# 8,11-Dihydroxy-6-[(aminoalkyl)amino]-7*H*-benzo[*e*]perimidin-7-ones with Activity in Multidrug-Resistant Cell Lines: Synthesis and Antitumor Evaluation[§]

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*Received April 26, 1999*

The synthesis of dihydroxybenzoperimidine derivatives, which are chromophore-modified dihydroxyanthracenediones with an additional pyrimidine ring incorporated into the chromophore, is reported. These derivatives are structurally related to the antitumor agent mitoxantrone. Their synthesis was carried out by the reaction of 6-amino-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (**5**) or 6,8,11-trihydroxy-7*H*-benzo[*e*]perimidin-7-one (**10**) with a number of respective (alkylamino)alkylamines. The dihydroxybenzoperimidine derivatives exhibited in vitro cytotoxic activity against murine leukemia L1210 and human leukemia HL60 cell lines comparable to that of mitoxantrone. These compounds also exhibited a range of in vitro activity against the human MDR-type resistant leukemia K562R cell line with the MDR phenotype. The most active compound of this series, namely **6a**, exhibited potent in vitro cytotoxic activity against a panel of human cell lines. Furthermore, in contrast to both mitoxantrone and doxorubicin, it displayed little cross-resistance in cell lines characterized by a MDR phenotype. Cell cycle analysis in the sensitive HT-29 and mitoxantrone-resistant HT-29/Mx (not identified resistance mechanism) cell lines has revealed that both mitoxantrone and **6a** induce a G2/M block. However, while the proportion of apoptotic cells after mitoxantrone treatment is similar for both sensitive and resistant cell lines, it is much lower for **6a**. Compound **6a** tested against P388 murine leukemia in vivo displayed a significant antitumor effect (%T/C 196 at an optimal dose of 10 mg/kg). The property of overcoming the cross-resistance was maintained also in in vivo efficacy studies, where no difference was observed in the antitumor activity of compound **6a** against the A2780 human tumor xenograft and its MDR A2780/Dx subline. We conclude that benzoperimidines, if properly substituted, constitute a novel class of compounds that can overcome multidrug resistance.

## Introduction

Mitoxantrone (**1**) (Chart 1), a synthetic anthracene-9,10-dione derivative, is an important antitumor agent with demonstrated clinical efficacy in the treatment of leukemia, lymphomas, and breast cancer.[1] However, some undesired side effects have been reported, principally cardiotoxicity, which is thought to be one consequence of the ability of anthracenediones to generate oxygen radicals.[2] In recent years the problem of multidrug resistance (MDR) toward numerous antitumor compounds has also become important.[3] Many efforts have been directed toward the design of new antitumor anthracenedione derivatives with increased effectiveness against MDR tumor cell lines. Several structural classes have been identified. Derivatives with unsymmetrical side chains[4] and aza analogues containing pyridine[5] or pyridazine[6] rings are noteworthy. It appears that the most common type of anthracenedione modification, which targets overcoming multidrug resistance, is the incorporation into the chromophore of a five- or six-membered heterocyclic ring. This has resulted in the design of anthrapyrazoles[7] and their aza analogues,[8] anthrapyridones and anthrapyridazones.[9] It is of interest that a similar positive effect on overcoming multidrug resistance has been reported for the structurally related acridones, such as the imidazoacridones,[10] triazoloacridones,[11] pyrazoloacridones,[12] and pyrimidoacridones.[13]

We have previously reported on the synthesis and biological activity of a novel group of antineoplastic agents, the benzoperimidines **2** (Chart 1), which are

[§] Polish Patent Application P317493, Dec 12, 1996.
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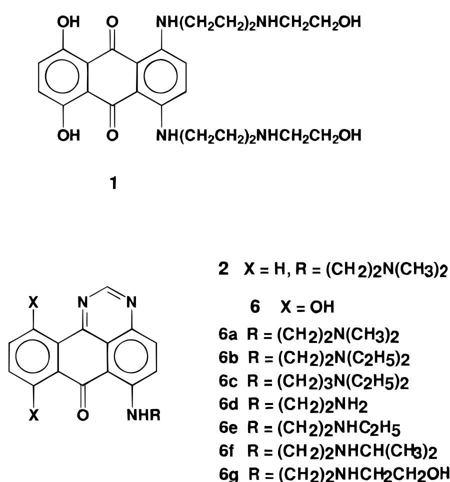
10.1021/jm980682p CCC: $18.00 © 1999 American Chemical Society
Published on Web 08/21/1999

**Chart 1**




chromophore-modified anthracenediones with a fused pyrimidine ring.[14] This class of compounds did not stimulate free radical formation, due to their poor enzyme substrate properties toward NADH dehydrogenase.[15] Our preliminary results have shown that these compounds exhibit in vitro cytotoxic activity toward multidrug resistant cell lines.[9b]

In this paper we present the synthesis and in vitro and in vivo evaluation of newer benzoperimidines along with data on their ability to overcome multidrug resistance. These studies include 6-[(aminoalkyl)amino]-7*H*-benzo[*e*]perimid-7-one derivatives **6a**−**g** with additional hydroxy groups incorporated into the C-8 and C-11 positions of the ring system. As noted earlier, the presence of hydroxy groups in related ring systems such as anthracenediones,[1a,b] anthrapyrazoles,[7a] and acridine derivatives[16,17] increases antitumor activity in these classes of compounds.

## Chemistry

8,11-Dihydroxy-6-[(aminoalkyl)amino]-substituted benzoperimidines **6a**−**g** were synthesized by two methods using 1,4-diamino-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**3**)[18] (method A) or 1-hydroxy-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**7**)[19] (method B) as starting materials (see Scheme 1). 6-Amino-8,11-bis(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (**4**) was obtained by refluxing 1,4-diamino-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**3**) with formamide in the presence of ammonium metavanadate in dimethylacetamide (method A). After removal of the benzyl groups with trifluoroacetic acid, the isolated product **5** was heated with appropriate aminoalkylamines, in a solvent mixture of *N*,*N*,*N'*,*N'*-tetramethylethylenediamine and generally a small amount of water.

1-Amino-4-hydroxy-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**8**) (method B) was prepared by reduction of 1-hydroxy-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**7**) using hydrazine and Pd/C. The product was cyclized with formamide in phenol to give **9**. After removal of the 8,11-benzyl groups, the derived 6,8,11-trihydroxy-7*H*-benzo[*e*]perimidin-7-one (**10**) was reacted with appropriate amines under similar conditions as described in method A.

The transamination reaction of **5** with ethylenediamine or N-monosubstituted ethylenediamines may be accompanied by a subsequent cyclization step to form side products.[14] However, when using 6,8,11-trihydroxybenzoperimidine as starting material (method B), we found that this reaction proceeds under milder conditions, thus diminishing the amount of the cyclized side product (reaction conditions are indicated in Table 1).

