A large-scale synthesis of the bioreductive drug 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide (AQ4N)

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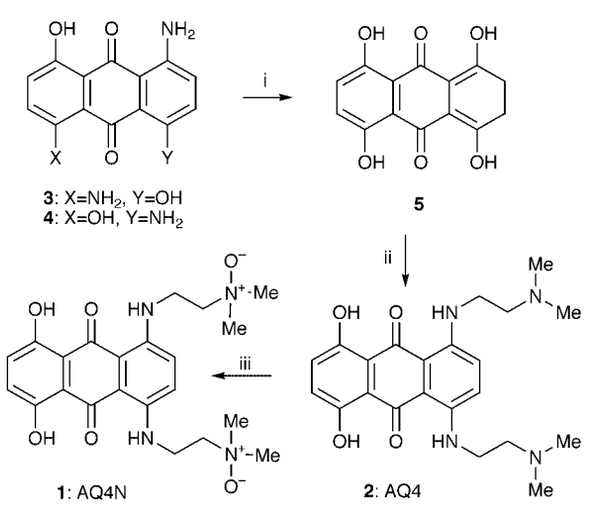
A large-scale synthesis of the bis-bioreductive drug 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide (AQ4N) has been developed. This six-step synthesis provides AQ4N in 20% overall yield from readily available tetrachlorophthalic anhydride. The key step was a KF–NaF-mediated conversion of 3,6-dichlorophthalic anhydride to 3,6-difluorophthalic anhydride, which could be achieved in 77% yield on a 100 g scale. A trace impurity in AQ4N was determined (by LC-MS and independent synthesis) to be the mono-*N*-oxide 1-amino-4-[2-(dimethylamino)ethyl]amino-5,8-dihydroxyanthracene-9,10-dione *N*-oxide. This is formed spontaneously from AQ4N under a number of conditions, including during HPLC on reversed-phase columns.

Introduction

1,4-Bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide **1** (AQ4N) is a bis-bioreductive agent developed as a potential hypoxia-selective cytotoxin for cancer therapy.[1,2] It is a member of the general class of pro-intercalators, where oxygen-inhibited cellular reduction of the *N*-oxides demasks cationic amine functions, greatly increasing DNA-binding affinity and thus cytotoxicity (the latter usually through topoisomerase inhibition).[3,4] Several studies[4–6] have shown that **1** has activity against hypoxic cells in a number of tumour models, and a clinical trial is planned. It has been prepared by oxidation of the bisamine precursor **2** (AQ4), which in turn has been prepared from either **3** or **4** by reduction to the corresponding leuco derivative **5**, reaction of this with excess of *N*,*N*-dimethylethylenediamine, and reoxidation of the product (usually by atmospheric oxygen)[7,8] (Scheme 1). Because this



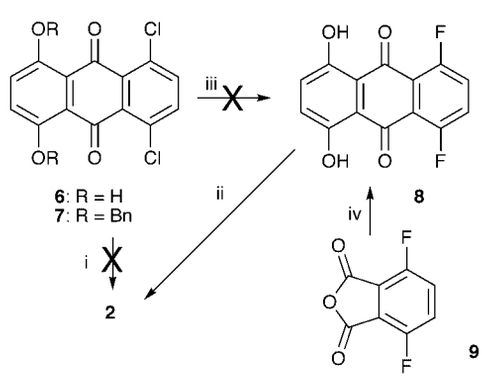
Scheme 1 Literature synthesis of AQ4N. *Reagents*: i, NaOH, $Na_2S_2O_4$, H_2O; ii, $Me_2N(CH_2)_2NH_2$, EtOH; then air oxidation; iii, MCPBA, CH_2Cl_2.

method, although direct, appeared to have limitations, we report here the development of an alternative synthetic route, able to provide **1** on a large scale.

Results and discussion

Initial studies of the preparation of **2** from 1,4-diamino-5,8-dihydroxyanthraquinone **4** by the method[7,8] of Scheme 1 showed that the leuco compound **5** is formed in low purity. Because it is difficult to purify further due to significant instability, it is usually converted directly to **2**, which is purified by extensive chromatography, followed by crystallisation. This gives material of 90–97% purity in about 30% overall yield from **3**.

