Anticancer Anthrapyrazoles. The Synthesis of 2-Substituted 5-Nitro and 5-Aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones

H. D. Hollis Showalter* and William R. Turner

Chemistry Department, Parke-Davis Pharmaceutical Research,
Division of Warner-Lambert Co., Ann Arbor, Michigan 48106

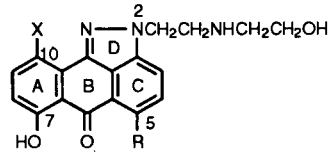
Recieved November 20, 1992

Synthetic methodologies for the preparation of substituted 5-nitro- and 5-aminoanthra[1,9-*cd*]-pyrazol-6(2*H*)-ones, **4** and **5** respectively, substituted with a basic side at N-2 and dioxy substitution in the A-ring, are reported. These compounds are essentially devoid of activity against *in vitro* L1210 leukemia and *in vivo* murine P388 leukemia.

J. Heterocyclic Chem., **30**, 493 (1993).

Introduction.

There have been numerous reports from our and other laboratories that have detailed the design rationale [1,2], synthesis [3-5], tumor biology [6], biochemical pharmacology [7-9], pharmacokinetics and toxicity [10,11], and Phase 1 clinical trials [12-17] of the anthrapyrazoles, a novel class of anticancer agents derived from chromophore modification of the anthracenediones related to the clinical agent mitoxantrone (Novantrone®). Many of these studies have been summarized in a recent review [18]. Three agents, **1-3**, from this class were entered into human trials, and indeed **3** has now progressed well into Phase 2 studies.



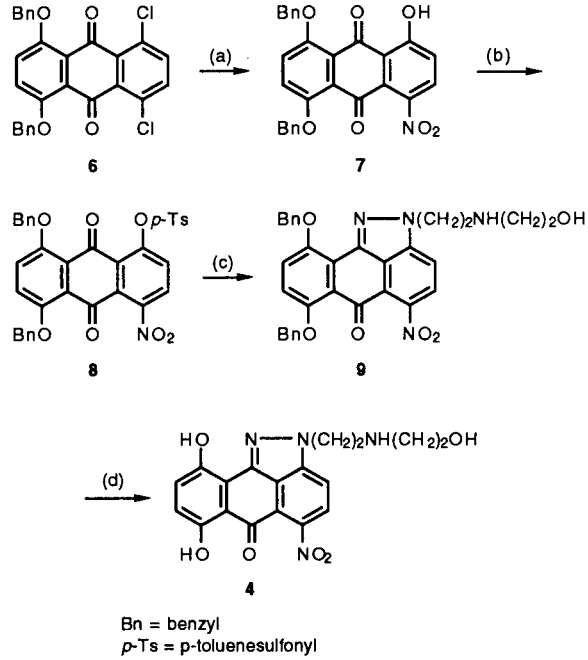
1 X = OH; R = $NHCH_2CH_2NHCH_3$
2 X = OH; R = $NHCH_2CH_2CH_2NH_2$
3 X = H; R = $NHCH_2CH_2NHCH_2CH_2OH$
4 X = OH; R = NO_2
5 X = OH; R = NH_2

Prompted by the biological results from studies carried in our laboratories on benzothiopyranoindazole [19] and pyrazoloacridine [20] anticancer agents, which are chromophore-modified anthracenediones closely related to the anthrapyrazoles, we decided to synthesize representative examples of 2-substituted 5-nitro- and 5-aminoanthrapyrazoles so that direct cross-series comparisons could be made. Highly substituted anthrapyrazoles with these simple 5-substituents have never been reported, and were inaccessible *via* the chemistry that we developed earlier. In this paper we detail synthetic pathways and biological results for two anthrapyrazoles, (**4** and **5**), which possess the same A-ring 7,10-dihydroxylation pattern and N-2 side chain found in **1-3**, and either a nitro or primary amino substituent at C-5, respectively.

Results and Discussion.

