A New Approach to the Synthesis of 4,5-Dioxoaporphine Alkaloids from Preformed Biaryl Bond Precursors.

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Abstract: The synthesis of cepharadione-B and 2-demethoxy analogues is described. Starting from fluorenones, ring C was formed by cyclization of (biphenyl-2-yl)acetyl morpholines under Bischler-Napieralsky conditions. The photochemistry of chloroacetamides was used to form ring B. The cytotoxicity of these compounds on several tumor cell lines was evaluated.

4,5-Dioxoaporphine alkaloids constitute a class of highly oxidized aporphinoids that exhibit DNA-modifying bioactivity and are chemically and biosynthetically related to aristolactams and aristolochic acids of recognized antitumoral activity. Their functionality is also distinctive of 4,5-dioxo-1-azaaporphinoids (imbiline-type alkaloids) and 3,4-dioxocularine alkaloids. It has been suggested that these highly coloured alkaloids might act as post-infectional phytoalexins, with the reduced aporphines as the immediate precursors. Their thermal and photochemical stability suggest that their antimicrobial activity might be related to their ability to act as singlet oxygen sensitizer.

The partial synthesis of dioxoaporphines has been accomplished by air oxidation and direct or sensitized photooxygenation of dehydroaporphines (in yields below 15%), as well as by chemical oxidation (with iodine or DDQ) of 4-hydroxyaporphines. The total synthesis of these alkaloids has been achieved by two different approaches (Scheme 1). One of them involves the biaryl bond formation in the last steps (route a) of the synthetic sequence. The photocyclization of 1-(2'-bromo-benzyl)isoquinoline-3-ones proceeds with moderate yield directly to the 4,5-dioxoaporphine. Yields have been improved in the radical cyclization of these intermediates to give 5-oxodehydroaporphines that are readily oxidized to the 4,5-dioxoaporphines. This approach is of interest when substituents at positions 1, 2, 9 and 10 of the aporphine nucleus are the same, since the key step in the preparation of the

Scheme 1

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bromobenzylisoquinoline is the self condensation of the appropriately substituted phenylacetic acid. The second approach (route b) uses a more convergent synthetic scheme and is based on the formation of two strategic bonds in a single step. The intermolecular benzyne cycloaddition to in situ generated 1-methylene-3,4-dioxoisouqinolines gave acceptable yields for the dioxoaporphine. Although the reaction exhibits good regioselectivity, it is specially useful for ring-D unsubstituted alkaloids; the overall yield depends on the accessibility of the isoquinoline component. An intramolecular aryne approach was recently developed, that failed in the cyclization of oxidized precursors, however.  

\[
\begin{align*}
R_1 &\quad \rightarrow \quad R_1 \\
R_2 &\quad \rightarrow \quad R_2
\end{align*}
\]

\[\text{la-c}\]
\[\text{2a-c}\]
\[\text{3d,e}\]

\[\text{a: } R_1 = R_2 = \text{OMe} \]
\[\text{b: } R_1 = \text{H}; R_2 = \text{OMe} \]
\[\text{c: } R_1 = \text{H}; R_2 = \text{OBn} \]
\[\text{d: } R_1 = \text{H} \]
\[\text{e: } R_1 = \text{OMe} \]

Scheme 2

We recently started a program aimed at testing the thermal, photochemical and biological properties of these alkaloids and their synthetic analogue. This paper reports on our exploratory work on the total synthesis of cepharadione-B (1a) and 2-demethoxy analogues (1b,c) by use of a new and simple approach based on fluorenones as the starting compounds (Scheme 2). The synthesis uses a cyclization related to the Bischler-Napieralsky reaction to assemble the phenanthrene ring system and the photocyclization of chloroacetamides to build up the isoquinolone fragment. Our preliminary results for the cytotoxic activity of these 4,5-dioxoisoporphines against human and murine tumor cell lines are also reported.

