_3$: C, 74.11; H, 5.21; N, 11.15.

2-Amino-6-(dimethylamino)-8,11-dihydroxy-7*H*-benzo[*e*]-perimidin-7-one (4b). A sample of **4a** was left in trifluoroacetic acid at room temperature for 18 h. The TFA was removed under reduced pressure by coevaporation with benzene. An analytical sample was dissolved in $CHCl_3$ and the solution was carefully washed with $NaHCO_3$ solution and then with water. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and the residue was solidified under Et_2O to yield **4b** (yield 95%) as blue powder. Mp > 300 °C. ^{1}H NMR (DMSO-d_6) δ 3.15 (s, 6H, 2×CH_3), 5.2 (br s, 2H, NH_2, ex), 7.3–7.7 (m, 4H, ar), 13.2 (s, 1H, ex), 13.8 (s, 1H, ex). MS *m*/*z* (relative intensity, %): 324 ([M]$^+$, 100).

2-Amino-6-[(2-dimethylamino)ethyl]amino]-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (4c). A sample of **4b** was treated with 2-(dimethylamino)ethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine under nitrogen atmosphere at 100 °C for 30 min. The reaction mixture

was worked up and purified by flash chromatography (eluent $CHCl_3$/MeOH 5:1) to afford **4c** (yield 65%) as a purple powder. Mp 282–285 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H, 2×CH_3), 2.8 (t, 2H, J = 6.2 Hz), 3.62 (q, 2H, J = 6.4 Hz), 5.2 (br s, 2H, NH_2, ex), 7.3–7.7 (m, 4H, ar), 11.1 (t, 1H, NH, J = 4.5 Hz, ex), 13.2 (s, 1H, ex), 13.8 (s, 1H, ex). MS m/z (relative intensity, %): 365 ($[M]^+$, 100). Found: C, 62.95; H, 5.18; N, 19.01; calcd for $C_{19}H_{19}N_5O_3$: C, 62.47; H, 5.24; N, 19.19.

2-Amino-6-[2(1-piperidinyl)ethyl]amino]-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (4d). The reaction of **4b** with 1-(2-aminoethyl)piperidine was carried out as described for **4c** for 30 min. The reaction mixture was worked up and then purified by chromatographed (eluent $CHCl_3$/MeOH 10:1) to afford **4d** (yield 70%) as a purple solid. Mp 270–273 °C. ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 6H), 2.5 (t, 4H, J = 4.9 Hz), 2.75 (t, 2H, J = 6.5 Hz), 3.6 (q, 2H, J = 6.5 Hz), 5.1 (s, 2H, NH_2, ex), 7.16 (d, 1H, ar, J = 9.0 Hz), 7.24–7.3 (m, 3H, ar), 7.44 (d, 1H, ar, J = 9.69 Hz), 7.7 (d, 1H, ar, J = 9.52 Hz), 10.8 (t, 1H, NH, J = 4.2 Hz, ex), 13.2 (s, 2H, ex). MS m/z (relative intensity, %): 405 ($[M]^+$, 100).

2-Amino-6-[2(4-morpholinyl)ethyl]amino]-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (4e). Compound **4e** was obtained in the reaction of **4b** with 4-(2-aminoethyl)morpholine by a procedure similar to that described for **4c**, as a pink solid (yield 94%). Mp 260–263 °C. ^{1}H NMR ($CDCl_3$) δ 2.6 (t, 4H, J = 4.68 Hz), 2.75 (t, 2H, J = 6.26 Hz), 3.65 (q, 2H, J = 4.19 Hz), 3.8 (t, 4H, J = 4.6 Hz), 5.05 (br s, 2H, NH_2, ex), 7.2 (d, 1H, ar, J = 9.65 Hz), 7.25–7.3 (m, 3H, ar), 7.45 (d, 1H, ar, J = 9.65 Hz), 7.75 (d, 1H, ar, J = 9.55 Hz), 10.8 (t, 1H, NH, J = 4.1 Hz, ex), 13.6 (s, 2H, ex), 13.6 (s, 2H, ex). MS (relative intensity, %): 407 ($[M]^+$, 100).