## Biological Results and Discussion

The 8,11-dihydroxy-6-[(aminoalkyl)amino]-substituted benzoperimidines **6a**−**g** were tested for the growth inhibition of L1210 murine and HL60 human leukemia cells in vitro. The results, including nonhydroxylated benzoperimidine **2** and reference compounds (mitoxantrone, ametantrone, and doxorubicin), are presented in Table 2. All the evaluated compounds **6a**−**g** exhibited significant in vitro cytotoxicity. Among them **6a** was shown to be the most active. Moreover all benzoperimidine derivatives demonstrated activity against the MDR cell line K562R (Table 2).

The compound most effective as a cytotoxic agent (**6a**) was tested in the NCI panel of human tumor cell lines.

**Scheme 1**

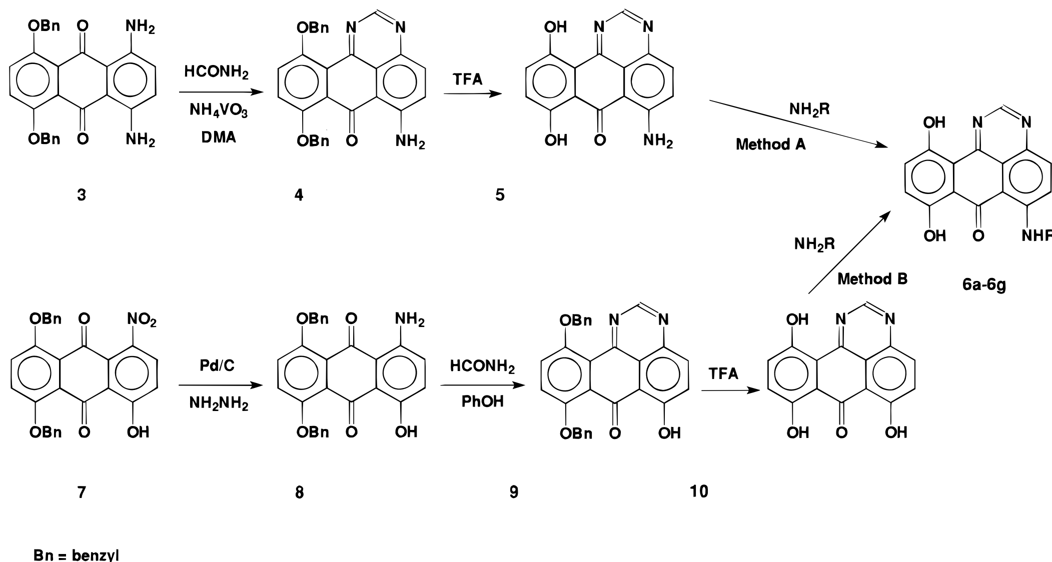


**Table 1.** Synthesis and Physicochemical Properties of 8,11-Dihydroxybenzoperimidines **6a**−**g**[a]

| compd | R | reaction conditions: method | time (h) | temp of the bath (°C) | yield (%) | mp (°C dec) | molecular formula |
|---|---|---|---|---|---|---|---|
| **6a** | $CH_2CH_2N(CH_3)_2$ | A | 5 | 130 | 36 | >300 | $C_{19}H_{18}N_4O_3$·HCl |
| | | B | 4 | 125 | 40 | | |
| **6b** | $CH_2CH_2N(C_2H_5)_2$ | A | 3 | 160 | 50 | >300 | $C_{21}H_{22}N_4O_3$·HCl |
| **6c** | $CH_2CH_2CH_2N(CH_3)_2$ | A | 3 | 135 | 42 | 295−300 | $C_{20}H_{20}N_4O_3$·HCl |
| **6d** | $CH_2CH_2NH_2$ | A | 1.5 | 65 | 24 | 280−285 | $C_{17}H_{14}N_4O_3$·HCl·$H_2O$[b] |
| | | B | 1.5 | 65 | 26 | | |
| **6e** | $CH_2CH_2NHC_2H_5$ | A | 12 | 100 | 24 | >300 | $C_{19}H_{18}N_4O_3$·HCl·0.5$H_2O$ |
| | | B | 6.5 | 80−85 | 30 | | |
| **6f** | $CH_2CH_2NHCH(CH_3)_2$ | A | 14 | 125−130 | 55 | >300 | $C_{20}H_{20}N_4O_3$·HCl·0.5$H_2O$ |
| | | B | 2.5 | 110 | 65 | | |
| **6g** | $CH_2CH_2NHCH_2CH_2OH$ | A | 4 | 135 | 30 | >300 | $C_{19}H_{18}N_4O_4$·HCl·$H_2O$[c] |
| | | B | 2 | 100 | 40 | | |

[a] The structures of compounds **6a**−**g** were confirmed by their spectral data ($^1$H NMR, IR, UV−vis, MS-FD) and by elemental analysis. [b] N: calcd, 14.87; found, 14.38. [c] H: calcd, 5.03; found, 5.56.

**Table 2.** In Vitro Cytotoxic Activity of 6-[(Aminoalkyl)amino]-7*H*-benzo[*e*]perimidine Derivatives

| compd | cell line $IC_{50}$ (nM) ± SEM: L1210[a] | HL60[b] | K562S[b] | K562R[b] (RI) |
|---|---|---|---|---|
| **6a** | 1.29 ± 0.26 | 1.67 ± 0.12 | 5.56 ± 0.21 | 10.41 ± 1.07 (1.87) |
| **6b** | 19.52 ± 6.27 | 16.58 ± 0.63 | 49.97 ± 1.71 | 78.31 ± 6.46 (1.57) |
| **6c** | 12.97 ± 6.49 | 21.04 ± 0.91 | 67.90 ± 5.46 | 140.0 ± 9.6 (2.06) |
| **6d** | 9.02 ± 1.86 | 11.68 ± 0.45 | 16.83 ± 1.57 | 45.41 ± 3.45 (2.70) |
| **6e** | 3.79 ± 1.52 | 14.45 ± 1.16 | 48.91 ± 2.15 | 89.12 ± 9.09 (1.82) |
| **6f** | 6.59 ± 2.68 | 8.38 ± 0.28 | 23.52 ± 0.48 | 53.41 ± 5.15 (2.27) |
| **6g** | 3.55 ± 0.95 | 6.62 ± 0.31 | 21.35 ± 1.30 | 42.51 ± 3.38 (1.99) |
| **2** | 203 ± 22 | 587 ± 34 | 1700 ± 340 | 1756 ± 316 (1.03) |
| ametantrone | 146 ± 47 | 15.9 ± 0.9 | 181 ± 18 | 12392 ± 886 (68.5) |
| mitoxantrone | 11.4 ± 5.7 | 0.81 ± 0.06 | 7.06 ± 0.32 | 133.5 ± 7.1 (18.9) |
| doxorubicin | NT[c] | 7.8 ± 0.8 | 15.6 ± 0.8 | 2474 ± 126 (158.6) |

[a] 48-h exposure to drug, method I. [b] 72-h exposure to drug, method II. [c] NT, not tested in these experiments.