**Results and Discussion**

The synthesis of dioxoisoporphines 1a-c is depicted in Schemes 3 and 4; it started with the Baeyer-Villiger\(^{14}\) oxidation of fluorenone 3d and 1-methoxyfluorenone 3e. The reaction was performed with sodium perborate in acetic acid,\(^{15}\) and lactones 4d and 4e were obtained in excellent yield with the expected regioselectivity.\(^{16}\) The heterocyclic oxygen introduced in this way became the oxygenated substituent at C-1 position of the target dioxoisoporphines. Direct homologation of the lactone 4d to the corresponding arylacetic acid via \(\alpha\)-bromo \(\alpha\)-keto dianion rearrangement\(^{17}\) was attempted. However, the unrearranged 6,7-dihydro-dibenz[b,d]oxepin-6-one (16) was the only product isolated from the reaction mixture, as inferred from the carbonyl resonance (\(\delta_{c} 203.7 \text{ ppm}\)) and chemical shift for the methylene protons (\(\delta_{H} 4.83 \text{ ppm}\)) observed in the NMR spectra. Consequently, the conventional multistep homologation was selected. Opening of the lactone ring with potassium hydroxide in the presence of methyl iodide or benzyl bromide afforded the biphenyl carboxylates 5a-c. In one pot reaction, the carboxylates were reduced to the benzyl alcohol, transformed to the benzyl chloride and substituted to the corresponding nitriles (6a-c).
The cyclization to phenanthrenes proved more difficult than expected. Thus, the Hoesch condensation of 6b was attempted, with both PPA and ZnCl₂/HCl; however, no cyclization to the phenanthrol 2b was observed. Attempts at cyclizing the acids 7a-e, as well as their acid chloride derivatives, under Friedel-Crafts conditions, also failed. However, the cyclization succeeded when the morpholine amides 8a-e were treated with phosphoryl chloride (an analogue of the Bischler-Napieralsky reaction used by McLean in the synthesis of dibenzoxepinones) and the 10-morpholinophenanthrenes 9a-e were obtained in good yields. Unfortunately, the cyclization of the amide 10b (R = H), which would allow direct access to the amine 11b, did not occur. The morpholines 9a-e were hydrolysed to the air sensitive phenanthrols 2a-e. The Bucherer amination reaction of the phenanthrols with methylamine or ammonia afforded the phenanthrylamines 11a-e and 12b, which were readily transformed to the
corresponding chloroacetamides under Schotten-Baumann conditions.

Two products (Scheme 4) can be expected from the photochemistry of these chloroacetamides, viz. that resulting from cyclization at C-1 to afford the desired aporphinoid lactam, or that involving the formation of a five member ring through C-10. In the former case, the photocyclization is known to occur via an electron transfer mechanism, so it exhibits a high solvent sensitivity; in the latter case, an alternative mechanism is suspected to operate. Since the formation of the new C-C bond at the para position of a methoxyl group is the preferred choice in aqueous solvents, 14b was irradiated (λ > 300 nm) in acetonitrile-water. After 3 h, photosolvolysis to the hydroxyacetamide 15b was the sole observed reaction. The absence of cyclization has occasionally been attributed to inefficient exciplex formation owing to geometrical restrictions. However, the amide geometry can be changed on conversion from N-monosubstituted to tertiary amides. In fact, the irradiation of the N-methyl chloroacetamides 13a-c proceeded as expected. The appearance of a strong yellow colour indicated the correct cyclization, followed by the air oxidation of the lactam intermediate to the 4,5-dioxo compounds 1a-c, in 31, 60 and 57% yields, respectively.

A thermal approach via the Bobbit modification of the Pomerantz-Fritz reaction as an alternative to the isoquinoline ring formation was also studied. However, no phenanthrene formation was observed when the amide 10b [R = CH2CH(OMe)2] was treated with phosphoryl chloride.

The above results support this novel synthetic approach to 4,5-dioxoaoporphinoids with the sequential formation of rings C and B. The synthesis of 4-alkoxy-10-phenanthrols from simple fluorenone precursors complements alternative choices. Furthermore, the photochemical cyclization step requires a tertiary chloroacetamide, so it does not give access to the N-nor-4,5-dioxoaoporphine alkaloids.