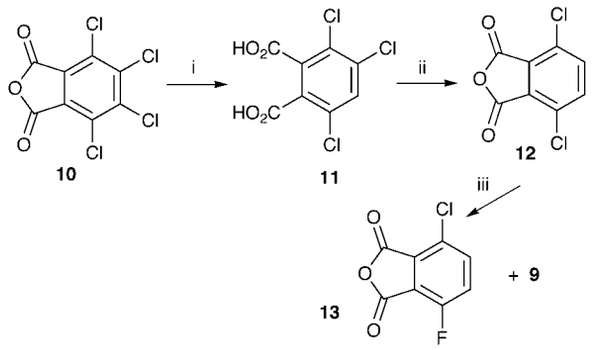
An alternative preparation of **2** from 1,4-difluoro-5,8-dihydroxy derivative **8** has been reported[9] (Scheme 2). We



Scheme 2 Synthesis of anthraquinone **8**. *Reagents*: i, $Me_2N(CH_2)_2NH_2$; ii, $Me_2N(CH_2)_2NH_2$, pyridine; iii, various; iv, hydroquinone, $AlCl_3$.

evaluated the more readily available 1,4-dichloroanthraquinone analogues **6** and **7**, but these gave only trace amounts of product. The only reported synthesis[10] of **8** is from the expensive difluorophthalic anhydride **9**; it could not be prepared from **6**, although such an exchange has been reported[11] for the corresponding deoxy analogues. Synthesis of **9** has been reported in six steps (overall 40% yield) from 2,5-difluorobenzoyl chloride, which is commercially available but relatively expensive.[10] A five-step synthesis of the diacid precursor of **9** from 2,3-dimethylaniline has also been reported,[12] but the overall yield was only 8%.

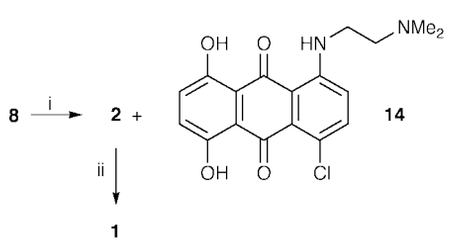
We therefore chose to prepare **9** from the cheap and readily



Scheme 3 Synthesis of anhydride **9**. *Reagents*: i, Zn (5 wt-%), aq. NaOH; ii, Zn (10 wt-%), NaOH; then azeotrope (toluene); iii, KF–NaF melt; then sublime.

available tetrachlorophthalic anhydride **10** (Scheme 3). This can be dechlorinated stepwise,[13] first to 3,4,6-trichlorophthalic acid **11** and then to 3,6-dichlorophthalic acid, which can be dehydrated[14] to give 3,6-dichlorophthalic anhydride **12** in 72% overall yield. Separate and well defined conditions are required to achieve a clean product in each reduction step, and combining these leads to less pure material.[15] A critical step was conversion of **12** to **9**. This had been reported once[16] on a very small scale using a KF melt. Several modifications to this method, including the use of a mixed KF–NaF reagent, allowed conversion in up to 77% yield on a large scale (up to 100 g). The chlorofluoro anhydride **13** was a minor (<10%) impurity; attempts to reduce the amount of this by longer reaction times led to a significant decrease in the yield of **9**.

Friedel–Crafts alkylation of **9** by modification of the reported method[10] gave **8** in 98% yield, suitable for direct use in the next step, on a large scale. Compound **8** was treated with *N*,*N*-dimethylethylenediamine (Aldrich, 95%) in pyridine at room temperature for 58 h, and the crude product (on a 100 g scale) was filtered through a short column of silica gel to separate the required AQ4 product **2** from a small amount of the 4-chloro monoamine **14**, presumably derived from the corresponding chlorofluoro anhydride **13** (Scheme 4). The AQ4 so