It showed significant cytotoxic activity not only against several leukemic cell lines but also against a number of other tumor cell lines, including solid tumors. Of 60 cell lines tested against, the response parameter log $GI_{50}$ was <−8.00 (0.01 μM) for 19 cell lines and −8.00 ÷ −5.23 (0.01−5.9 μM) for the remaining cell lines (Table 3).

A comparison of the pattern of cytotoxic activity of the compound **6a** against the reference agents mitoxantrone, ametantrone, and doxorubicin in a panel of sensitive and resistant human cell lines of different origin (ovarian, colon, and prostate) is given in Table 4. Compound **6a** showed cytotoxic activity similar to that of mitoxantrone against the sensitive cell lines and retained activity toward the HT29 cells resistant to mitoxantrone (Mx). Moreover, **6a** showed a similar effect toward the LoVo/Dx and A2780/Dx cell lines.

The comparative effects of **6a** and mitoxantrone on the cell cycle parameters and apoptosis were studied in HT29-sensitive and HT29/Mx-resistant cell lines. As shown in Figure 1, in the sensitive cell line **6a** and mitoxantrone induced similar levels of toxicity, and at the $IC_{80}$, both drugs induced 34−37% of apoptosis. In contrast, in the resistant cell line treated at the $IC_{80}$ concentrations of drugs, **6a** induced a lower apoptotic response than mitoxantrone (15% and 37%, respectively). Both drugs induced a cell cycle accumulation in the G2/M phase in both the sensitive and resistant cell lines (Table 5). Moreover, there is an increase also in the S phase without a complete depletion of cells in G1 phase, suggesting that HT29 and HT29/Mx cells are actively cycling after 72 h of treatment.

On the basis of the results of cytotoxicity studies, compound **6a** was selected for in vivo evaluation. The

**Table 3.** Selected Growth Inhibition Data for **6a** from the NCI in Vitro Screen

| panel/cell line | log $GI_{50}$ | panel/cell line | log $GI_{50}$ |
|---|---|---|---|
| leukemia | | melanoma | |
| CCRF-CEM | <−8.00 | LOX IMVI | <−8.00 |
| HL-60(TB) | <−8.00 | MALME-3M | −7.31 |
| K-562 | <−8.00 | M14 | −7.65 |
| MOLT-4 | <−8.00 | SK-MEL-2 | −6.00 |
| RPMI-8226 | −7.60 | SK-MEL-28 | −5.73 |
| SR | <−8.00 | SK-MEL-5 | −7.47 |
| prostate cancer | | UACC-257 | −6.28 |
| DU-145 | −7.83 | UACC-62 | −7.54 |
| PC-3 | −7.42 | ovarian cancer | |
| CNS cancer | | IGROV1 | −7.45 |
| SF-268 | −7.50 | OVCAR-3 | −6.21 |
| SF-295 | <−8.00 | OVCAR-4 | −6.00 |
| SF-539 | −7.61 | OVCAR-5 | −6.51 |
| SNB-19 | −7.88 | OVCAR-8 | −7.46 |
| SNB-75 | <−8.00 | SK-OV-3 | −7.86 |
| U251 | −8.00 | breast cancer | |
| non-small-cell | | BT-549 | −6.79 |
| lung cancer | | HS 578T | −7.38 |
| A549/ATCC | <−8.00 | MCF7 | <−8.00 |
| EKVX | −6.24 | MCF7/ADR-RES | −6.62 |
| HOP-62 | <−8.00 | MDA-MB-231/ATCC | −5.90 |
| HOP-92 | −7.26 | MDA-MB-435 | −6.78 |
| NCI-H226 | <−8.00 | MDA-N | −6.54 |
| NCI-H23 | −7.59 | T-47D | −6.86 |
| NCI-H322M | −6.37 | renal cancer | |
| NCI-H460 | <−8.00 | 786-0 | <−8.00 |
| NCI-H522 | −7.51 | A498 | −5.74 |
| colon cancer | | ACHN | <−8.00 |
| COLO 205 | <−8.00 | CAKI-1 | <−8.00 |
| HCC-2998 | −7.05 | RXF-393 | −6.18 |
| HCT-116 | −7.89 | SN12C | <−8.00 |
| HCT-15 | −7.53 | TK-10 | −6.04 |
| HT29 | −7.61 | UO-31 | <−8.00 |
| KM12 | −6.51 | | |
| SW-620 | −7.88 | | |

**Table 4.** In Vitro Cytotoxic Activity of Compound **6a** in Comparison with Reference Compounds in a Panel of Human Sensitive and Resistant Cell Lines

| cell line | compound $IC_{50}$ (nM) ± SEM | | | |
|---|---|---|---|---|
| | **6a** | ametantrone | mitoxantrone | doxorubicin |
| DU145[a] | 5.7 ± 0.8 | 1020 ± 278 | 5.6 ± 1.6 | 52.8 ± 2.8 |
| PC3[a] | 11.6 ± 2.6 | 1848 ± 143 | 7.1 ± 0.7 | 102.8 ± 9.1 |
| HT/29[a] | 49.1 ± 25.6 | NT[d] | 19.6 ± 0.4 | 56.4 ± 8.1 |
| HT29/Mx[a] (RI) | 56.9 ± 20.7 (1.2) | NT | 2025 ± 890 (103) | 231.2 ± 15.5 (4.1) |
| LoVo[b] | 11.1 ± 2.3 | 641 ± 134 | 17.1 ± 3.8 | NT |
| LoVo/Dx[b] (RI) | 12.7 ± 2.1 (1.1) | 36802 ± 4835 (57.4) | 461.6 ± 87.4 (27) | NT[c] |
| A2780[a] | 8.3 ± 1.2 | 180 ± 30 | 6.11 ± 0.55 | 19.9 ± 5.4 |
| A2780/Dx[a] (RI) | 9.5 ± 1.4 (1.1) | 1108 ± 20 (6.2) | 25.1 ± 4.1 (4.1) | 466 ± 109 (23.4) |

[a] 72-h exposure to drug following 24 h of preincubation, method II. [b] 72-h exposure to drug, method I. [c] The RI = 30.4 after 24-h exposure to drug.[25] [d] NT, not tested in these experiments.

