

Synthesis of Symmetrical 1,5-bis-thio-Substituted Anthraquinones for Cytotoxicity in Cultured Tumor Cells and Lipid Peroxidation

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The synthesis of a series of anthraquinone moieties bearing symmetrical sulfur-linked substituents in the 1 and 5 positions is described. These compounds were evaluated for their ability to inhibit the growth of suspended rat glioma C6 cells and human hepatoma G2 cells, respectively. In addition, the redox property of the compounds was determined based on the inhibition of lipid peroxidation in model membranes. Compounds **2a** and **2h** in this series compared favorably and exhibited the most potent cytotoxicity (0.02, 0.05 μM) against C6 cells in the XTT colorimetric assay. As far as redox properties are concerned, all bis-thio-anthraquinones show potential lipid peroxidation in model membranes very close to that of mitoxantrone (MX), and **2a**, **2d**, **2e**, **2i**, **2j**, and **2k** have more potential than that of MX. The lack of cytotoxicity of compound **2i** cannot be related to lipid peroxidation, but the steric and electronic properties of the side-chain substituent may impair effective recognition of the cleavable complex. In contrast to MX, **2a** and **2h** are cytotoxic in rat glioma C6 cells and do not enhance lipid peroxidation in model membranes.

Key words anthraquinone; cytotoxicity; lipid peroxidation; rat glioma C6 cell; human hepatoma G2 cell

The anthraquinone mitoxantrone (MX) has been shown to have outstanding antitumor activities but a much narrower spectrum of activity in comparison with those of the anthracyclines.¹⁾ Its planarity allows an intercalation between base pairs of DNA in the conformation, while its redox properties are linked to the production of radical species in biological systems.²⁾ The chemical and biological activity exhibited by anthraquinone compounds is greatly affected by the different substituents of the planar ring system.^{3–6)} It appears that the relative location of the planar and side-chain groups plays a major role in affecting enzyme function and sequence specificity.^{7–9)} In MX and active congeners, the substituents are linked to the anthraquinone structure *via* a C–S linkage.

We have recently started to explore the changes in the physicochemical and biological properties of unsymmetrical substituted anthracenes and symmetrical substituted anthraquinones, which exhibit unsymmetrical and symmetrical bis-substituted C–O–C and C–S–C linkage between the planar ring system and the side-chain substituents.^{10–15)} Structure–activity relationship studies led to the discovery of symmetrical thio-substituted anthraquinones, which showed potent cytotoxicity with in terms of mean IC_{50} value in cultured tumor cells and lipid peroxidation, respectively. Since some of the compounds retained remarkable biological activity, this class appears to be worthy of further examination.

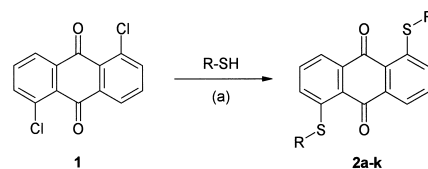
Compounds **2a** and **2h** showed potent cytotoxicity against rat glioma C6 cells, with IC_{50} values of 0.02 and 0.05 μM , respectively, comparable to that of MX (IC_{50} value of 0.07 μM). The goal of the present study was an evaluation of the importance of the symmetrical bis-thio-substituted side-arm patterns at the 1 and 5 positions of the anthraquinone ring system. Not only would the nature of the side arms influence the binding of the anthraquinone–DNA intercalant, but they also might be intimately involved in lipid peroxidation. This has enabled us to address the issue of how, and to what extent, the position of side-chain substituents affects biological ac-

tivity.

Chemistry

We report here a convenient synthetic pathway that leads to symmetrically substituted 1,5-bis-thio-anthraquinone derivatives. Moreover, the preliminary ESR studies revealed that the reduction power of anthracenones substituted with an electron-withdrawing group in the C-10 position is impeded, whereas that of 10-thio derivatives is increased.^{16,17)} The reaction undergoes a nucleophilic substitution at the 1 and 5 positions with the appropriate thiols in the presence of sodium methoxide and THF at room temperature or after reflux for 1 to 2 h to generate this structural class of anthraquinones. The mechanism for the reaction may be rationalized assuming that thiols are ionized by sodium methoxide as nucleophiles undergo nucleophilic substitution.