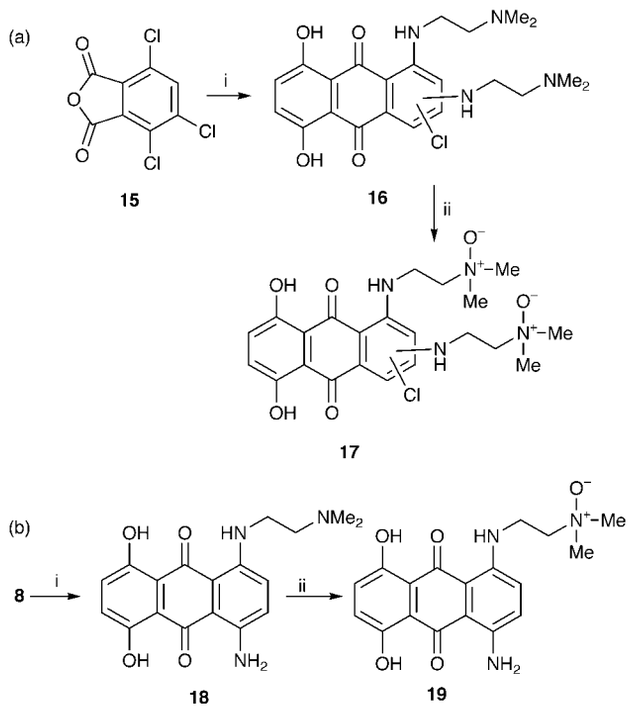
Scheme 4 Synthesis of AQ4N **1**. *Reagents*: i, $H_2N(CH_2)_2NMe_2$, pyridine; ii, 3-phenyl-2-(phenylsulfonyl)oxaziridine, CH_2Cl_2–MeOH.

obtained was contaminated with a trace of a chloro diamine analogue, presumably derived from 3,4,6-trichlorophthalic anhydride (**15**) originally present in **12**.

Reaction of authentic **15** (ref. 13) with hydroquinone as above, and reaction of the crude product with *N*,*N*-diethylenediamine, gave a mixture of three isomers **16**, barely resolved by HPLC (Scheme 5a). The fact that three isomers were generated suggests that the additional chlorine group activates the halogens so that any two can be displaced by the amine. The isomer mixture had the same retention time as the contaminant in AQ4 **2**, but the exact composition of the latter could not be determined. Oxidation of **2** with 3-phenyl-2-(phenylsulfonyl)-oxaziridine in dichloromethane–methanol gave AQ4N **1**, which was converted to the dihydrochloride salt and recrystallised from aq. ethanol.

Table 1 Cytotoxicities of AQ4 analogues and side products in murine P388 leukaemia cells

Compound	IC_{50} (nM)
1 (AQ4N)	330
2 (AQ4)	6.6
16	880
17	>20 000



Scheme 5 (**a**) Synthesis of chloro impurity **17**. *Reagents*: i, hydroquinone, NaCl, $AlCl_3$; then $H_2N(CH_2)_2NMe_2$; ii, 3-phenyl-2-(phenylsulfonyl)oxaziridine, CH_2Cl_2. (**b**) Synthesis of impurity **19**. *Reagents*: i, NH_3 (gas), pyridine; then $H_2N(CH_2)_2NMe_2$; ii, 3-phenyl-2-(phenylsulfonyl)oxaziridine, CH_2Cl_2–MeOH.

Analysis of this material by LC-MS showed it contained trace amounts of two impurities. The more polar (0.45%) had the same retention time as the mixture of chloro bis-*N*-oxides **17** derived from the mixture of amines **16**. The less polar (0.33%) was the 4-amino derivative **19**, authenticated by independent synthesis from **8** (Scheme 5b). Sequential fluorine displacement with ammonia and *N*,*N*-dimethylethylenediamine gave the 1-amino analogue **18**, which was oxidised to give **19**. The precursor amine **18** was not observed in the samples of AQ4 **2**, and mono-*N*-oxide **19** is suggested to arise from **1**, which is unstable under some conditions. A pure sample of **1** was prepared by collecting the major peak from HPLC, but when this was re-injected, it was found to contain 1% of **19** (and no **17**). When the oxidation of **2** to give **1** was carried out for longer times, larger amounts of **19** were seen.