2-[(2-Dimethylamino)ethyl]amino]-6-(dimethylamino)-8,11-bis(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (6a). The reaction of **4a** with sodium amide followed by treatment of obtained sodium salt of **4a** with 2-(dimethylamino)ethyl chloride was performed similar to that described for **5a** to afford **6a** (yield 22%) as a dark purple powder. Mp 170–172 °C. ^{1}H NMR (CDC_3) δ 2.3 (s, 6H, 2×CH_3), 2.5 (t, 2H, J = 6.1 Hz), 3.1 (s, 6H, 2×CH_3), 3.5 (q, 2H, J = 5.6 Hz), 5.24 (s, 2H), 5.26 (s, 2H), 5.55 (t, 1H, NH, J = 5.4 Hz, ex), 7.4 (d, 1H, ar, J = 6.2 Hz), 7.34–7.66 (m, 12H, ar), 7.72 (d, 1H, ar, J = 9.5 Hz), MS m/z (relative intensity, %): 574 ($[M+1]^+$, 100). Found: C, 72.89; H, 6.05; N, 12.01; calcd for $C_{35}H_{35}N_5O_3$: C, 73.28; H, 6.15; N, 12.21.

2-[(2-Dimethylamino)ethyl]amino]-6-(dimethylamino)-8,11-dihydroxy-7*H*-benzo[*e*]peri-midin-7-one (6b). A sample of **6a** was treated with trifluoroacetic acid at room temperature for 18 h. The TFA was removed under reduced pressure. An analytical sample was worked up as described for **4b,** to afford **6b** (yield 95%) as blue solid. Mp 200–202 °C (dec). ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 6H, 2×CH_3), 2.7 (t, 2H, J = 6.0 Hz), 3.2 (s, 6H, 2×CH_3), 3.72 (q, 2H, J = 5.7 Hz), 5.6 (t, 1H, NH, J = 5.1 Hz, ex), 7.4–7.6 (m, 2H, ar), 8.3 (m, 2H, ar), 13.3 (s, 1H, ex), 13.6 (s, 1H, ex). MS m/z (relative intensity, %): 373 ($[M]^+$, 100).

2,6-Di[(2-dimethylamino)ethyl]amino]-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (6c). The reaction of **6b** with 2-(dimethylamino)ethylamine was performed similar to that described for **5b** to afford **6c** (yield 80%), as a dark purple solid. Mp 242–244 °C. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H, 2×CH_3), 2.38 (s, 6H, 2×CH_3), 2.6–2.8 (two overlapping t, 4H), 3.6 (q, 4H, J = 3.2 Hz), 6.0 (m, 1H, ex), 7.12 (d, 1H, ar, J = 9.0 Hz), 7.2–7.28 (m, 1H, ar), 7.36 (d, 1H, ar, J = 9.6 Hz), 7.68 (d, 1H, ar, J = 9.5 Hz), 10.6 (m, NH, ex), 13.6 (br s, 1H, ex), 14.0 (br s, 1H, ex). MS m/z (relative intensity, %): 422 ($[M]^+$, 100). Found: C, 64.87; H, 6.57; N, 16.01; calcd for $C_{23}H_{28}N_6O_3$: C, 65.38; H, 6.68; N, 16.58.

1,4-Diamino-5-(phenylmethoxy)-9,10-anthracenedione (14). Compound **13**[24] in the reaction with potassium phthalimide, in the presence of Cu_2I_2, according to literature[26] was transformed into 1,4-diphthalyloamino-5-(phenylmethoxy)-9,10-anthracenedione; an orange solid, mp 251–253 °C, MS m/z (relative intensity, %): 712 ($[M]^+$, 100). The following reaction with hydrazine hydrate in pyridine solution, performed at 110 °C for 10 min, gave **14** as a dark blue solid (yield 45%). Mp 143–144 °C. ^{1}H NMR ($CDCl_3$) δ 5.4 (s, 2H), 6.85 (d, 1H, ar, J = 9.1 Hz), 6.9 (d, 1H, ar, J = 9.1 Hz), 6.92–6.98 (br s, 4H, NH_2, ex), 7.3–7.35 (m, 1H, ar), 7.4 (t, 1H, ar, J = 3.2 Hz), 7.54–7.62 (m, 5H, ar), 8.02 (d, 1H, ar, J = 7.6 Hz). MS m/z (relative intensity, %): 343 ($[M]^+$, 100).