The synthesis of 1,5-bis-thio-anthraquinone derivatives shown in Chart 1 was accomplished using procedures somewhat modified from those described elsewhere.^{10–12)} The structural assignments for the symmetrical products as **2a–k** are based on ¹H- and ¹³C-NMR data. Compound **2a** has absorption for the H-4,8 protons at δ 8.11 (t, 2H), H-3,7 protons at δ 7.66 (t, 2H), and H-2,6 protons at δ 7.60 (d, 2H), respectively. Furthermore, the ¹³C-NMR spectra of these compounds exhibited carbonyl resonance of an anthra-



(a) Sodium methoxide/methanol; tetrahydrofuran, reflux. R is defined in Table 1.

Chart 1

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quinone chemical shift in the δ 183.3—182.4 region for C-9,10. In addition, ^1H - and ^{13}C -NMR correlations of these and other data allowed for assignments and are included in the experimental protocols.

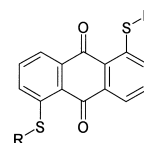
A study of the effects of substituents on the electronic spectrum of anthraquinone showed that the nature and number of the substituents, as well as their position in the anthraquinone nucleus, play a major role. Anthraquinone may be considered to consist of two isolated benzoyl chromogens in which little or no interaction would be expected between substituents located in different benzene rings. In contrast, electron-donating substituents can have a significant effect (bathochromic shift), producing dyes that vary in color from yellow through red.¹⁸⁾

Results and Discussion

Anthracyclines and anthraquinones form ternary complexes with DNA and the enzyme and stimulate DNA cleavage in a sequence-specific manner.¹⁹⁾ The cytotoxicity of a series of symmetrical *bis*-substituted anthraquinone derivatives were studied against murine and human cultured tumor cells using the lipid peroxidation assay. Their cytotoxicity properties were compared with that of MX as documented by the reactivity of the novel compounds and potent *in vitro* activity against C6 cells and hep G2 cells over a wide range of structural variants. There did not appear to be any correlation between these two cell lines. In addition, the redox property of the compounds for the inhibition of lipid peroxidation in model membranes was determined. Compounds **2a** and **2h** possess potent cytotoxicity with IC_{50} values of 0.02 and 0.05 μM in the inhibition of rat glioma C6 cell growth in culture by using the XTT colorimetric assay, while their antiproliferative activity is markedly enhanced and comparable to that of the positive control MX, which had an IC_{50} value of 0.07 μM .

The degree of peroxidation is related to the formation of thiobarbituric acid-reactive compounds and is expressed relative to that of malondialdehyde (MDA).²⁰⁾ It is also a measure of hydroxyl radical generation and thus reflects the prooxidant properties of the anthraquinones. Various data have emphasized the significance of free radicals and end-products derived from the lipid peroxidation of compounds that may help prevent tissue injury. Most of the anthraquinones examined, *e.g.*, **2a**, **2d**, **2e**, **2j**, and **2k**, exhibit greater lipid peroxidation than that of MX and, except for **2a** and **2h** cytotoxic properties, considerably better than that of MX. The *bis*-ethyl-thio analogue **2a** showed potential cytotoxicity against the C6 cell line when compared with MX; the *bis*(*p*-aminophenyl-thio) substitution of **2h** also showed good cytotoxicity. Compound **2i** exhibited stronger antioxidant activity than ascorbic acid, (+)- α -tocopherol, anthrurufin, and MX as further evaluated at several concentrations against positive controls with lipid peroxidation (Table 2). At the concentration of 0.01 mM, **2i** retained activity of 24%, the activity of ascorbic acid decreased to 10%, and (+)- α -tocopherol had no effect at 0.1 mM. In conclusion, this study confirms the dual-function activity the tested compounds against both suspended rat glioma C6 cells and human hepatoma G2 cells and lipid peroxidation and suggests that some symmetrical anthraquinone derivatives may be useful for the treatment of tumors.

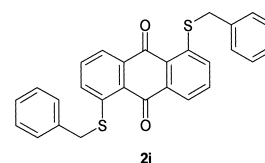
Table 1. Cytotoxicity against the Growth of Suspended Murine and Human Tumor Cell Lines and Inhibitory Effect of Anthraquinone Derivatives on Iron-Induced Lipid Peroxidation in Rat Brain Homogenates