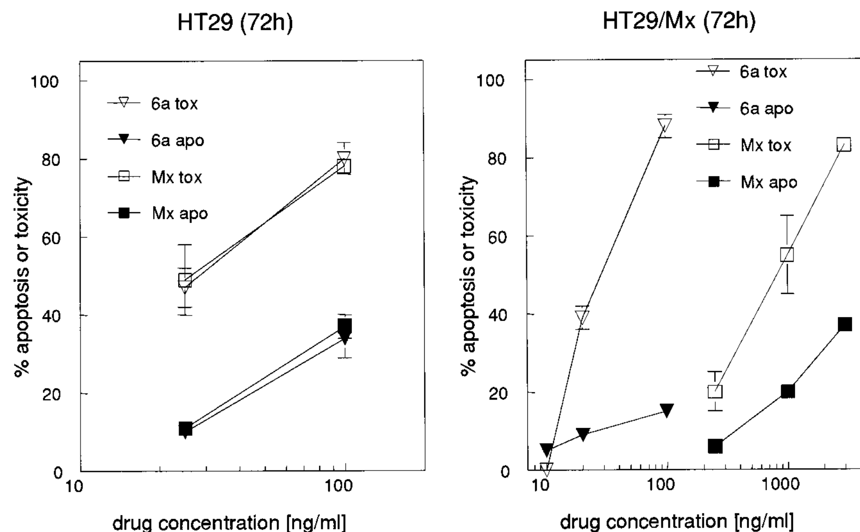



**Figure 1.** Apoptosis and toxicity of compound **6a** and mitoxantrone in HT29 and HT29/Mx cell lines. The percent apoptosis for the control (not treated cells) was 8 ± 1 and 4 ± 1 for HT29 and HT29/Mx cell lines, respectively. Apoptosis was assessed by fluorescence microscopy. The percentage of the apoptotic cells refers to the cell number of the whole population (floating + adherent cells). Toxicity was measured by cell counting. The percentage of toxicity refers to the cell number in control, not treated, cells.

**Table 5.** Effects of Compound **6a** and Mitoxantrone in HT29 and HT29/Mx Cell Lines

| cell line, compd (ng/mL) | % survival | cell cycle | | |
|---|---|---|---|---|
| | | %G1 | %S | %G2/M |
| HT29 (72 h) | | | | |
| control | | 56 ± 1 | 18 ± 1 | 25 ± 1 |
| **6a** | | | | |
| 25 | 53 ± 5 | 48 ± 3 | 19 ± 2 | 29 ± 4 |
| 100 | 20 ± 4 | 23 ± 3 | 21 ± 2 | 57 ± 2 |
| mitoxantrone | | | | |
| 25 | 51 ± 9 | 46 ± 1 | 20 ± 1 | 34 ± 1 |
| 100 | 22 ± 1 | 29 ± 2 | 24 ± 1 | 47 ± 1 |
| HT29/Mx (72 h) | | | | |
| control | | 62 ± 1 | 12 ± 4 | 26 ± 4 |
| **6a** | | | | |
| 10 | 100 | 63 | 7 | 30 |
| 20 | 61 ± 3 | 58 ± 2 | 11 ± 3 | 31 ± 3 |
| 100 | 12 ± 3 | 25 ± 3 | 17 ± 3 | 58 ± 5 |
| mitoxantrone | | | | |
| 250 | 80 ± 5 | 62 ± 1 | 8 ± 1 | 30 ± 2 |
| 1000 | 45 ± 10 | 47 ± 7 | 17 ± 5 | 37 ± 7 |
| 3000 | 17 | ND[a] | ND | ND |

[a] ND, not detectable.

antitumor activity of **6a** was examined initially in intraperitoneally (ip) growing murine P388 leukemia. When given as a single dose on day 1 or in a divided-dose schedule (5 consecutive days), **6a** resulted in significant life prolongation, which in the total dose range of 5−20 mg/kg was comparable in both applied schedules. Similar effects were observed upon single-dose administration of mitoxantrone; however, it displayed activity at much lower doses (Table 6). Preliminary evaluation of the toxicity of compound **6a** (ip) showed that 50% lethality in healthy CD2F1 mice was attained at the single dose of ca. 46 mg/kg (in the range of 43−49 mg/kg).[20] This $LD_{50}$ value was 2 times higher than that observed for mitoxantrone (20−22 mg/kg).

Compound **6a** was also tested in subcutaneously growing human ovarian carcinoma xenografts (Table 7). Against the A2780 tumor, good inhibition was achieved after 3 iv injections of 10 mg/kg. In keeping with the in vitro results, the antitumor efficacy of compound **6a** was maintained even in the MDR A2780/Dx tumor. Under the same experimental conditions, the activity of **6a** was comparable to that of mitoxantrone. In contrast, compound **6a** showed no activity in the human colon tumor HT29/Mx, even though higher doses were used (14 and 20 mg/kg). This was not surprising since the parent line of this tumor is known for being generally resistant to cytotoxic drugs. Thus, despite the low resistance index shown by this compound in the in vitro cell system (Table 4), no activity could be detected in the in vivo studies.

We have carried out mechanistic studies showing that the ability of benzoperimidine derivatives to overcome multidrug resistance in tumor cells is due to increased drug uptake relative to active P-gp-dependent and $MRP_1$-dependent efflux. As a consequence, the cellular

**Table 6.** In Vivo Antileukemic Activity of Compound **6a** toward P388 Murine Leukemia in Comparison with Mitoxantrone

| compd | schedule | total dose (mg/kg) | %T/C[a] | BWC[b] | toxic deaths[c] | LTS[d] |
|---|---|---|---|---|---|---|
| **6a** | D1 only | 1.0 | 118 | 1.05−1.06 | | 0/12 |
| | | 5.0 | 147 | 1.01−1.04 | | 1/18 |
| | | 10.0 | 172 | 0.98 | | 0/12 |
| | | 20.0 | 205 | 0.90 | | 0/6 |
| | | 30.0 | 171 | 0.88 | 2/6 | 0/6 |
| | | 35.0 | −110 | 0.73 | 5/6 | 0/6 |
| | QD1−5 | 2.5 | 145 | 0.98−1.04 | | 0/12 |
| | | 5.0 | 167 | 1.02−1.04 | | 1/18 |
| | | 10.0 | 196 | 0.98−1.00 | | 0/18 |
| | | 20.0 | 207 | 0.94 | | 0/6 |
| | | 22.5 | 215 | 0.88 | 1/6 | 0/6 |
| mitoxantrone | D1 only | 0.1 | 173 | 0.98 | | 0/12 |
| | | 0.2 | 174 | 1.02 | | 0/6 |
| | | 0.4 | 158 | 1.05 | | 0/12 |
| | | 0.8 | 229 | 1.02 | | 0/6 |

[a] The ratio of average survival times of treated to control mice, expressed as a percentage. Long-term survivors were not included in the calculation. [b] Relative change in mean body weight on day 5, expressed as a fraction of pretreatment body weight (day 1). Control mice BWC ranged from 1.07 to 1.1 in separate experiments. [c] Number of toxic deaths/total number of mice. [d] Number of long-term survivors (cures)/total number of mice.