6-Amino-8-(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (isomer 15) and 6-Amino-11-(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (isomer 16). A mixture of 205 mg (0.6 mmol) of **14**, 1.8 mL (45 mmol) of formamide and 4.8 g of phenol was heated at 160 °C for about 95 min. The course of the reaction was monitored by TLC ($CHCl_3$/MeOH 20:1). The reaction mixture was diluted with $CHCl_3$ and extracted several times with 2 N NaOH and then with water. The organic phase was dried over Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (eluent $CHCl_3$/MeOH 70:1) to afford 115 mg (yield 54 %) of **15** and **16** isomers mixture, showing by ^{1}H NMR a ca. 1.4:1 ratio of **15**:**16**. Repeated flash chromatography with using of the same eluent gave **15** (the slower eluting component) as a yellow solid with strong fluorescence at 366 nm. Mp 232–234 °C (subl), ^{1}H NMR of **15** (DMSO-d_6) δ 5.39 (s, 2H), 7.3–77 (m, 8H, ar), 8.06 (d, 1H, ar, J = 9.4 Hz), 8.2 (d, 1H, ar, J = 6.7 Hz), 9.4 (s, 1H). MS m/z (relative intensity, %): 353 ($[M]^+$, 100). Compound **16** (the faster eluting component), a dark yellow solid with fluorescence at 366 nm. Mp 246–248 °C (subl), ^{1}H NMR of **16** ($CDCl_3$-d_6) δ 5.41 (s, 2H), 7.3–7.48 (m, 5H, ar), 7.6–7.75 (m, 3H), 8.0 (d, 1H, ar, J = 9.32 Hz), 8.74 (dd, 1H, ar, J = 9.7, J = 1.1 Hz), 9.3 (s, 1H). MS m/z (relative intensity, %): 353 ($[M]^+$, 100).

6-Amino-8-hydroxy-7*H*-benzo[*e*]perimidin-7-one (17). A sample of **15** was treated with trifluoroacetic acid at room temperature for 18 h. The TFA was removed under reduced pressure by coevaporation with benzene. An analytical sample was worked up as described for **4b**. The flash chromatography (eluent $CHCl_3$/MeOH 70:1) gave **17** as an orange solid (yield 95%). Mp 264–265 °C.

^{1}H NMR (DMSO-d_6) δ 7.34 (dd, 1H, ar, J = 8.0, 1.2 Hz), 7.58 (d, 1H, ar, J = 9.6 Hz), 7.65 (t, 1H, ar, J = 8.0 Hz), 8.06 (dd, 1H, ar, J = 4.6, 1.2 Hz), 8.1 (dd, 1H, ar, J = 3.0, 1.2 Hz), 9.2 (s, 1H), 14.2 (s, 1H, ex).

6-Amino-11-hydroxy-7*H*-benzo[*e*]perimidin-7-one (18). A sample of **16** was treated with trifluoroacetic acid and worked up in the same manner as described for **17**. Obtained **18** (brown solid), melted at 307–309 °C. ^{1}H NMR (DMSO-d_6) δ 7.15 (dd, 1H, ar, J = 9.0, 1.0 Hz), 7.4 (d, 1H, ar, J = 9.6 Hz), 7.7 (t, 1H, ar, J = 8.0 Hz), 8.0 (d, 1H, ar, J = 9.8 Hz), 8.43 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.25 (s, 1H), 13.8 (s, 1H, ex).

6-[2-(Dimethylamino)ethyl]amino]-8-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (7a). A crude sample of **17** with 2-(dimethylamino)ethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was heated at 115 °C under nitrogen atmosphere for 16 h. The progress of the reaction was followed by TLC ($CHCl_3$/MeOH 10:1). The reaction mixture was diluted with $CHCl_3$ and carefully washed with diluted HCl followed by washed with water. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and the residue was flash chromatographed (eluent $CHCl_3$/MeOH 20:1 then 5:1) to afford **7a** as a brown powder (yield 30%). ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H), 2.8 (t, 2H, J = 6.5 Hz), 3.6 (q, 2H, J = 6.5 Hz), 7.35 (dd, 1H, ar, J = 8.0, 1.2 Hz), 7.58 (d, 1H, ar, J = 9.6 Hz), 7.7 (t, 1H, ar, J = 8.0 Hz), 8.06 (dd, 1H, ar, J = 9.0, 1.0 Hz), 8.1 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.45 (br s, 1H, ex), 14.2 (s, 1H, ex). Found: C, 59.87; H, 5.28; N, 13.92; calcd for $C_{19}H_{19}N_4O_2Cl \times 0.5\ H_2O$: C, 60.04; H, 5.31; N, 14.76.

6-[2-(piperazin-1-yl)ethyl]amino]-8-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (7b). The reaction of **17** with 1-(2-aminoethyl)-piperazine was carried out under conditions described for **7a** (reaction time 9 h). Then the reaction mixture was worked up and purified by flash chromatography with using of deactivated silica gel (eluent $CHCl_3$/MeOH 5:1 then $CHCl_3$/MeOH/NH_4OH 5:1:0.1) to afford **7b** (yield 50%). Mp 155–157 °C. ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 4H), 2.8 (t, 2H, J = 6.5 Hz), 2.9 (m, 4H), 3.55 (q, 2H, J = 6.5 Hz), 7.35 (dd, 1H, ar, J = 8.0, 1.2 Hz), 7.58 (d, 1H, ar, J = 9.6 Hz), 7.7 (t, 1H, ar, J = 8.0 Hz), 8.06 (dd, 1H, ar, J = 9.0, 1.0 Hz), 8.1 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.45 (br s, 1H, ex), 14.2 (s, 1H, ex). MS m/z (relative intensity, %): 375 ($[M]^+$, 100).