The results for the cytotoxicity of cepharadione-B (1a) and their 2-demethoxy analogues (1b,c) are shown in Table 1. The potency of these 4,5-dioxoaoporphines as cytotoxic agents varied among the different cell lines tested. The compounds exhibited significant activity against both wild-type, and adriamycin-resistant P-388 cell lines, and were also active against H-29 human colon adenocarcinoma and the MDA-MB-231 human breast carcinoma cells.

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The IC50 values obtained are consistent with those reported for artabotrine and suggest that 4,5-dioxoaoporphines may function as antineoplastic agents.
EXPERIMENTAL

General Methods

M.p.s. were determined on a Gallenkamp instrument and are given uncorrected. UV spectra were recorded on a Hewlett-Packard 8452A spectrophotometer, and IR spectra on a Perkin-Elmer 883 spectrophotometer. Low- and high-resolution mass spectra were recorded on HP-MS 5988A and Kratos MS 50 spectrometers, respectively, both operating at 70 eV. NMR spectra were obtained on Bruker WP-200 SY instrument at 200 MHz for 1H and 50 MHz for 13C. 1H Chemical shifts (δH) are given relative to either residual CHCl3 (δ 7.24 ppm) in CDCl3 or residual CD3SOCD3 (δ 2.50 ppm) in DMSO-d6. J values are in Hz. 13C Chemical shifts (δC) are given relative to either CDCl3 (δ 77.0 ppm) in CDCl3 or CD3SOCD3 (δ 39.7 ppm) in DMSO-d6. Irradiations were conducted with a 125 W medium-pressure mercury lamp (General Electric H125/27) in Pyrex tubes at RT. TLC analyses were performed on silica gel 60 F 256 plates, and column chromatography was carried out on silica gel 60 (70-230 mesh). 3'-Methoxy-biphenyl-2,2'-carbolactone (4e) and biphenyl-2,2'-carbolactone (4d) were prepared from 1-methoxy-9-fluorenone (3e) and 9-fluorenone (3d), respectively, by oxidation with sodium perborate/acetic acid, following the procedure described by McKillop.15

Synthesis of biphenyl-2-carboxylates 5a-c:

To a mixture of powdered KOH (255 mmol) and methyl iodide (510 mmol) in acetonitrile (300 ml), the biphenyl carbolactone 4d,e (51 mmol) was added. After stirring for 12 h at RT, the solvent was evaporated and H2O added to the residue. Extraction with CHCl3, drying the organic layer with MgSO4 and evaporation, afforded compounds 5a,b. Under identical conditions, the reaction of 4d with benzyl bromide gave 5c.

Methyl 2',3'-dimethoxy-biphenyl-2-carboxylate 5a: Yield: 93%; yellowish syrup; ν (film) cm⁻¹ 1730 (C=O); δH (CDCl3) 7.89 (1H, dd, J=7.5 and 1.7, H-3), 7.52 (1H, ddd, J=7.5, 7.5 and 1.7, H-5), 7.37 (1H, ddd, J=7.5, 7.5 and 1.5, H-4), 7.34 (1H, dd, J=7.5 and 1.5, H-6), 7.08 (1H, t, J=7.7, H-5'), 6.92 (1H, dd, J=7.7 and 1.7, H-4'), 6.81 (1H, dd, J=7.7 and 1.7, H-6'), 3.88 (3H, s, 3'-OMe), 3.66 (3H, s, COOMe), 3.47 (3H, s, 2'-OMe); δC (CDCl3) 168.2 (CO), 152.3 (C-3'), 145.9 (C-2'), 138.6, 135.8, 131.1 (C), 131.2, 131.2, 129.6, 127.2, 123.7, 121.9, 111.8 (CH), 60.2 (2'-OMe), 55.8 (3'-Ome), 51.8 (COOMe); m/z (%) 272 (M⁺, 32), 241 (100); Anal. Calcd. for C16H16O4: C, 70.56; H, 5.93%, found C, 70.09; H, 5.88.