**Table 7.** Antitumor Effects of Compound **6a** Administered iv to Nude Mice Bearing sc Human Tumor Xenografts

| tumor | treatment[a] schedule days | treatment[a] dose (single) (mg/kg) | %TVI[b] | toxic deaths[c] |
|---|---|---|---|---|
| A2780 | 4, 11, 18 | 6.7 | 32 | 0/4 |
| ovarian carcinoma | | 10 | 62 | 0/4 |
| A2780/Dx | 7, 14, 21 | 6.7 | 46 | 0/5 |
| ovarian carcinoma | | 10 | 80 | 0/4 |
| HT29/Mx | 4, 11, 18 | 14 | 7 | 0/4 |
| colon carcinoma | | 20 | 22 | 0/4 |

[a] Treatments started with just measurable tumors (80−100 mm$^3$) in all tested models. [b] Tumor volume inhibition percent in treated over control tumors, assessed 3−10 days after the last treatment. [c] Number of toxic deaths/total number of mice.

accumulation of benzoperimidines is not reduced in resistant cells compared to sensitive parent lines.[21]

Compound **6a** has also been evaluated as a substrate toward the enzyme NADH dehydrogenase, a property which correlates with the peroxidating ability of a compound. The generation of toxic oxygen radicals is thought to be responsible for the cardiotoxicity of anthraquinone antitumor drugs. We found that **6a** exhibits low efficiency in stimulating oxygen radical formation due to its poor enzyme substrate properties.[22]

The obtained results allow us to draw the following conclusions. One can postulate that the structural feature of the benzoperimidine class that is essential for the appearance of high cytotoxic activity against multidrug resistant tumor cell lines (resistance index close to 1) is the condensed pyrimidine heterocycle. Anthracenediones not bearing this moiety and having the same side chains (e.g., mitoxantrone) do not exhibit this highly desirable property. The kind of substitution at the benzoperimidine chromophore (side chain, phenolic groups) does not influence the ability of compounds to overcome multidrug resistance but affects their cytotoxic potency.

The overcoming multidrug resistance by benzoperimidine derivatives augments our suggestion, put forward in the introductory part of this paper, that the presence of a heterocyclic ring condensed with the anthracenedione or the related acridone chromophore determines cytotoxic activity toward the multidrug resistant cell lines.

## Experimental Section

Melting points were determined with a Boeticus PHMK05 apparatus and are uncorrected. Analyses are within ±0.4% of the theoretical values and were carried out on a Carlo Erba CHNS-O-EA1108 instrument. 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 spectrometer using tetramethylsilane as an internal standard. Molecular weights were determined by mass spectrometry (field desorption technique, MS-FD) on a Varian MAT 711 instrument. Column chromatography was performed on silica gel Merck (70−230 mesh) and on Sephadex LH-20 (Pharmacia).

**6-Amino-8,11-bis(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (4).** A sample of 0.45 g (1 mmol) of 1,4-diamino-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**3**),[18] 0.45 g of ammonium metavanadate, and 1.5 mL of formamide in 15 mL of *N*,*N*-dimethylacetamide was stirred under reflux for 30 min. The course of the reaction was followed by TLC in the solvent system toluene−acetone, 2:1. The reaction mixture was cooled, the crude product was precipitated with cold water, and the precipitate was collected and washed well with hot water. The crude product was purified using silica gel column chromatography ($CHCl_3$/MeOH, 50:1) to give the pure product **4** as an orange powder: yield 140 mg, 31%; mp 231−233 °C. $^1$H NMR ($CDCl_3$): $\delta$ 5.22 (s, 2H); 5.3 (s, 2H); 7.25 (d, 1H, $J = 9.19$ Hz); 7.3−7.5 (m, 10H); 7.6−7.7 (m, 2H); 8.05 (d, 1H, $J = 9.24$ Hz); 9.4 (s, 1H). MS-FD *m*/*z* (relative intensity, %): 458 ([M − 1]$^+$, 50); 459 ([M]$^+$, 100); 460 ([M + 1]$^+$, 50). Anal. ($C_{29}H_{21}N_3O_3$) C,H,N.

**6-Amino-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7one (5).** A solution of 460 mg of **4** in 5 mL of trifluoroacetic acid was left at room temperature for 10 h. The TFA was removed under reduced pressure by coevaporation with benzene to afford crude 6-amino-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (265 mg, 95%). An analytical sample was purified by use of column chromatography (silica gel) using the solvent system $CHCl_3$/MeOH (50:1). The pure compound was obtained as a dark-cherry powder: mp > 300 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.2 (d, 1H, $J = 9.0$ Hz); 7.35 (d, 1H, $J = 9.05$ Hz); 7.65 (d, 1H, $J = 8.0$ Hz); 8.1 (d, 1H, $J = 8.1$ Hz); 9.2 (s, 1H); 13.5 (s, 1H, exchangeable with $D_2O$); 13.82 (s, 1H, exchangeable with $D_2O$). MS-FD *m*/*z* (relative intensity, %): 279 ([M]$^+$, 100). Anal. ($C_{15}H_9N_3O_3$) C,H,N.

**1-Amino-4-hydroxy-5,8-bis(phenylmethoxy)-9,10-anthracenedione (8).** To a stirred solution of 2.4 g (5 mmol) of 1-hydroxy-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**7**) [19] in 100 mL of $CHCl_3$/MeOH (1:1) was added 30 mg of Pd/C (10%); then the reaction mixture was cooled to 0 °C and treated dropwise with 15 mL (0.5 mol) of hydrazine hydrate 98% during 20 min. After additional stirring for 0.5 h at room temperature the reaction mixture was filtered, diluted with

$CHCl_3$, and extracted with diluted HCl and water. The $CHCl_3$ layer was dried over $Na_2SO_4$, evaporated under reduced pressure to a small volume, and chromatographed utilizing $CHCl_3$/MeOH (100:1) as eluant. Crystallization from $CHCl_3$/petroleum ether afforded 1.7 g (80%) of **8**: mp 127–130 °C. $^1$H NMR ($CDCl_3$): $\delta$ 5.21 (s, 2H); 5.26 (s, 2H); 6.80 (br s, exchangeable with $D_2O$), 6.97 (d, 1H, $J = 9.4$ Hz), 7.14 (d, 1H, $J = 9.4$ Hz), 7.2–7.6 (m, 12H), 13.7 (s, 1H, exchangeable with $D_2O$). Anal. ($C_{28}H_{21}NO_5$) C,H,N.