The synthesis of the target 5-nitroanthrapyrazole **4** is given in Scheme 1. Key to the successful completion of this target was to utilize a modification of a patent procedure [21] that describes the synthesis of 1-nitro-4,5,8-trihydroxyanthraquinone by the treatment of 1,4-dichloro-5,8-dihydroxyanthraquinone with sodium nitrite in *N*,*N*-dimethylformamide. In our case, we applied this reaction for the conversion of the less activated bis(benzyloxy) starting material **6** to give adduct **7**, which is a double displacement product with

Scheme 1
Synthesis of 5-Nitroanthrapyrazole **4**



Bn = benzyl
p-Ts = p-toluenesulfonyl

Reagents: (a) $NaNO_2$, KF, DMA, 155°. (b) *p*-TsCl, *i*-$PrNEt_2$, CH_3CN, 5°. (c) $NH_2NH(CH_2)_2NH(CH_2)_2OH$, DMA, 5-25°. (d) BCl_3, CH_2Cl_2, 5°.

nitrite anion acting as an ambient nucleophile. Activation of the phenolic functionality *via* tosylation followed by condensation with a monosubstituted

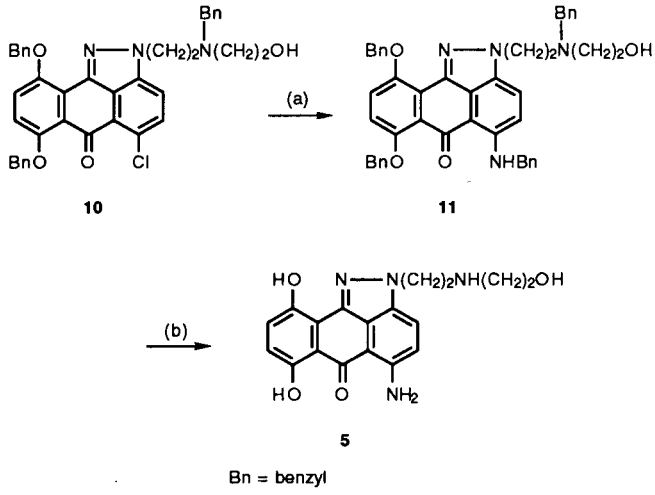
hydrazine then resulted in the selective nucleophilic displacement of the tosyloxy group to provide an entry into the desired 5-nitroanthrapyrazole system. Thus, reaction of 1,4-dichloro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**6**) [3] with sodium nitrite catalyzed with potassium fluoride in *N*,*N*-dimethylacetamide at 155° gave the 1-hydroxy-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione **7** in 65% yield. Phenolic activation *via* tosylation was carried out in 81% yield to give intermediate **8**, which was condensed with 2-[(hydrazinoethyl)amino]ethanol [5] under our previously reported conditions [22] to provide the "one-armed" 7,10-bis(benzyloxy)-5-nitroanthrapyrazole **9** in 69% yield. Because of the likely sensitivity of the nitro functionality to benzylic hydrogenolysis, the benzyl protecting groups were removed by standard treatment with boron trichloride to give the target 7,10-dihydroxy-5-nitroanthrapyrazole **4** as the monohydrochloride salt in 53% yield.

While in principle the synthesis of the target 5-aminoanthrapyrazole **5** could be carried out by reduction of the 5-nitroanthrapyrazole **4** of Scheme 1, we decided to start from the known 5-chloroanthrapyrazole intermediate **10** [5] (Scheme 2). Attempted chloride displacement with ammonia under a number of conditions, or by utilizing ammonia surrogates such as 1,1,1,3,3,3-hexamethyldisilazane, resulted in recovered starting material. However, condensation with neat benzyl amine at 140° afforded the tetrabenzyl product in 55% isolated yield. Removal of benzyl protecting groups *via* catalytic hydrogenolysis over 20% palladium on charcoal as previously described [5] gave the target 5-aminoanthrapyrazole **5** in 49% yield as the monohydrochloride salt. It should be noted that the 5-amino functionality is conjugated to the 6-carbonyl group of the central ring, and thus is of very low basicity. Most of the reactions of Schemes 1 and 2 have been carried out on a multigram scale. No attempt has been made to optimize yields. The structures of all the intermediates and target compounds follow directly from the spectral and microanalytical data, and are fully consistent with the proposed structures, as reported earlier [3-5].