Methyl 2'-methoxy-biphenyl-2-carboxylate 5b: Yield: 95%; yellowish syrup; ν (film) cm⁻¹ 1729 (C=O); δH (CDCl3) 7.87 (1H, dd, J=7.6 and 1.4, H-3), 7.54 (1H, ddd, J=7.6, 7.6 and 1.4, H-5), 7.43-7.23 (4H, m, Ar-H), 7.04 (1H, ddd, J=7.4, 7.4 and 1.0, H-3'), 6.90 (1H, dd, J=7.4 and 1.0, H-3), 3.71 (3H, s, OMe), 3.64 (3H, s, COOMe); δC (CDCl3) 168.4 (CO), 155.9 (C-2'), 138.6, 131.5, 130.4 (C), 131.4, 131.2, 129.8, 129.2, 128.7, 126.9, 120.6, 110.0 (CH), 55.0 (OMe), 51.5 (COOMe); m/z (%) 242 (M⁺, 74), 211 (100); Anal. Calcd. for C15H14O3: C, 70.56; H, 5.93%, found C, 70.09; H, 5.88.

Benzyl 2'-benzoxyl-biphenyl-2-carboxylate 5c: Yield: 83%, syrup that crystallized on standing; m.p. 74-76 °C; ν (KBr) cm⁻¹ 1718 (C=O); δH (CDCl3) 7.97 (1H, d, J=7.5, H-3), 7.6-6.8 (16H, m, Ar-H), 6.87 (1H,
d, J=8.2, H-3′), 5.05 (2H, s, OCH2Bn), 4.88 (2H, s, CO2CH2Bn); δC (CDCl3) 167.7 (CO), 155.3 (C-2′), 139.2, 137.1, 135.6, 131.6, 131.3 (C), 131.5, 131.5, 130.0, 129.7, 128.6, 128.2, 128.1, 127.8, 127.4, 127.1, 126.6, 121.1, 112.6 (CH), 70.1 (OCH2Bn), 66.5 (CO2CH2Bn); m/z (%) 394 (M+, 2), 303 (11), 91 (100); Anal. Calcd. for C27H22O3: C, 82.21; H, 5.62%; found C, 82.30; H, 5.82.

Homologation reaction. Synthesis of 6a-c:

A N2 purged solution of 5a-e (45 mmol) in dry THF (120 ml), was supplied with LiAlH4 (68 mmol) in small portions over a period of 30 min. After stirring the reaction medium for 1 h at RT, excess hydride was decomposed by the addition of 1M H2SO4, and the resulting suspension treated with more dilute acid and extracted with TBME. The extract was carefully dried (MgSO4) and the volume reduced to 50 ml. This ether solution was ice cooled and thionyl chloride (68 mmol) was added dropwise. The mixture was stirred at RT for 30 min. TBME and excess thionyl chloride were removed in vacuo without heating. The crude products were dissolved in acetonitrile (400 ml), NaCN (450 mmol) was added and the mixture refluxed for 48 h. After evaporation of the solvent, the residue was dissolved in H2O, extracted with CHCl3 and dried over MgSO4, and concentrated under reduced pressure to give the corresponding cyanides, 6a-c.

(2',3'-Dimethoxy)biphenyl-2-yl acetonitrile 6a: Yield: 82%; yellowish syrup; ν (film) cm⁻¹ 2255 (CN); δH (CDCl3) 7.6-7.2 (4H, m, Ar-H), 7.13 (1H, t, J=8.0, H-5′), 6.97 (1H, dd, J=8.0 and 1.4, H-4′), 6.78 (1H, dd, J=8.0 and 1.4, H-6′), 3.90 (3H, s, 3′-OMe), 3.79 (1H, d, J=18.5, HCH), 3.50 (1H, d, J=18.5, HCH), 3.46 (3H, s, 2′-OMe); δC (CDCl3) 152.8 (C-3′), 145.9 (C-2′), 137.6, 134.0, 129.1 (C), 118.3 (CN), 130.3, 128.1, 127.9, 127.7, 124.5, 122.6, 112.1 (CH), 60.7 (2′-OMe), 55.8 (3′-OMe), 21.8 (CH2); m/z (%) 253 (M+, 100), 238 (57), 210 (37); Anal. Calcd. for C15H13NO2: 75.86; H, 5.97; N, 5.53%; found C, 75.69; H, 6.09; N, 5.53.