**6-Hydroxy-8,11-bis(phenylmethoxy)-7*H*-benzo[*e*]perimidin-7-one (9).** A mixture of 225 mg (0.5 mmol) of **8** and 1 mL (25 mmol) of formamide was heated in 5 g of phenol for 30–40 min at 165 °C. The course of the reaction was monitored by TLC ($CHCl_3$/MeOH, 10:1). The reaction mixture was diluted with $CHCl_3$ and extracted several times with 2 N NaOH and then with water. The $CHCl_3$ layer was dried over $Na_2SO_4$, evaporated to a small volume, and purified by column chromatography utilizing the solvent system $CHCl_3$/MeOH (50:1). For analytical data a sample of **9** was further purified by additional chromatography on a silica gel column (benzene/acetone, 10:1). The product **9** was obtained in the yield of 30 mg (12%): mp 225–227 °C. $^1$H NMR ($CDCl_3$): $\delta$ 5.25 (s, 2H); 5.3 (s, 2H); 7.2 (d, 1H, $J = 9.0$ Hz); 7.3–7.5 (m, 10H); 7.6–7.7 (m, 2H); 8.0 (d, 1H, $J = 9.1$ Hz); 9.35 (s, 1H); 13.6 (s, 1H, exchangeable with $D_2O$). MS-FD *m*/*z* (relative intensity, %): 459 ($[M - 1]^+$, 30), 460 ($[M]^+$). Anal. ($C_{29}H_{20}N_2O_4$) C,H,N.

**6,8,11-Trihydroxy-7*H*-benzo[*e*]perimidin-7-one (10).** 460 mg (1 mmol) of **9** in 5 mL of trifluoroacetic acid was left at room temperature for 20 h. The product was isolated and purified similarly as described for **5** to give 260 mg (95%) of the crude product **10.** An analytical sample was purified on a silica gel column utilizing the solvent system $CHCl_3$/MeOH (50:1): mp > 300 °C dec. $^1$H NMR (TFA): $\delta$ 7.32 (d, 2H, $J = 1.4$ Hz); 7.61 (d, 1H, $J = 9.4$ Hz); 8.03 (d, 1H, $J = 9.4$ Hz); 8.85 (s, 1H).

**General Procedure for the Synthesis of Compounds 6a–g. Method A. 8,11-Dihydroxy-6-[[2-(dimethylamino)-ethyl]amino]-7*H*-benzo[*e*]perimidin-7-one Hydrochloride (6a).** A sample of 280 mg (1 mmol) of 6-amino-8,11-dihydroxy-7*H*-benzo[*e*]perimidin-7-one (**5**) in a mixture of 6 mL of 2-(dimethylamino)ethylamine, 2 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and 0.8 mL of water was stirred and refluxed under a nitrogen atmosphere for 5 h. The progress of the reaction was followed by TLC (silica gel) in the solvent system $CHCl_3$/MeOH (10:1). The reaction mixture was diluted with $CHCl_3$ and washed with diluted HCl followed by water to remove the excess of amines. The organic layer was dried over $Na_2SO_4$ and evaporated under reduced pressure, and the residue was chromatographed on a silica gel column using gradient elution with $CHCl_3$/MeOH (10:1, 5:1) and then with $CHCl_3$/MeOH–25% $NH_4OH$ (5:1:0.1). The pure 8,11-dihydroxy-6-[[2-(dimethylamino)ethyl]amino]-7*H*-benzo[*e*]perimidin-7-one (**6a**) was converted into its hydrochloride salt, using an etheral solution of hydrogen chloride, which was then crystallized from MeOH/$H_2O$ (30:1), to give **6a** as a dark brown powder (150 mg, 36%): mp > 300 °C dec. UV–vis (MeOH): $\lambda_{max}$ nm ($\epsilon$) 540 (17343). IR (KBr, major peaks, cm$^{-1}$): 1460, 1565, 1620. $^1$H NMR (as free base $CDCl_3$): $\delta$ 2.4 (s, 6H); 2.8 (t, 2H, $J = 6.5$ Hz); 3.65 (q, 2H, $J = 6.5$ Hz); 7.16 (d, 1H, $J = 9.0$ Hz); 7.3 (d, 1H, $J = 9.0$ Hz); 7.5 (d, 1H, $J = 9.1$ Hz); 8.0 (d, 1H, $J = 9.5$ Hz); 9.1 (s, 1H); 10.8 (m, 1H, $D_2O$ exchangeable); 13.4 (s, 1H, exchangeable with $D_2O$); 14.0 (s, 1H, exchangeable with $D_2O$). MS-FD *m*/*z* (relative intensity, %): 349 ($[M - 1]^+$, 30); 350 ($[M]^+$, 100). Anal. ($C_{19}H_{18}N_4O_3 \cdot HCl$) C,H,N.

**Method B.** A sample of **10** (280 mg, 1 mmol) in 5 mL of 2-(dimethylamino)ethylamine, 5 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and 0.4 mL of water was stirred and heated at 125° C under nitrogen for 4 h. The course of the reaction was monitored by TLC (silica gel) in the solvent system $CHCl_3$/MeOH/25% $NH_4OH$ (5:1:0.1). The isolation and purification procedures of the crude products were the same as described in method A.

**Biological Tests. 1. 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 HL60 promyelocytic leukemia cells were grown in RPMI 1640 medium supplemented with 10% FBS. Human myelogenous leukemia-sensitive cell line K562S and doxorubicine-resistant subline K562R (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 colon adenocarcinoma-sensitive cell line LoVo and doxorubicin-resistant subline LoVo/Dx (Farmitalia Carlo Erba, Nerviano, Italy) were grown as monolayer cultures in Ham's F-12 medium supplemented with 10% FBS, penicillin G (100 000 units/L), streptomycin (100 mg/L), 1% of a 200 mM L-glutamine solution, and 1% of a MEM vitamin solution 100X. Human colon adenocarcinoma-sensitive cell line HT29 and mitoxantrone-resistant subline HT29/Mx (Boehringer Mannheim, Monza, Italy) were grown as monolayer cultures in McCoy's 5A medium supplemented with 10% FBS, and 1% 1 M Hepes. Human ovarian carcinoma-sensitive cell line A2780 and doxorubicin-resistant subline A2780/Dx, human prostate carcinoma DU145, and human prostate adenocarcinoma PC3 were grown as monolayer cultures in RPMI 1640 medium supplemented with 10% FBS. Cell lines were grown in a controlled (air–5% $CO_2$) humidified atmosphere at 37 °C and were transplanted twice a week. For the experiments the cells in logarithmic growth were suspended in the growth medium to give a final required density.

**2. In Vitro Cytotoxic Evaluation.** Cells of required density were seeded, and different concentrations of the drugs were added after or without preincubation of the cells in drug-free medium. The experiments were carried out at times indicated in a controlled (air–5% $CO_2$) humidified atmosphere at 37 °C. The cytotoxic activity ($IC_{50}$ values) of the compounds were defined as their in vitro concentrations causing 50% inhibition of cell growth after exposure to the drug, as measured by the protein content of the cells according to the Lowry method as described previously[23] (method I) or by cell counting with a ZbI Coulter Counter (Coulter Electronics, Ltd., U.K.) (method II). 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 the resistant cell line to $IC_{50}$ value for the sensitive cell line.