When tested *in vitro* against murine L1210 leukemia as described by Baguley and Nash [23], 5-nitroanthrapyrazole **4** gave an IC_{50} = 1.6 x 10^{-7} *M* and 5-aminoanthrapyrazole **5** an IC_{50} = < 10^{-6} *M* (inactive). When these compounds were tested *in vivo* against P388 leukemia in mice (ip/ip; D1,5; maximum tolerated dose) utilizing the NCI protocol [24], compound **4** demonstrated a T/C = 132% (25 mg/kg/injection) and compound **5** a T/C = 133% (75 mg/kg/injection). Hence, both of these agents showed only marginal efficacy over controls, and were much less active than related "two-armed" anthrapyrazoles and benzothiopyranoindazoles as well as the "one-armed" benzothiopyranoindazoles and pyrazoloacridines. Thus further synthesis to incorporate other side chains at the N-2 position was not pursued.

Scheme 2

Synthesis of 5-Aminoanthrapyrazole **5**



10 **11** **5**

Bn = benzyl

Reagents: (a) $PhCH_2NH_2$, 130°. (b) H_2, 20% Pd/C, 50% aq acetic acid, 25°

In summary, we have developed short, good-yielding routes for the synthesis of substituted 5-nitro and 5-amino anthrapyrazoles. While we demonstrate this methodology for compounds with A-ring dioxy substituents, we are confident that the chemistry in Schemes 1 and 2 can be extended to the synthesis of other substitution patterns. We also have shown that two target compounds incorporating this functionality are essentially devoid of activity against *in vitro* L1210 leukemia and *in vivo* murine P388 leukemia.

EXPERIMENTAL

Melting points (mp) were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (ir) spectra were determined on a Digilab FTS-14 or Nicolet MX-1 FT-IR spectrometer system. Proton magnetic resonance (pmr) spectra were recorded on a Varian EM-390 or XL-200 spectrometer operating at 90 MHz or 200 MHz, respectively, for ^1H. Chemical shifts are reported as δ units in parts per million downfield from internal tetramethylsilane. Combustion analyses were performed on a Perkin-Elmer 240 elemental analyzer. Water of crystallization was determined by Karl Fischer titration.

Chromatography was carried out with (a) E. Merck products utilizing silica gel 60 catalog No. 5760 for tlc, catalog No. 7734 for open column chromatography and catalog No. 9385 for flash chromatography.

All solvents and reagents were "reagent grade." Charcoal refers to activated "Darco" G-60. *In vacuo* refers to 1.0-1.5 torr. All solvents were concentrated on a rotary evaporator at 30-40° (15-20 torr) unless noted otherwise.

1-Hydroxy-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**7**).

A stirred mixture of 150 g (307 mmoles) of 1,4-dichloro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**6**) [3], 85 g (1.23 moles) of anhydrous sodium nitrite, 17.8 g (307 mmoles) of anhydrous potassium fluoride and 550 ml of *N,N*-dimethylacetamide was heated at 155° under nitrogen for 5 hours. The mixture was cooled and poured into 2 liters of 10% aqueous acetic acid. The solids were collected by filtration, washed sequentially with water and methanol, and air dried. The solids were dissolved in 500 ml of methylene chloride and the solution was diluted with 500 ml of 2-propanol then cooled at 5° for 18 hours. The precipitated red solids were collected by filtration, washed with 2-propanol, and dried to give 117 g of crude **7**. Recrystallization from 3 liters of 3:1 chloroform:2-propanol gave 96 g (65%) of pure **7**, mp 174-175°, after drying at 200 mm/50°/18 hours; ir (potassium bromide): 1680, 1640, 1540, 1455, 1280, 1215, 960 cm^{-1}; pmr (deuterochloroform + d_6-dimethyl sulfoxide): δ 5.15 (s, 2H), 5.17 (s, 2H), 7.14 (d, J=9 Hz, 1H), 7.18-7.58 (m, 12H), 7.72 (d, J=9 Hz, 1H), 10.10 (s, 1H, exchanges with deuterium oxide).

Anal. Calcd. for $C_{28}H_{19}NO_7$: C, 69.85; H, 3.98; N, 2.91. Found: C, 69.75; H, 3.98; N, 2.79.

1-[{(4-Methylphenyl)sulfonyl}oxy]-4-nitro-5,8-bis(phenylmethoxy)-9,10-anthracenedione (**8**).