The cytotoxicities of AQ4N **1**, AQ4 **2** and the trace impurity **17** (together with its amine precursor **16**) were measured in P388 cells (Table 1). As has been shown previously,[4] *N*-oxidation of **2** to give **1** results in very considerable loss of cytotoxicity (6.6 to 330 nM in this cell line). The chloro analogues **16** and **17**, where the side chains may not be in the preferred 1,4-arrangement, were even less cytotoxic.

In summary, the six-step synthesis **10** ⟶ **11** ⟶ **12** ⟶ **9** ⟶ **8** ⟶ **2** ⟶ **1** (Schemes 2–4) is a viable route for large-scale preparation of high-purity **1** (AQ4N). It provided **1** in an overall yield of ≈20% from a cheap and available starting material, and required only one straightforward filtration

chromatography step near the end of the synthesis (to remove a small amount of the monochloro compound **14** from **2**).

Experimental

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Mps were determined on an Electrothermal 2300 melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker DRX-400 spectrometer, and are referenced to Me_4Si. Thin-layer chromatography was carried out on aluminium-backed silica gel (Merck 60 F_{254}) or alumina plates. Column chromatography was carried out on Merck silica gel (230–400 mesh). Petroleum ether refers to the fraction boiling at 40–60 °C. Mass spectra were determined using a VG 7070 spectrometer at nominal 5000 resolution. Purity of the final products AQ4 and AQ4N was analysed by reversed-phase HPLC, with UV detection at 254 nm.

3,4,6-Trichlorophthalic acid 11

This compound was prepared by modifications to a literature method.[13] A mixture of 3,4,5,6-tetrachlorophthalic anhydride **10** (Aldrich Chemicals) (200 g, 0.70 mol) and NaOH (100 g, 2.50 mol) in water (2000 mL) was stirred at 50–60 °C (bath) for 45 min under a nitrogen atmosphere. Zinc dust (140 g, 2.14 mol) was then added portionwise over a period of 10 min, and the mixture was stirred at 70–80 °C for a further 6 h. The reaction mixture was cooled to room temperature and filtered through a bed of Celite, and the filter and residue were washed successively with 0.1 M NaOH (2 × 100 mL) and H_2O (2 × 100 mL). The combined filtrate was acidified with conc. HCl to pH ≤ 1, and the colourless precipitate was collected by filtration and washed with 0.1 M HCl (3 × 100 mL). The damp solid was stirred with EtOAc (1200 mL) and acidified with conc. HCl until all the solids had dissolved. The EtOAc layer was separated and the aqueous portion further extracted with the same solvent (2 × 200 mL). The combined EtOAc solution was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 3,4,6-trichlorophthalic acid **11** (189 g, 100%) as a colourless solid, mp (without recrystallisation) 150 °C (lit.,[13] 150–153 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 7.90 (s).

3,6-Dichlorophthalic anhydride 12

This compound was prepared by modifications to the method of ref. 14. Zinc dust (165 g, 2.52 mol) was added portionwise (over a period of 15 min) to a homogenous mixture of **11** (118 g, 0.437 mol) and NaOH (120 g) in water (1200 mL) stirred at 90 °C (bath) under a nitrogen atmosphere. The resulting heterogeneous mixture was further stirred at 95–100 °C for 5 h, then cooled to room temperature and filtered through a bed of Celite. The filter and residue were washed with water (3 × 100 mL), and the combined filtrate was acidified with conc. HCl (250 mL) and extracted with EtOAc (2 × 300 mL). The combined EtOAc solution was dried (Na_2SO_4), filtered and evaporated under reduced pressure to give crude 3,6-dichlorophthalic acid (100 g). Toluene (1000 mL) was added to this solid, and the mixture was distilled until the distillate was clear (after about 600 mL had been collected). The hot concentrate was gravity-filtered, and the residue was washed with hot toluene (3 × 50 mL). The combined filtrate was seeded and chilled to give 3,6-dichlorophthalic anhydride **12** as a colourless solid (67.0 g, 72%), mp 187–190 °C (lit.,[14] 188–191 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 8.03 (s).