| Compound | R | IC_{50} (μM) ^{a)} | | LP (%) (10 mM) ^{b)} |
|-----------|---|--|------------------------|------------------------------|
| | | Hep G2 ^{c)} | C6 cells ^{d)} | |
| 2a | CH_2CH_3 | 12.2 \pm 1.1 | 0.02 \pm 0.01 | 83 \pm 2.2 |
| 2b | $\text{CH}_2\text{CH}_2\text{OH}$ | 36.4 \pm 1.5 | 21.5 \pm 0.8 | 16 \pm 2.2 |
| 2c | $\text{CH}_2\text{CH}_2\text{CH}_3$ | 75.1 \pm 2.5 | 29.9 \pm 2.1 | 15 \pm 1.5 |
| 2d | $\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$ | 34.3 \pm 1.8 | 38.5 \pm 1.5 | 83 \pm 1.1 |
| 2e | $(\text{CH}_2)_6\text{OH}$ | 49.3 \pm 2.1 | 31.7 \pm 1.6 | 54 \pm 1.9 |
| 2f | 2- $\text{NH}_2\text{C}_6\text{H}_4$ | 34.0 \pm 1.7 | 15.1 \pm 1.7 | 5 \pm 0.5 |
| 2g | 3- $\text{NH}_2\text{C}_6\text{H}_4$ | 21.5 \pm 1.2 | 26.3 \pm 2.8 | 6 \pm 0.9 |
| 2h | 4- $\text{NH}_2\text{C}_6\text{H}_4$ | 17.4 \pm 1.5 | 0.05 \pm 0.01 | 20 \pm 1.4 |
| 2i | $\text{CH}_2\text{C}_6\text{H}_5$ | 41.5 \pm 2.5 | 38.2 \pm 4.4 | >100 |
| 2j | $\text{CH}_2\text{C}_6\text{H}_4(\text{OCH}_3)(p)$ | 28.6 \pm 1.2 | 25.1 \pm 2.8 | 67 \pm 2.9 |
| 2k | $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$ | 36.9 \pm 1.5 | 32.9 \pm 3.3 | 69 \pm 1.5 |
| | Mitoxantrone | 2.0 \pm 0.5 | 0.07 \pm 0.01 | 54 \pm 1.5 |
| | Ascorbic acid | | | >100 |
| | (+)- α -Tocopherol | | | >100 |
| | Anthrurufin | | | -36 \pm 1.9 |

a) IC_{50} , drug concentration inhibiting 50% of cellular growth following 48 h of drug exposure. Values are in μM and represent an average of three experiments. The variance for the IC_{50} values was less than $\pm 20\%$. Inhibition of cell growth was significantly different with respect to that of the control; $n=3$ or more, $p<0.01$. b) Relative percentage of inhibition. Inhibition was compared with that of the control (ascorbic acid, α -tocopherol and mitoxantrone-HCl), $p<0.01$, mean \pm S.E., $n=4$. Values are mean percent inhibition at the indicated concentration (mM), and standard errors. c) Hep G2, human hepatoma G2 cells. d) C6 cells, rat glioma C6 cells.

Table 2. Inhibitory Effects of **2i** on Iron-Induced Lipid Peroxidation in Rat Brain Homogenates



| Compound | Inhibition (%) ^{a)} | | | |
|---------------------------|------------------------------|--------------|--------------|--------------|
| | 10 mM | 1 mM | 0.1 mM | 0.01 mM |
| 2i | >100 | 95 | 60 \pm 2.0 | 24 \pm 0.8 |
| Ascorbic acid | 100 | 75 \pm 1.5 | 32 \pm 1.2 | 10 \pm 0.6 |
| (+)- α -Tocopherol | 100 | 55 \pm 1.7 | 0 | 0 |
| Mitoxantrone-HCl | 100 | 54 \pm 2.1 | 22 \pm 3.5 | 5 \pm 0.3 |

a) Relative percentage of inhibition. Inhibition was compared to that of the control (ascorbic acid, (+)- α -tocopherol and mitoxantrone-HCl), $p<0.01$, mean \pm S.E., $n=4$. Values are mean percent inhibition at the indicated concentration (mM) with standard errors.

Experimental

Melting points were determined with a Büchi B-545 melting point apparatus and are uncorrected. All reactions were monitored by TLC (silica gel 60 F₂₅₄), flash-column chromatography: silica gel (E. Merck, 70—230 mesh) with CH_2Cl_2 as the eluent. ^1H -NMR: Varian GEMINI-300 (300 MHz) and Bruker AM-500 (500 MHz); δ values are in ppm relative to TMS as an internal standard. Fourier-transform IR spectra (KBr): Perkin-Elmer 983G spectrometer. The UV spectra were recorded on a Shimadzu UV-160A. Mass spectra (EI, 70 eV, unless otherwise stated): Finnigan MAT TSQ-46 and Finnigan MAT TSQ-700 (Universität Regensburg, Germany). Typical

experiments illustrating the general procedures for the preparation of the anthraquinones are described below.