A 5° stirred suspension of 50 g (104 mmoles) of anthracenedione **7**, 23.3 ml (134 mmoles) of *N,N*-diisopropylethylamine, and 350 ml of acetonitrile was treated in one portion with 26.7 g (140 mmoles) of *p*-toluenesulfonyl chloride. The mixture was maintained at 5° for 18 hours, then the red solids were collected by filtration, washed with acetonitrile, and dried at 200 mm/60°/5 hours to give 53.4 g (81%) of **7**, mp 184-187°; ir (potassium bromide): 1686, 1545, 1267, 1236, 1180 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 2.31 (s, 3H), 5.25 (s, 4H), 7.1-7.8 (m, 17H), 8.10 (d, J=9 Hz, 1H).

Anal. Calcd. for $C_{35}H_{25}NO_9S$: C, 66.13; H, 3.96; N, 2.20; S, 5.04. Found: C, 65.83; H, 4.13; N, 2.20; S, 5.10.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-nitro-7,10-bis-(phenylmethoxy)anthra[1,9-*cd*]pyrazol-6(2*H*)-one (**9**).

A 5° stirred solution of 20.0 g (31.5 mmoles) of anthracenedione tosylate **8** in 50 ml of *N,N*-dimethylacetamide was treated slowly with a solution of 3.9 g (32.8 mmoles) of 2-[(hydrazinoethyl)amino]ethanol [5] in 10 ml of *N,N*-dimethylacetamide. The solution was maintained at 5° for 16 hours, then at 25° for 2 hours. The mixture was poured into 300 ml of 10% aqueous sodium carbonate solution. The solids were collected by filtration, washed sequentially with water then 2-propanol, and air dried. Recrystallization from acetonitrile followed by drying gave 12.5 g (69%) of **9** as deep red needles, mp 139-142°; ir (potassium bromide): 1660, 1534, 1273, 1224, 741 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 2.53 (t, J=6 Hz, 2H), 3.08 (br t, J=6 Hz, 2H), 3.29 (t, J=6 Hz, 2H), 4.57 (br t, J=6 Hz, 2H), 5.10 (s, 2H), 5.20 (s, 2H), 7.15-7.57 (m, 10H), 7.60-7.77 (m, 2H), 7.80 (d, J=9 Hz, 1H), 8.03 (d, J=9 Hz, 1H).

Anal. Calcd. for $C_{32}H_{28}N_4O_6 \cdot 0.5\,H_2O$: C, 67.01; H, 5.10; N, 9.77; H_2O, 1.57. Found: C, 66.92; H, 4.91; N, 9.79; H_2O, 1.67.

7,10-Dihydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]-5-nitroanthra[1,9-*cd*]pyrazol-6(2*H*)-one (**4**).

A 5° stirred suspension of 9.0 g (15.7 mmoles) of bisbenzyl nitroanthrapyrazole **9** in 100 ml of dichloromethane was treated dropwise under nitrogen with 96 ml of a 1-molar solution of boron trichloride in dichloromethane. The mixture was maintained at 5° for 16 hours then treated carefully over 30 minutes with 200 ml of methanol. The suspension was stirred at 25° for 4 hours, then the solids were collected by filtration, washed sequentially with methanol and dichloromethane, and air dried. The solids were dissolved in ca. 2 liters of boiling methanol, the solution filtered, and the filtrate treated with 20 ml of a 7.5-molar solution of hydrogen chloride in 2-propanol. The volume was reduced to 200 ml and the suspension was ice cooled. The solids were collected by filtration, washed sequentially with 2-propanol and ether, and dried to leave 3.6 g (53%) of **4** as the hydrochloride salt, mp 248-251°; ir (potassium bromide): 1619, 1603, 1537, 1293, 1212, 1161 cm^{-1}; pmr (deuterium oxide): δ 3.34 (m, 2H), 3.71 (m, 2H), 3.92 (m, 2H), 4.7 (m, 2H), 6.23 (d, J=8.9 Hz, 1H), 6.60 (d, J=9 Hz, 1H), 7.53 (d, J=8.3 Hz, 1H), 7.76 (d, J=8.7 Hz, 1H).

Anal. Calcd. for $C_{18}H_{16}N_4O_6 \cdot 1.1$ HCl 0.4 H_2O: C, 50.09; H, 4.18; N, 12.98; Cl^-, 9.03; H_2O, 1.67. Found: C, 49.77; H, 3.79; N, 12.64; Cl^-, 8.58; H_2O, 1.50.