3,6-Difluorophthalic anhydride 9

This compound was prepared as reported previously without details,[16] but well defined conditions and operating procedures were developed. In a 1 L round-bottomed flask was placed a layer of **12** (100 g, 0.47 mol) over a layer of powdered mixed anhydrous KF (400 g) and NaF (80 g). This packing was not disturbed, but dried in a vacuum oven at 130–140 °C at 20 mmHg for 7 h. The flask was transferred to an oil-bath such that the oil level was about 1 cm above the solid layer. The flask was evacuated again by a water-pump, and then filled with nitrogen gas. The bath was then heated to 260–270 °C and held at this temperature. After about 20 min, a considerable amount of solid sublimed onto the top of the reaction flask, and the flask was lowered gently into the oil-bath until the oil level reached to the neck of the flask. When all the sublimed solid melted and flowed back onto the solid layer, the flask was returned to its original level in the oil-bath. This operation was repeated at about 20 min intervals, until a light brown layer of KF was observed (after 1.75–3 h). The reaction mixture was then sublimed at 140–170 °C/0.3 mmHg in a Kugelrohr apparatus, giving a solid product that contained mainly 3,6-difluorophthalic anhydride **9** (≈90% by NMR) (65 g, 77%), mp 211–214 °C (from toluene) (lit.,[16] 212 °C). ^{1}H NMR spectrum identical with that of an authentic sample (Aldrich Chemical).

The only significant impurity (≈5–10%) present in the sublimed product is considered to be the intermediate 3-chloro-6-fluorophthalic anhydride **13**. However, the above material was used for the next step without further purification.

1,4-Difluoro-5,8-dihydroxyanthracene-9,10-dione 8

This compound was prepared by modifications to the literature[10] method. A mixture of **9** (100 g, 0.55 mol), hydroquinone (63.7 g, 0.58 mol), NaCl (1270 g, 2.22 mol) and powdered anhydrous $AlCl_3$ (830 g, 6.26 mol) was placed in a 5 L flask equipped with a condenser. The reactants were well mixed by shaking, then heated over a period of 1–2 h to 200 ± 5 °C (bath) under a nitrogen atmosphere (there was very large gas evolution during the heating process). After a further 2 h at 200 ± 5 °C, the melt was poured onto ice, and conc. HCl (1600 mL) was added. The mixture was stirred at room temperature overnight and the reddish brown precipitate was collected, washed with H_2O and dried to give crude 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione **8** (151 g, 98%), mp 301–304 °C (lit.,[10] 318–319 °C). This crude product was virtually insoluble in all solvents, and showed (by TLC in EtOAc–petroleum ether 1 : 3) only one minor impurity (probably 1-chloro-4-fluoro-5,8-dihydroxyanthracene-9,10-dione). The bulk material was used for the next step without further purification.

1,4-Bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione 2 (AQ4)

This was prepared by modifications to the literature[9] method. A mixture of crude **8** (100 g, 0.36 mol) and *N*,*N*-dimethylethylenediamine (199 mL, 1.81 mol) in pyridine (1500 mL) was stirred at room temperature under a nitrogen atmosphere for 58 h. The mixture was then poured into brine (3000 mL) and stirred at 0 °C for 2 h. The blue precipitate was collected by filtration, washed with 1 M NH_4OH (5000 mL), and dried under vacuum over KOH–silica for 15 h. This crude product (86.8 g) was dissolved in CH_2Cl_2 and transferred to a short silica gel filtration column. Elution with CH_2Cl_2–MeOH (99 : 1) gave a pink impurity, mainly 1-chloro-4-{[2-(dimethylamino)-ethyl]amino}anthracene-9,10-dione **14** (13.8 g, 10%), which was purified by recrystallisation from CH_2Cl_2, mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 12.97 (s, 1 H, exchangeable with D_2O, OH), 12.92 (s, 1 H, exchangeable with D_2O, OH), 10.04 (s, 1 H, exchangeable with D_2O, NH), 7.50 (d, *J* 9.5 Hz, 1 H, H-3), 7.24 (d, *J* 9.2 Hz, 1 H, H-6), 7.20 (d, *J* 9.2 Hz, 1 H, H-7), 6.98 (d, *J* 9.5 Hz, 1 H, H-2), 3.40 (q, *J* 6.3 Hz, collapse to t after D_2O, 2 H, $NHCH_2$), 2.67 (t, *J* 6.3 Hz, 2 H, $NHCH_2CH_2$), 2.35 (s, 6 H, NCH_3) (Found: C, 58.5; H, 4.7; N, 7.5. Calc. for $C_{18}H_{17}ClN_2O_4 \cdot \frac{1}{2}H_2O$: C, 58.5; H, 4.9, N, 7.6%).