General Procedure for the Preparation of 1,5-bis-thio-Anthraquinones To a solution of 1,5-dichloroanthraquinone (1.0 g, 3.6 mmol) in dry THF (100 ml) a solution of an appropriate thiols (28.8 mmol) in sodium methoxide (1.56 g, 28.8 mmol) and dry methanol (30 ml) under N₂ was added dropwise. The reaction mixture was refluxed for 1 h. Water (250 ml) was added, and then the mixture was extracted with dichloromethane. The combined organic extracts were washed with water, dried (MgSO₄), and concentrated. The resulting precipitate was collected by filtration, washed with water and further purified by chromatography and crystallization.

1,5-bis-Ethylthio-anthraquinone (2a): 66% yield. mp 235–236 °C (THF). ¹H-NMR (CDCl₃) δ: 1.45 (6H, t, *J*=7.4 Hz), 3.01 (4H, q, *J*=7.4 Hz), 7.60 (2H, d, *J*=8.0 Hz), 7.66 (2H, t, *J*=7.8 Hz), 8.11 (2H, t, *J*=7.6, 0.9 Hz). ¹³C-NMR (CDCl₃) δ: 12.77, 25.96, 123.47, 127.89, 129.26, 133.14, 136.09, 145.03, 183.33. IR (KBr) cm⁻¹: 1651, 1202. UV λ_{max} (CHCl₃) nm (log ε): 503 (2.41). MS *m/z*: 328 (M⁺), 299, 267, 239, 139. Anal. Calcd for C₁₈H₁₆O₂S₂: C, 65.82; H, 4.91. Found: C, 65.65; H, 4.88.

1,5-bis-Hydroxyethylthio-anthraquinone (2b): 45% yield. mp 261–262 °C (DMSO). ¹H-NMR (CDCl₃) δ: 3.12 (4H, t, *J*=6.5 Hz), 3.70 (4H, q, *J*=6.2 Hz), 5.04 (2H, t, *J*=5.5 Hz), 7.78 (2H, d, *J*=7.6 Hz), 7.82–7.80 (2H, m), 7.94 (2H, dd, *J*=6.8, 1.5 Hz). ¹³C-NMR (CDCl₃) δ: 34.04, 59.06, 122.84, 127.42, 129.88, 133.57, 135.55, 144.07, 182.35. IR (KBr) cm⁻¹: 1638, 1204. UV λ_{max} (CHCl₃) nm (log ε): 513 (2.48). MS *m/z*: 360 (M⁺), 324. Anal. Calcd for C₁₈H₁₆O₄S₂: C, 59.98; H, 4.47. Found: C, 59.81; H, 4.38.

1,5-bis-Propylthio-anthraquinone (2c): 69% yield. mp 232–233 °C (THF). ¹H-NMR (CDCl₃) δ: 1.13 (6H, t, *J*=7.4 Hz), 1.83 (4H, m), 2.96 (4H, t, *J*=7.4 Hz), 7.60 (2H, d, *J*=7.9 Hz), 7.65 (2H, t, *J*=7.8 Hz), 8.11 (2H, d, *J*=6.9 Hz). ¹³C-NMR (CDCl₃) δ: 13.96, 21.33, 34.04, 123.46, 127.99, 129.32, 133.12, 136.14, 145.20, 183.35. IR (KBr) cm⁻¹: 1649, 1199. UV λ_{max} (CHCl₃) nm (log ε): 485 (2.25). MS *m/z*: 356 (M⁺), 313, 271, 239, 139. Anal. Calcd for C₂₀H₂₀O₂S₂: C, 67.38; H, 5.65. Found: C, 67.55; H, 5.78.