(2'-Methoxy)biphenyl-2-yl acetonitrile 6b: Yield: 95%; yellowish syrup; ν (film) cm⁻¹ 2257 (CN); δH (CDCl3) 7.6-6.9 (8H, m, Ar-H), 3.78 (3H, s, OMe), 3.63 (1H, d, J=18.7, HCH), 3.47 (1H, d, J=18.7, HCH); δC (CDCl3) 156.1 (C-2′), 138.2, 129.2, 128.5 (C), 118.4 (CN), 131.1, 130.7, 129.6, 128.1, 128.0, 120.9, 110.7 (CH), 55.3 (OMe), 21.6 (CH2); m/z (%) 223 (M+, 100), 208 (82), 190 (62); HRMS calcd. for C15H13NO (M+) m/z 223.0997, found 223.1000; Anal. Calcd. for C15H13NO: C, 80.68; H, 5.87; N, 6.28%; found C, 80.19; H, 5.91; N, 6.54.

(2'-Benzyloxy)biphenyl-2-yl acetonitrile 6c: Yield: 88%; white syrup that crystallized on standing; m.p. 40-41°C; ν (KBr) cm⁻¹ 2255 (CN); δH (CDCl3) 7.6-7.0 (13H, m, Ar-H), 5.09 (1H, d, J=11, HCHBn), 5.00 (1H, d, J=11, HCHBn), 3.67 (1H, d, J=18.6, HCHCN), 3.49 (1H, d, J=18.6, HCHCN); δC (CDCl3) 155.3 (C-2′), 138.2, 136.7, 129.2 (C), 118.3 (CN), 131.1, 130.7, 129.5, 128.4, 128.0, 127.9, 127.7, 126.7, 121.5, 113.3 (CH), 70.4 (CH2Bn), 21.8 (CH2CN); m/z (%) 299 (M+, 6), 91 (100); Anal. Calcd. for C21H17NO: C, 84.25; H, 5.72; N 4.68%; found C, 83.73; H, 5.77; N 4.76.
Synthesis of 4-(biphenyl-2-yl)acetyl morpholines 8a-c

To a solution of 6a-c (36.3 mmol) in ethanol (400 ml), KOH (363 mmol) and water (170 ml) were added. After refluxing the mixture for 24 h, most of the ethanol was removed in vacuo. The resulting suspension was diluted with 1M NaOH, and washed with CHCl₃. The aqueous layer was acidified with concentrated HCl and extracted with CHCl₃. The extracts were dried over anhydrous MgSO₄ and the solvent evaporated.

(2',3'-Dimethoxy)biphenyl-2-yl acetic acid 7a: Yield: 93%; white solid; m.p. 89-91 °C (CHCl₃); ν (KBr) cm⁻¹ 3600-2400 (OH), 1707 (C=O); δH (CDCl₃) 7.5-7.2 (4H, m, Ar-H), 7.09 (1H, t, J = 7.8, H-5'), 6.94 (1H, dd, J = 7.8 and 1.5, H-4'), 6.72 (1H, dd, J = 7.8 and 1.5, H-6'), 3.88 (3H, s, 3'-OMe), 3.52 (3H, s, 2'-OMe), 3.37 (2H, s, CH₂); δC (CDCl₃) 174.9 (CO), 152.7 (C-3'), 145.7 (C-2'), 138.0, 135.3, 133.7 (C), 130.1, 129.4, 128.1, 126.8, 124.4, 122.7, 111.9 (CH), 60.7 (2'-OMe), 55.7 (3'-OMe), 40.7 (CH₂); m/z (%) 272 (M⁺, 32), 271 (66), 228 (18), 196 (79), 195 (100); Anal. Calcd. for C₁₆H₁₆O₄ ½ H₂O: C, 68.30; H, 6.10%, found C, 68.65; H, 6.14.