Elution of the column with CH_2Cl_2–MeOH–Et_3N (90:10:1) gave 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione **2** (63 g, 42%), 97% pure by HPLC; mp 240–242 °C (without recrystallisation) (lit.,[10] 236–238 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 13.53 (s, 2 H, exchangeable with D_2O, OH), 10.42 (t, *J* 4.8 Hz, 2 H, exchangeable with D_2O, NH), 7.22 (s, 2 H, H-6, -7), 7.12 (s, 2 H, H-2, -3), 3.50 (br q, collapsed to t after D_2O, *J* 6.5 Hz, 4 H, NHCH_2CH$_2$), 2.67 (t, *J* 6.5 Hz, 4 H, CHCH$_2$CH_2), 2.35 (s, 12 H, CH_3).

1,4-Bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide dihydrochloride 1 (AQ4N.diHCl)

A stirred solution of **2** (63.1 g, 0.15 mol) in CH_2Cl_2–MeOH (3:1; 1600 mL) was treated dropwise over a period of 1 h with a solution of 3-phenyl-2-(phenylsulfonyl)oxaziridine[17] (84 g, 0.32 mol) in CH_2Cl_2 (500 mL). After this addition, the mixture was stirred at 0 °C in the dark for a further 60 min. Anhydrous HCl gas was then passed through the solution at 0 °C until the pH was ≈1. The mixture was then diluted with EtOAc (2000 mL) and kept at 0 °C for a further 30 min. The resulting blue precipitate was collected by filtration, washed with EtOAc–MeOH (1:1; 4 × 100 mL), and dried under vacuum over silica gel to give the crude dihydrochloride salt of **1** (70.6 g, 90%), 97.6% pure by HPLC. Recrystallisation from EtOH–water (2:1; 600 mL) gave material of mp 243–245 °C (decomp.) (66 g, 84%), of purity >99% (HPLC) (Found: C, 50.1; H, 6.1; N, 10.7; Cl, 13.1. Calc. for $C_{22}H_{28}N_4O_6$·2HCl·0.5H_2O: C, 50.2; H, 5.9; N, 10.6; Cl, 13.5%).

Synthesis of mixture of chlorodiamines 16

Reaction of 3,4,6-trichlorophthalic anhydride[13] **15** with hydroquinone as above gave crude 4,5,8-trichloro-1,4-dihydroxyanthraquinone-9,10-dione. A mixture of this (1.0 g, 2.91 mmol) and *N*,*N*-dimethylethylenediamine (5 mL) was stirred at 20 °C for 6 days, and worked up as above. Chromatography of the crude product on silica gel, and elution with CH_2Cl_2 and CH_2Cl_2–MeOH (20:1 ⟶ 10:1), gave a solid (410 mg) that was a single spot on TLC, but by HPLC was resolved into three very closely running peaks, suggested to be a mixture of chlorodiamines **16** (Found: C, 59.1; H, 5.5. Calc. for $C_{22}H_{27}ClN_4O_4$: C, 59.1; H, 6.1%).

Oxidation of the mixture **16** with 3-phenyl-2-(phenylsulfonyl)-oxaziridine as above gave a mixture of the corresponding bis-*N*-oxides **17**, which co-eluted with the more polar minor impurity detected in **1** (Found: C, 43.5; H, 5.1; N, 9.8; Cl, 20.8. Calc. for $C_{22}H_{27}ClN_4O_6$·2HCl·0.5H_2O: C, 43.6; H, 5.6; N, 9.2; Cl, 20.5%).