1,5-bis-Dihydroxypropylthio-anthraquinone (2d): 45% yield. mp 238–239 °C (DMSO). ¹H-NMR (CDCl₃) δ: 2.93 (2H, t, *J*=10.1 Hz), 3.20 (2H, dd, *J*=12.7, 4.2 Hz), 3.41–3.50 (4H, m), 3.73 (2H, m), 4.79 (2H, t), 5.12 (2H, d, *J*=5.3 Hz), 7.79 (2H, t, *J*=7.7 Hz), 7.82 (2H, d, *J*=7.4 Hz), 7.94 (2H, t, *J*=7.3, 0.8 Hz). ¹³C-NMR (CDCl₃) δ: 35.53, 65.05, 69.91, 122.75, 127.39, 130.02, 133.56, 135.55, 144.71, 182.41. IR (KBr) cm⁻¹: 1647, 1202. UV λ_{max} (CHCl₃) nm (log ε): 507 (2.48). MS *m/z*: 420 (M⁺), 348. Anal. Calcd for C₂₀H₂₀O₆S₂: C, 57.12; H, 4.79. Found: C, 57.35; H, 4.98.

1,5-bis-Hydroxyhexylthio-anthraquinone (2e): 79% yield. mp 195–196 °C (DMSO). ¹H-NMR (CDCl₃) δ: 1.37 (4H, q, *J*=6.9 Hz), 1.46 (4H, q, *J*=6.9 Hz), 1.49 (4H, q, *J*=7.5 Hz), 1.70 (4H, q, *J*=7.3 Hz), 3.00 (4H, t, *J*=7.2 Hz), 3.41 (4H, q, *J*=5.9 Hz), 4.10 (2H, t, *J*=5.1 Hz), 7.77–7.80 (4H, m), 7.95 (2H, d, *J*=6.5 Hz). ¹³C-NMR (CDCl₃) δ: 24.79, 27.31, 28.10, 30.77, 32.04, 60.41, 122.41, 127.18, 129.64, 133.13, 135.34, 144.10, 181.98. IR (KBr) cm⁻¹: 1643, 1259. UV λ_{max} (DMSO) nm (log ε): 564 (0.32). MS *m/z*: 472 (M⁺), 474. Anal. Calcd for C₂₆H₃₂O₄S₂: C, 66.06; H, 6.82. Found: C, 66.35; H, 6.98.

1,5-bis(o-Aminophenylthio)-anthraquinone (2f): 55% yield. mp 283–284 °C (DMSO). ¹H-NMR (CDCl₃) δ: 5.37 (4H, s), 6.66 (2H, t, *J*=7.5 Hz), 6.85 (2H, d, *J*=8.1 Hz), 7.01 (2H, d, *J*=8.2 Hz), 7.25 (2H, t, *J*=7.6, 0.9 Hz), 7.34 (2H, d, *J*=7.5 Hz), 7.66 (2H, t, *J*=7.9 Hz), 8.00 (2H, d, *J*=7.4 Hz). ¹³C-NMR (CDCl₃) δ: 111.35, 115.06, 117.03, 123.67, 127.77, 130.43, 131.77, 133.41, 135.51, 137.08, 143.20, 150.66, 182.65. IR (KBr) cm⁻¹: 1651, 1256. UV λ_{max} (DMSO) nm (log ε): 508 (2.36). MS *m/z*: 454 (M⁺), 361. Anal. Calcd for C₂₆H₁₈N₂O₂S₂: C, 68.69; H, 3.99. Found: C, 68.55; H, 3.78.

1,5-bis(m-Aminophenylthio)-anthraquinone (2g): 65% yield. mp 292–293 °C (DMSO). ¹H-NMR (CDCl₃) δ: 5.40 (4H, s), 6.71–6.73 (4H, m), 6.80 (2H, s), 7.16 (2H, d, *J*=8.3 Hz), 7.19 (2H, t, *J*=7.8 Hz), 7.67 (2H, t, *J*=7.9 Hz), 7.97 (2H, d, *J*=7.5 Hz). ¹³C-NMR (CDCl₃) δ: 115.41, 120.04, 122.24, 123.57, 126.59, 130.74, 130.94, 131.01, 133.49, 135.10, 145.53, 150.44, 182.42. IR (KBr) cm⁻¹: 1653, 1202. UV λ_{max} (DMSO) nm (log ε): 535 (2.48). MS *m/z*: 454 (M⁺), 125. Anal. Calcd for C₂₆H₁₈N₂O₂S₂: C, 68.69; H, 3.99. Found: C, 68.49; H, 3.69.