(2'-Methoxy)biphenyl-2-yl acetic acid 7b: Yield: 94%; colourless crystals; m.p. 140-141 °C (ether); ν (KBr) cm⁻¹ 3400-2200 (OH), 1705 (C=O); δH (CDCl₃) 9.7 (1H, br s, COOH), 7.5-6.9 (8H, m, Ar-H), 3.73 (1H, d, J = 16.8, HCH), 3.71 (3H, s, OMe), 3.49 (1H, d, J = 16.8, HCH); δC (CDCl₃) 177.5 (CO), 156.2 (C-2'), 138.8, 132.4, 129.8 (C), 131.2, 130.3, 130.0, 128.9, 127.4, 127.1, 120.5, 110.4 (CH), 54.9 (OMe), 38.5 (CH₂); m/z (%) 242 (M⁺, 73), 224 (35), 198 (31), 165 (100); HRMS calcd. for C₁₅H₁₄O₃ (M⁺) m/z 242.0943, found 242.0940; Anal. Calcd. for C₁₅H₁₄O₃: C, 74.35; H, 5.83%, found C, 74.60; H, 5.87.

(2'-Benzyloxy)biphenyl-2-yl acetic acid 7c: Yield: 85%; colourless prisms; m.p. 95-97 °C (MeOH); ν (film) cm⁻¹ 3475-2400 (OH), 1707 (C=O); δH (CDCl₃) 7.4-6.9 (13H, m, Ar-H), 4.98 (2H, s, CH₂Bn), 3.50 (2H, s, CH₂COOH); δC (CDCl₃) 177.7 (CO), 155.4 (C-2'), 138.9, 137.0, 132.3, 130.4 (C), 131.4, 130.5, 129.8, 128.9, 128.3, 127.5, 127.4, 127.1, 126.5, 121.1, 113.1 (CH), 70.1 (CH₂Bn), 38.7 (CH₂COOH); m/z (%) 318 (M⁺, 4), 300 (9), 91 (100); Anal. Calcd. for C₂₁H₁₈O₃ ½ H₂O: C, 78.48; H, 5.75%, found C, 78.28; H, 5.83.

Over an ice cooled solution of compound 7a-c (12.4 mmol) and pyridine (1 ml) in benzene (200 ml), thionyl chloride (248 mmol) (oxalyl chloride for 7a) was added dropwise. The ice-bath was removed and the mixture stirred at RT for 45 min. The solvent and excess reagent were distilled and the resulting acid chloride was dissolved in CHCl₃ (5 ml). This solution was added to a cooled mixture of morpholine (124 mmol), triethylamine (4.6 ml) and CHCl₃ (50 ml) with stirring. The reaction mixture was stirred at RT for 30 min and washed sequentially with 1M NaOH, 1M HCl, and water. The organic solution was dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was purified by crystallization.

4-(2',3'-Dimethoxybiphenyl-2-yl)acetyl morpholine 8a: Yield: 85%; white solid; m.p. 78-80 °C (MeOH); ν (KBr) cm⁻¹ 1643 (C=O); δH (CDCl₃) 7.4-7.2 (4H, m, Ar-H), 7.06 (1H, t, J = 7.8, H-5'), 6.9-6.7 (2H, m, Ar-H), 3.86 (3H, s, OMe), 3.67-3.09 (10H, m, morpholine-H, CH₂CO), 3.45 (3H, s, OMe); δC (CDCl₃) 170.0 (CO), 152.5, 145.9 (C-2', C-3'), 137.6, 135.1, 133.7 (C), 129.9, 128.4, 127.6, 126.3, 124.0, 122.8, 111.4
(CH), 66.5, 66.2 (CH₂OCH₂), 60.5 (OMe), 55.6 (OMe), 46.0, 41.8 (CH₂NCH₂), 37.9 (CH₂); m/z (%): 341 (M⁺, 49), 310 (23), 227 (78), 209 (75), 196 (75), 195 (96), 114 (100); Anal. Calcd. for C₂₀H₂₃NO₄: C, 70.35; H, 6.79; N 4.10%; found C, 70.22; H, 6.83; N 4.00.