1-Amino-4-{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione 18

Anhydrous NH_3 gas was bubbled for 5 min through a solution of **8** (1.0 g, 3.62 mmol) in pyridine (55 mL), and the mixture was then stirred in a bomb at 50–55 °C (bath temperature) for 5 h. The mixture was cooled to 20 °C, *N*,*N*-dimethylethylenediamine (4 mL) was added, and the mixture was stirred at 20 °C in the open atmosphere for 90 h. The mixture was poured into a mixture of brine (150 mL) and conc. NH_4OH (50 mL) at 0 °C, and kept for 3 h. The resulting blue precipitate was collected, washed several times with 1 M NH_4OH, and dried. Chromatography of the crude material on silica gel, and elution with CH_2Cl_2 and CH_2Cl_2–MeOH (50:1 ⟶ 20:1) gave **18** (0.34 g, 55%); mp 167–169 °C (from EtOAc–MeOH); ^{1}H NMR [$(CD_3)_2SO$] δ 13.66 and 13.64 (2 s, 2 H, exchangeable with D_2O, OH), 10.60 (t, *J* 5.0 Hz, 1 H, exchangeable with D_2O, NH), 7.47 (d, *J* 9.8 Hz, 1 H, H-6), 7.33 (d, *J* 9.8 Hz, 1 H, H-7), 7.15 (d, *J* 9.4 Hz, 1 H, H-3), 7.13 (d, *J* 9.4 Hz, 1 H, H-2), 3.53 (td, *J* 6.1, 5.0 Hz, 2 H, NHCH_2), 3.38 (s, 2 H, exchangeable with D_2O, NH_2), 2.56 (t, *J* 6.1 Hz, 2 H, NHCH$_2$CH_2), 2.24 [s, 6 H, N$(CH_3)_2$] (Found: C, 62.9; H, 5.2; N, 12.2. Calc. for $C_{18}H_{19}N_3O_4$: C, 63.3; H, 5.6; N, 12.2%).

1-Amino-4-{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione *N*-oxide 19

A solution of **18** in CH_2Cl_2–MeOH (5:1; 12 mL) was treated with a solution of 3-phenyl-2-(phenylsulfonyl)oxaziridine (0.19 g, 0.55 mmol) in CH_2Cl_2 (6 mL) at 0 °C for 1 h. The mixture was then treated with anhydrous HCl and diluted with EtOAc, to give a precipitate of **19** as the hydrochloride salt (80 mg, 37%), mp 225 °C (decomp.); ^{1}H NMR [$(CD_3)_2SO$] δ 13.63 and 13.52 (2 s, 2 H, exchangeable with D_2O, OH), 12.59 (s, 1 H, exchangeable with D_2O, HCl), 10.60 (t, *J* 5.5 Hz, 1 H, NH), 7.56 (d, *J* 9.8 Hz, 1 H, H-6), 7.42 (d, *J* 9.8 Hz, 1 H, H-7), 7.20 (s, 2 H, H-2, -3), 4.07 (q, *J* 5.5 Hz, collapse to t with D_2O, 2 H, NHCH_2), 3.95 (t, *J* 5.5 Hz, 2 H, NHCH$_2$CH_2), 3.54 [s, 6 H, N$(CH_3)_2$] (Found: C, 50.3; H, 4.8. Calc. for $C_{18}H_{21}Cl_2N_3O_5$: C, 50.2; H, 4.9%). The compound was only 76% pure by HPLC, but all attempts to purify it futher by recrystallisation led to a lower purity product. It co-eluted on HPLC with the less polar minor impurity found in **1**.

Cell-line assay

Murine P388 leukaemia cells were obtained and cultured as described previously.[18] Growth inhibition assays were performed by culturing cells at 4.5×10^3 cells per well in microculture plates for three days in the presence of drug. Cell growth was determined by [^{3}H]TdR uptake.[19] IC_{50}-Values are the averages of at least duplicate determinations.

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