6-[2-(1-piperidinyl)ethyl]amino]-8-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (7c). The reaction of **17** with 1-(2-aminoethyl)-piperidine was carried out for 9 h. The workup was similar to that described for **7a** (yield 35%). Mp 135–136 °C. ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 6H), 2.5 (t, 4H, J = 4.9 Hz), 2.75 (t, 2H, J = 6.5 Hz), 3.6 (q, 2H, J = 6.5 Hz), 7.35 (dd, 1H, ar, J = 8.0, 1.2 Hz), 7.58 (d, 1H, ar, J = 9.6 Hz), 7.7 (t, 1H, ar, J = 8.0 Hz), 8.06 (dd, 1H, ar, J = 9.0, 1.0 Hz), 8.1 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.45 (br s, 1H, ex), 14.2 (s, 1H, ex). MS m/z (relative intensity, %): 374 ($[M]^+$, 100).

6-[2-(Dimethylamino)ethyl]amino]-11-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (8a). The reaction of **18** with 2-(dimethylamino)ethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine was carried out for 12 h under conditions as described for **7a**. Then the reaction mixture was diluted with $CHCl_3$ and carefully washed with diluted HCl was worked up and purified by flash chromatography (eluent $CHCl_3$/MeOH 20:1 then 5:1) to afford **8a** as a brown solid (yield 43%). Mp 275–280 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 6H), 2.8 (t, 2H, J = 6.5 Hz), 3.6 (q, 2H, J = 6.5 Hz), 7.18 (dd, 1H, ar, J = 9.0, 1.0 Hz), 7.45 (d, 1H, ar, J = 9.6 Hz), 7.65 (t, 1H, ar, J = 8.0 Hz), 7.98 (d, 1H, ar, J = 9.8 Hz), 8.42 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.0 (br s, 1H, ex), 13.6 (s, 1H, ex). Found: C, 67.87; H, 5.20; N, 16.51; calcd for $C_{19}H_{18}N_4O_2$: C, 68.19; H, 5.43; N, 16.76.

6-[2-(piperazin-1-yl)ethyl]amino]-11-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (8b). The reaction of **18** with 1-(2-aminoethyl)-piperazine was carried out for 5 h. The workup was similar to that described for **7b**. Yield 50%, mp 205–207 °C. ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 4H), 2.8 (t, 2H, J = 6.5 Hz), 2.9 (m, 4H), 3.55 (q, 2H, J = 6.5 Hz), 7.18 (dd, 1H, ar, J = 9.0, 1.0 Hz), 7.45 (d, 1H, ar, J = 9.6 Hz), 7.65 (t, 1H, ar, J = 8.0 Hz), 7.98 (d, 1H, ar, J = 9.8 Hz), 8.42 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.0 (br s, 1H, ex), 13.6 (s, 1H, ex). MS m/z (relative intensity, %): 375 ($[M]^+$, 100).

6-[2-(1-piperydyl)ethyl]amino]-11-hydroxy-7*H*-benzo[*e*]-perimidin-7-one (8c). The reaction of **18** with 1-(2-aminoethyl)-piperidine was carried out for 6 h and then worked up as described for **7c**. Yield 55%, mp. 137–139 °C. ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 6H), 2.5 (t, 4H, J = 4.9 Hz), 2.75 (t, 2H, J = 6.5 Hz), 3.6 (q, 2H, J = 6.5 Hz), 7.18 (dd, 1H, ar, J = 9.0, 1.0 Hz), 7.45 (d, 1H, ar, J = 9.6 Hz), 7.65 (t, 1H, ar, J = 8.0 Hz), 7.98 (d, 1H, ar, J = 9.8 Hz), 8.42 (dd, 1H, ar, J = 9.0, 1.0 Hz), 9.2 (s, 1H), 11.0 (br s, 1H, ex), 13.6 (s, 1H, ex). MS m/z (relative intensity, %): 374 ($[M]^+$, 100).

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 proportion 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.

Biological Evaluation

Cell lines

Murine L1210 lymphocytic leukemia cells were grown in RPMI 1640 medium supplemented with 5% FBS (foetal 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. 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). 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.

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) or by the protein content of the cells according to Lowry method as described previously.[29] 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.

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