2-[2-[(2-Hydroxyethyl)(phenylmethyl)amino]ethyl]-7,10-bis-(phenylmethoxy)-5-[(phenylmethyl)amino]anthra[1,9-*cd*]-pyrazol-6(2*H*)-one (**11**).

A mixture of 5 g (7.7 mmoles) of 5-chloro-2-[2-[(2-hydroxyethyl)-(phenylmethyl)amino]ethyl]-7,10-bis-(phenylmethoxy)anthra[1,9-*cd*]pyrazol-6(2*H*)-one (**10**) [5] and 17 g of benzylamine were heated under nitrogen at 130° for 4 hours. The mixture was cooled and concentrated at 1 mm/50° to leave an oily residue that was dissolved in a minimum volume of 2-propanol. The solution was stored for one day at 25°, then the precipitated solids were filtered off, washed with 2-propanol, and air dried. Crystallization from 1:1 chloroform:2-propanol gave 3.2 g (55%) of **11**, mp 152-155°; ir (potassium bromide): 1663, 1606, 1581, 1565, 1454, 1266, 1205, 1027 cm^{-1}; pmr (deuteriochloroform): δ 2.78 (t, J=4.5 Hz, 2H), 2.97 (t, J=5.3 Hz, 2H), 3.55 (s overlapping m, 4H), 4.25 (br s, 1H, exchanges with deuterium oxide), 4.55 (t, J=5.1 Hz, 2H), 4.70 (d, J=6.4 Hz, 2H, collapses to s with deuterium oxide), 5.31 (s, 2H), 5.47 (s, 2H), 6.7-7.8 (m, 24H), 10.00 (t, J=6.0 Hz, 1H, exchanges with deuterium oxide).

Anal. Calcd. for $C_{46}H_{42}N_4O_4 \cdot 1.9\,H_2O$: C, 73.76; H, 6.16; N, 7.48. Found: C, 74.03; H, 5.81; N, 7.60.

5-Amino-7,10-dihydroxy-2-[2-[(2-hydroxyethyl)amino]-ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one, Hydrochloride (**5**).

A 50° mixture of 2.25 g (3 mmoles) of tetrabenzyl compound **11** [5], 100 mg of 20% palladium on carbon, and 27 ml of 50% aqueous acetic acid was hydrogenated at one atmosphere of hydrogen pressure over a 5-hour period. The mixture was filtered through Celite and the filtrate was concentrated to a solid residue that was suspended in 75 ml of methanol. The suspension was treated with 0.5 ml of a 7.5-molar solution of hydrogen chloride in 2-propanol, then boiled for 10 minutes. The solids were filtered, washed with 2-propanol, and dried to leave 583 mg (49%) of **5** as the hydrochloride salt, mp 270-275° dec; ir (potassium bromide): 3333, 1682, 1607, 1574, 1517, 1304, 1226, 825 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 3.08 (br s, 2H), 3.4-3.8 (m, 4H), 4.94 (t, J=6.1 Hz, 2H), 6.86 (d, J=8.9 Hz, 1H), 7.13 (d, J=9.3 Hz, 1H), 7.29 (d, J=8.9 Hz, 1H), 7.9-8.1 (m, 3H; collapses to d, J=9.1 Hz, 1H with deuterium oxide), 8.87 (br s, 1H, exchanges with deuterium oxide), 9.18 (br s, 2H, exchanges with deuterium oxide), 13.71 (s, 1H, exchanges with deuterium oxide).

Anal. Calcd. for $C_{18}H_{18}N_4O_4 \cdot HCl\ 0.2\ H_2O$: C, 54.81; H, 4.96; N, 14.20; Cl^-, 8.99. Found: C, 55.11; H, 5.22; N, 14.09; Cl^-, 9.28.

REFERENCES AND NOTES

[1] H. D. H. Showalter, D. W. Fry, W. R. Leopold, J. W. Lown, J. A. Plambeck and K. Reszka, *Anti-Cancer Drug Des.*, **1**, 73 (1986).

[2] H. D. H. Showalter, L. M. Werbel, W. R. Leopold, D. W. Fry, W. D. Klohs and R. C. Jackson, in "Anthracycline and Anthracenedione-Based Anticancer Agents," J. W. Lown, ed, Elsevier Science Publishers B.V., Amsterdam, 1988, 201-243.