**3. 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 U.S. National Cancer Institute.[24] For experimental purposes, $10^6$ cells were injected ip to CD2F1 (Balb/c × DBA/2) mice. 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. Human tumor xenografts were maintained by sc passages of tumor fragments in Swiss nude athymic mice (Charles River Laboratories, Calco, Italy). For experimental purposes, tumor fragments were grafted sc in both flanks of athymic mice. Each group included 4–5 mice (for a total of 8–10 tumors). Tumor growth was followed by biweekly measurements of tumor diameters. Tumor volume (TV) was calculated according to the formula: TV (mm$^3$) = $d^2 \times D/2$, where $d$ and $D$ are the shortest and longest diameters, respectively. Drug treatment started when mean TV was ca. 80–100 mm$^3$.

**4. Assessment of Apoptosis.** Apoptosis was assessed by fluorescence microscopy. After 72 h of treatment (HT29 and HT29/Mx cell lines), floating and adherent cells were collected, washed in PBS, and fixed in 70% ice-cold ethanol. Samples were then stored at −20 °C until analysis (2–5 days). After rehydratation in PBS, cells were stained with propidium iodide solution (30 $\mu$g/mL propidium iodide and 66 units/mL RNase A in PBS) and stored in the dark for 30 min. At least 100 cells in two different smears were examined for their nuclear morphology changes (condensed nuclei and/or fragmented chromatin). The percentage of the apoptotic cells refers to the cell number of the whole population (floating + adherent cells).

**5. Cell Cycle Analysis.** Cell cycle perturbations were studied with a FACScan flow cytometer (Becton Dickinson, CA), equipped with an argon laser. In brief, after 72 h of treatment fixed cells (HT29 and HT29/Mx cell lines) were washed twice and resuspended in PBS containing 30 $\mu$g/mL propidium iodide and 66 units/mL RNase A. At least $10^4$ cells were collected and evaluated for the DNA content. The cell cycle distributions were calculated by LYSYS II software (Becton Dickinson, CA).

**Acknowledgment.** Financial support from the State Committee for Scientific Research, Warszawa, Poland (Grant No. PBZ 040-07), and, in part, Italian MURST (Fondi 40%), Roma, Italy, and the Chemical Faculty Technical University of Gdańsk, Poland, is gratefully acknowledged. Part of the in vitro work was supported by a fellowship (M.M.B.-G.) from the International Union Against Cancer (UICC). The authors thank Dr. V. L. Narayanan from the National Cancer Institute, Bethesda, MD, for arranging the preliminary in vitro screening.

## References

(1) (a) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. Antitumor Agents 1. 1,4-Bis[(aminoalkyl)amino]-9,10-anthracenediones. *J. Med. Chem.* **1979**, *22*, 1024−1030. (b) Cheng, C.; Zee-Cheng, R. K. Y. The Design, Synthesis and Development of a New Class of Potent Antineoplastic Anthraquinones. *Prog. Med. Chem.* **1983**, *20*, 83−118. (c) Shenkenberg, T. D.; Von Hoff, D. D. Mitoxantrone: A New Anticancer Drug with Significant Clinical Activity. *Ann. Int. Med.* **1986**, *105*, 67−81. (d) Koeller, J.; Eble, M. Mitoxantrone: A Novel Anthracycline Derivative. *Clin. Pharm.* **1988**, *7*, 574−581. (e) Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. Mitoxantrone. A Review of its Pharmacodinamic and Pharmacokinetic Properties, and Therapeutic Potential in the Chemotherapy of Cancer. *Drugs* **1991**, *41*, 400−449.

(2) (a) Tumminello, F. M.; Leto, G.; Gebbia, N.; Gebbia, V.; Russo, A.; Rausa, L. Acute Myocardial Effects of Mitoxantrone in the Rabbit. *Cancer Treat. Rep.* **1987**, *71*, 529−531. (b) Perkins, W. E.; Schroeder, R. L.; Carrano, R. A.; Imondi, A. R. Myocardial Effects of Mitoxantrone and Doxorubicin in the Mouse and Guinea Pig. *Cancer Treat. Rep.* **1984**, *68*, 841−847.

(3) (a) Selassie, C. D.; Hansch, C.; Khwaja, T. A. Structure−Activity Relationships of Antineoplastic Agents in Multidrug Resistance. *J. Med. Chem.* **1990**, *33*, 1914−1919. (b) Bradley, G.; Juranka, P. F.; Ling, V. Mechanism of Multidrug Resistance. *Biochim. Biophys. Acta* **1988**, *948*, 87−128. (c) Endicott, J. A.; Ling, V. The Biochemistry of P-Glycoprotein-Mediated Multidrug Resistance. *Annu. Rev. Biochem.* **1989**, *58*, 137−171.

(4) Krapcho, A. P.; Getahun, Z.; Avery, K. L., Jr.; Vargas, K. J.; Hacker, M. P.; Spinelli, S.; Pezzoni, G.; Manzotti, C. Synthesis and Antitumor Evaluation of Symmetrically and Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones and 1,4-Bis[(aminoalkyl)amino]-5,8-dihydroxy-anthracene-9,10-diones. *J. Med. Chem.* **1991**, *34*, 2373−2380.

(5) Krapcho, A. P.; Petry, M. E.; Getahun, Z.; Landi, J. J., Jr.; Stallman, J.; Polsenberg, J. F.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P.; Giuliani F. C.; Beggolin, G.; Pezzoni, G.; Menta, E.; Manzotti, C.; Oliva, A.; Spinelli, S.; Tognella, S. 6,9-Bis[(aminoalkyl)amino]benzo[*g*]isoquinoline-5,10-diones. A Novel Class of Chromophore-Modified Antitumor Anthracene-9,10-diones: Synthesis and Antitumor Evaluation. *J. Med. Chem.* **1994**, *37*, 828−837.

(6) Gandolfi, C. A.; Beggiolin, G.; Menta, E.; Palumbo, M.; Sissi, C.; Spinelli, S.; Johnson, F. Chromophore-Modified Antitumor Anthracenediones: Synthesis, DNA Binding, and Cytotoxic Acivity of 1,4-Bis[(aminoalkyl)amino]benzo[*g*]-phthalazine-5,10-diones. *J. Med. Chem.* **1995**, *38*, 526−536.

(7) (a) 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. Anthrapyrazole Anticancer Agents. Synthesis and Structure−Activity Relationships Against Murine Leukemias. *J. Med. Chem.* **1987**, *30*, 121−131. (b) Showalter, H. D. H.; Werbel, L. M.; Leopold, W. R.; Fry, D. W.; Klohs, W. D.; Jackson, R. C. Design, Tumor Biology, and Biochemical Pharmacology of Anthrapyrazoles. In *Anthracycline and Anthracenedione-based Anticancer Agents*; Lown, J. W., Ed.; Elsevier Science Publishers B.V.: Amsterdam, The Netherlands, 1988; pp 201−243. (c) Klohs, W. D.; Steinkampf, R. W.; Havlick, M. J.; Jackson, R. C. Resistance to Anthrapyrazoles and Anthracyclines in Multidrug-resistant P388 Murine Leukemia Cells: Reversal by calcium Blockers and Calmodulin Antagonists. *Cancer Res.* **1986**, *46*, 4352−4356.