1,5-bis(p-Aminophenylthio)-anthraquinone (2h): 66% yield. mp 364–365 °C (DMSO). ¹H-NMR (CDCl₃) δ: 5.64 (4H, s), 6.70 (4H, t, *J*=8.3 Hz), 7.07 (2H, d, *J*=8.3 Hz), 7.20 (4H, d, *J*=8.3 Hz), 7.63 (2H, t, *J*=7.9 Hz), 7.94 (2H, d, *J*=7.5 Hz). ¹³C-NMR (CDCl₃) δ: 114.01, 115.19, 123.28, 126.46, 130.57, 133.29, 135.19, 137.02, 147.69, 150.64, 182.41. IR (KBr) cm⁻¹: 1649, 1283. UV λ_{max} (DMSO) nm (log ε): 557 (2.48). MS *m/z*: 454 (M⁺), 124. Anal. Calcd for C₂₆H₁₈N₂O₂S₂: C, 68.69; H, 3.99. Found: C, 68.49; H, 3.68.

1,5-bis-Benzylthio-anthraquinone (2i): 78% yield. mp 281–282 °C (THF). ¹H-NMR (CDCl₃) δ: 4.23 (4H, s), 7.27 (2H, t, *J*=7.3 Hz), 7.33 (4H, d, *J*=7.4 Hz), 7.45 (4H, d, *J*=7.4 Hz), 7.62 (2H, d, *J*=8.0 Hz), 7.66 (2H, t, *J*=7.4 Hz), 8.10 (2H, d, *J*=7.1 Hz). ¹³C-NMR (CDCl₃) δ: 37.35, 123.76, 127.58, 127.91, 128.78, 129.10, 129.59, 133.32, 135.41, 135.86, 144.92, 183.31. IR (KBr) cm⁻¹: 1653, 1261. UV λ_{max} (CHCl₃) nm (log ε): 476 (1.50). MS *m/z*: 452 (M⁺), 361, 270, 91. Anal. Calcd for C₂₈H₂₀O₂S₂: C, 74.30; H, 4.55. Found: C, 74.55; H, 4.78.

1,5-bis(p-Methoxybenzylthio)-anthraquinone (2j): 62% yield. mp 297–299 °C (THF). HR-FAB-MS *m/z*: 512.6472 (Calcd for C₃₀H₂₄O₄S₂: 512.6492).

1,5-bis-Phenylethylthio-anthraquinone (2k): 69% yield. mp 209–210 °C (THF). ¹H-NMR (CDCl₃) δ: 3.08 (4H, t, *J*=8.0 Hz), 3.25 (4H, t, *J*=8.1 Hz), 7.28 (2H, t, *J*=7.0 Hz), 7.30 (2H, t, *J*=8.3 Hz), 7.32 (2H, d, *J*=7.4 Hz), 7.62 (2H, d, *J*=7.4 Hz), 7.66 (2H, t, *J*=7.7 Hz), 8.12 (2H, d, *J*=6.2 Hz). ¹³C-NMR (CDCl₃) δ: 33.64, 34.28, 123.65, 126.68, 128.02, 128.43, 128.69, 129.31, 133.22, 136.07, 140.04, 144.64, 183.29. IR (KBr) cm⁻¹: 1653, 1204. UV λ_{max} (CHCl₃) nm (log ε): 512 (0.60). MS *m/z*: 480 (M⁺), 285. Anal. Calcd for C₃₀H₂₄O₂S₂: C, 74.96; H, 5.03. Found: C, 74.75; H, 4.91.

Cytotoxic Evaluations (XTT Colorimetric Assay) Tumor cell lines used were rat glioma C6 cells and human hepatoma G2 cells. The cells (2.5×10⁴ cells/ml) were placed into 96-well plates and preincubated for 24 to 72 h in complete medium. The drug concentration inhibiting 50% of cellular growth (IC₅₀, mg/ml) was determined using the XTT assay following 72 h of drug exposure.²¹ The results are the means of at least three independent experiments unless otherwise indicated.

Assay of Lipid Peroxidation Rat brain homogenate was prepared from the brains of freshly killed Wistar rats, and its peroxidation in the presence of iron ions was measured using the thiobarbituric acid method as previously described.^{10–13,22} The extent of lipid peroxidation was estimated in terms of thiobarbituric acid-reactive substances and was read at 532 nm on a spectrophotometer (Shimadzu UV-160). The results of this assay are shown in Tables 1 and 2.

Acknowledgments This research was partially supported by grants from the National Defense Medical Center. The authors are indebted to Dr. Klaus K. Mayer (Universität Regensburg, Germany) for the mass spectrometry analytical determinations.

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