[3] H. D. H. Showalter, J. L. Johnson and J. M. Hoftiezer, *J. Heterocyclic Chem.*, **23**, 1491 (1986).

[4] H. D. H. Showalter, J. L. Johnson, J. M. Hoftiezer, W. R. Turner, L. M. Werbel, W. R. Leopold, J. L. Shillis, R. C. Jackson and E. F. Elslager, *J. Med. Chem.*, **30**, 121 (1987).

[5] V. G. Beylin, N. L. Colbry, O. P. Goel, J. E. Haky, D. R. Johnson, J. L. Johnson, G. D. Kanter, R. L. Leeds, B. Leja, E. P. Lewis, C. D. Rithner, H. D. H. Showalter, A. D. Sercel, W. R. Turner and S. E. Uhlendorf, *J. Heterocyclic Chem.*, **26**, 85 (1989).

[6] W. R. Leopold, J. M. Nelson, J. Plowman and R. C. Jackson, *Cancer Res.*, **45**, 5532 (1985).

[7] D. W. Fry, T. J. Boritzki, J. A. Besserer and R. C. Jackson, *Biochem. Pharmacol.*, **34**, 3499 (1985).

[8] M. A. Graham, D. R. Newell, J. Butler, B. Hoey and L. H. Patterson, *Biochem. Pharmacol.*, **36**, 3345 (1987).

[9] D. W. Fry, *Pharmac. Ther.*, **52**, 109 (1991).

[10] M. A. Graham, D. R. Newell, B. J. Foster and A. H. Calvert, *Cancer Chemother. Pharmacol.*, **23**, 8 (1989).

[11] S. K. Frank, D. A. Mathiesen, M. Szurszewski, M. J. Kuffel and M. M. Ames, *Cancer Chemother. Pharmacol.*, **23**, 213 (1989).

[12] A. Hantel, R. C. Donehower, E. K. Rowinsky, E. Vance, B. V. Clarke, W. P. McGuire, D. S. Ettinger, D. A. Noe and L. B. Grochow, *Cancer Res.*, **50**, 3284 (1990).

[13] M. M. Ames, C. L. Loprinzi, J. M. Collins, C. van Haelst-Pisani, R. L. Richardson, J. Rubin and C. G. Moertel, *Cancer Res.*, **50**, 3905 (1990).

[14] C. Erlichman, M. Moore, I. G. Kerr, B. Wong, E. Eisenhauer, B. Zee and L. R. Whitfield, *Cancer Res.*, **51**, 6317 (1991).

[15] D. C. Talbot, I. E. Smith, J. L. Mansi, I. Judson, A. H. Calvert and S. E. Ashley, *J. Clin. Oncol.*, **9**, 2141 (1991).

[16] M. A. Graham, D. R. Newell, B. J. Foster, L. A. Gumbrell, K. E. Jenns and A. H. Calvert, *Cancer Res.*, **52**, 603 (1992).

[17] B. J. Foster, D. R. Newell, M. A. Graham, L. A. Gumbrell, K. E. Jenns, S. B. Kaye and A. H. Calvert, *Eur. J. Cancer*, **28**, 463 (1992).

[18] I. R. Judson, *Anti-Cancer Drugs*, **2**, 223 (1991).

[19] H. D. H. Showalter, M. M. Angelo, E. M. Berman, G. D. Kanter, D. F. Ortwine, S. G. Ross-Kesten, A. D. Sercel, W. R. Turner, L. M. Werbel, D. F. Worth, E. F. Elslager, W. R. Leopold and J. L. Shillis, *J. Med. Chem.*, **31**, 1527 (1988).

[20] J. S. Sebolt, S. V. Scavone, C. D. Pinter, K. L. Hamelehle, D. D. von Hoff and R. C. Jackson, *Cancer Res*, **47**, 4299 (1987).

[21] I. Cheetham, British Patent 1,358,172 (1972); *Chem. Abstr.*, **78**, 99054r (1973).

[22] H. D. H. Showalter, E. M. Berman, J. L. Johnson, J. L. Atwood and W. E. Hunter, *Tetrahedron Letters*, **26**, 157 (1985).

[23] B. C. Baguley and R. Nash, *Eur. J. Cancer* **17**, 671 (1981).

[24] R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).