(8) 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. Synthesis and Antitumor Evaluation of 2,5-Disubstituted-Indazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones (9-Aza-anthrapyrazoles). *J. Med. Chem.* **1998**, *41*, 5429−5444.

(9) (a) Stefańska, B. Unpublished data. (b) Bontemps-Gracz, M. M.; Stefańska, B.; Dzieduszycka, M.; Borowski, E.; Antonini, I.; Martelli, S.; Supino, R.; Zunino, F. Biological Characterization of Novel Anthracenedione Analogues and Related Compounds with Fused Heterocyclic Rings. Effectiveness against Multidrug Resistant Tumor Cell Lines. Multidyscyplinarna Konferencja Nauki o Leku (Multidisciplinary Conference of Medicinal Science), March 3−5, 1999, Muszyna, Poland; Abstract Book, Abstract P-80.

(10) (a) Chołody, W. M.; Martelli, S.; Paradziej-Łukowicz, J.; Konopa, J. 5-[(Aminoalkyl)amino]imidazo[4,5,1-*de*]acridine-6-ones as a Novel Class of Antineoplastic Agents: Synthesis and Biologcal Activity. *J. Med. Chem.* **1990**, *33*, 49−52. (b) Berger, B.; Marquardt, H.; Westendorf, J. Pharmacological and Toxicological Aspects of New Imidazoacridinone Antitumor Agents. *Cancer Res.* **1996**, *56*, 2094−2104.

(11) (a) Chołody, W. M.; Martelli, S.; Konopa, J. 8-Substituted 5-[(Aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acidin-6-ones as Potential Antineoplastic Agents. Synthesis and Biological Activity. *J. Med. Chem.* **1990**, *33*, 2852−2856. (b) Składanowski, A. Unpublished data.

(12) Sugaya, T.; Mimura, Y.; Shida, Y.; Osawa, Y.; Matsukuma, I.; Ikeda, S.; Akinaga, S.; Morimoto, M.; Ashizawa, T.; Okabe, M.; Ohno, H.; Gomi, K.; Kasai, M. 6*H*-Pyrazolo[4,5,1-*de*]acridin-6-ones as a Novel Class of Antitumor Agents. Synthesis and Biological Activity. *J. Med. Chem.* **1994**, *37*, 1028−1032.

(13) Antonini, I.; Cola, D.; Polucci, P.; Bontemps-Gracz, M.; Borowski, E.; Martelli, S. Synthesis of (Dialkylamino)alkyl-disubstituted Pyrimido[5,6,1-*de*]acridines, a Novel Group of Anticancer Agents Active on a Multidrug Resistant Cell Line. *J. Med. Chem.* **1995**, *38*, 3282−3286.

(14) Stefańska, B.; Dzieduszycka, M.; Martelli, S.; Tarasiuk, J.; Bontemps-Gracz, M.; Borowski, E. 6-[(Aminoalkyl)amino]-Substituted 7*H*-Benzo[*e*]perimidin-7-ones as Novel Antineoplastic Agents. Synthesis and Biological Evaluation. *J. Med. Chem.* **1993**, *36*, 38−41.

(15) Tarasiuk, J.; Garnier-Suillerot, A.; Stefańska, B.; Borowski, E. The Essential Role of Anthraquinones as Substrates for NADH Dehydrogenase in Their Redox Cycling Activity. *Anti-Cancer Drug Des.* **1992**, *7*, 329−340.

(16) Chołody, W. M.; Martelli, S.; Konopa, J. Chromophore-Modified Antineoplastic Imidazoacridinones. Synthesis and Activity Against Murine Leukemias. *J. Med. Chem.* **1992**, *35*, 378−382.

(17) Kato, N.; Sugaya, T.; Mimura, T.; Ikuta, M.; Kato, S.; Kuge, Y.; Tomioka, S.; Kasai, M. Facile and Efficient Synthesis of 7,10-Dihydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one via Hypervalent Iodine Oxidation. *Synthesis* **1997**, 625−627.

(18) Dzieduszycka, M.; Stefańska, B.; Borowski, E. Synthesis of 1,4 Diamino-5,8-dihydroxy-9,10-anthracenedione via Gabriel Reaction. *Pol. J. Chem.* **1994**, *68*, 1669−1671.

(19) Showalter, H. D. H.; Turner, W. R. Anticancer Anthrapyrazoles. The Synthesis of 2-Substituted 5-Nitro and 5-Aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones. *J. Heterocycl. Chem.* **1993**, *30*, 493−496.

(20) Matuszyk, J.; Kuœnierczyk, H.; Radzikowski, C. Biological Evaluation of Mitoxantrone Dihydrochloride Synthesized by a New Methodol. I. Acute Toxicity and Antitumor Activity in Mouse Transplantable Tumor Systems. *Arch. Immunol. Ther. Exp.* **1989**, *37*, 77−87.

(21) Tkaczyk-Gobis, K.; Tarasiuk, J.; Stefańska, B.; Dzieduszycka, M.; Seksek, O.; Borowski, E.; Garnier-Suillerot, A. Mechanism of Action of Benzoperimidines Active on Multidrug Resistant Tumor Cell Lines. Multidyscyplinarna Konferencja Nauki o Leku (Multidisciplinary Conference of Medicinal Science), March 3−5, 1999, Muszyna, Poland; Abstract Book, Abstract P-81.

(22) Tarasiuk, J.; Tkaczyk-Gobis, K.; Stefańska, B.; Dzieduszycka, M.; Priebe, W.; Martelli, S.; Borowski, E. The Role of Structural Factors of Anthraquinones and Their Heteroatomic Analogues in NADH Dehydrogenase Catalysed Oxygen Radical Formation. *Anti-Cancer Drug Des.* **1998**, *13*, 923−939.

(23) Bontemps-Gracz, M.; Milewski, S.; Borowski, E. The Influence of L-Norvalyl-N3−4-methoxyfumaroyl-L-2,3-diaminopropanoic Acid, an Antifungal Agent, on Mammalian Cells in Tissue Culture. *Acta Biochim. Pol.* **1991**, *38*, 229−239.

(24) (a) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems. *Cancer Chemother. Rep.* **1972**, *3*, 1−88. (b) *In vivo Cancer Models*; NIH Publication No. 84-2635, 1984; p 27.

(25) Grandi, M.; Geroni, C.; Giuliani, F. C. Isolation and Characterization of a Human Colon Adenocarcinoma Cell Line Resistant to Doxorubicin. *Br. J. Cancer* **1986**, *54*, 515−518.

JM980682P