4-(2'-Methoxybiphenyl-2-yl)acetyl morpholine 8b: Yield: 99%; white needles; m.p. 111-113 °C (EtOH); ν (KBr) cm⁻¹ 1639 (C=O); 6H (CDCl₃) 7.4-6.9 (8H, m, Ar-H), 3.73 (3H, s, OMe), 3.7-3.0 (8H, m, morpholine-H), 3.59 (1H, d, J=14.0, HCHCO), 3.43 (1H, d, J=14.0, HCHCO); δc (CDCl₃) 169.6 (CO), 155.9 (C-2'), 137.6, 133.4, 128.9 (C), 130.6, 130.0, 128.7, 127.8, 127.2, 126.2, 120.2, 110.3 (CH), 66.1, 65.8 (CH₂OCH₂), 54.8 (OMe), 45.5, 41.5 (CH₂NCH₂), 37.9 (CH₂CO); m/z (%) 311 (M⁺, 17), 197 (18), 182 (18), 165 (30), 114 (100); HRMS calcd. for C₁₉H₂₁NO₃ (M⁺) m/z 311.1521, found 311.1517.

Cyclization to 4-(10-phenanthryl)morpholines 9a-c

A mixture of the amides 8a-c (11.7 mmol), dry toluene (34 ml), dry benzene (16 ml) and phosphoryl chloride (117 mmol) was refluxed for 1 h. The cold mixture was diluted with ether and a 1M NaOH aqueous solution was added. The organic layer was washed with water, dried over anhydrous MgSO₄ and concentrated in vacuo. Compound 9e was purified by column chromatography (SiO₂, 20:2 hexane-EtOAc as eluent).

4-[10-(3,4-Dimethoxy)phenanthryl]morpholine 9a: Yield: 74%; amorphous solid; λ (CHCl₃) nm (log ε): 366 (3.28), 348 (3.35), 318 (4.01), 262 (4.59); δH (CDCl₃) 9.58 (1H, m, H-5), 8.16 (1H, d, J=9.0, H-1), 7.34 (1H, d, J=9.0, H-2), 7.77 (1H, m, H-8), 7.54 (2H, m, H-6, -7), 7.16 (1H, s, H-9), 4.03 (3H, s, OMe), 3.95 (3H, s, OMe), 3.95-4.03 (4H, m, CH₂OCH₂), 3.13 (4H, br s, CH₂NCH₂); δc (CDCl₃) 151.5, 147.3, 147.1 (C-3, C-4, C-10), 133.4, 127.3, 126.1, 124.5 (C), 127.6, 127.5, 126.6, 126.5, 121.2, 112.3 (CH), 67.3 (CH₂OCH₂), 59.7 (OMe), 56.3 (OMe), 53.3 (CH₂NCH₂); m/z (%) 323 (M⁺, 100), 322 (26), 308 (26); HRMS calcd. for C₂₀H₂₁NO₃ (M⁺) m/z 323.1521, found 323.1527.

4-[10-(4-Methoxy)phenanthryl]morpholine 9b: Yield: 75%; colourless prisms; λ (CHCl₃) nm (log ε): 358 (3.06), 342 (3.03), 308 (3.79), 244 (4.18); δH (CDCl₃) 9.56 (1H, m, H-5), 8.03 (1H, d, J=8.0, H-1), 7.78 (1H, m, H-8), 7.58-7.49 (3H, m, Ar-H), 7.31 (1H, s, H-9), 7.16 (1H, d, J=8.0, H-3), 4.11 (3H, s, OMe), 3.99 (4H, br t, CH₂OCH₂), 3.14 (4H, br s, CH₂NCH₂); δc (CDCl₃) 159.2 (C-4), 147.1 (C-10), 131.2, 127.9, 122.2 (C), 128.4, 127.5, 126.3, 126.1, 125.2, 115.6, 115.6, 108.5 (CH), 67.4 (CH₂OCH₂), 55.7 (OMe), 53.3 (CH₂NCH₂); m/z (%) 293 (M⁺, 100), 235 (31), 204 (19), 165 (23), 114 (28); HRMS calcd. for C₁₉H₁₉NO₂ (M⁺) m/z 293.1416, found 293.1416.
4,5-Dioxoaporphine alkaloids

4-(10-(4-Benzoyloxy)phenanthryl)morpholine 9c: Yield: 66%; colourless crystals; m.p. 126-127 °C (hexane-EtOAc); λ (CHCl₃) nm (log ε): 360 (3.34), 342 (3.36), 312 (3.92), 272 (4.19); δH (CDCl₃) 9.70 (1H, d, J=8.3, H-5), 8.10 (1H, d, J=7.7, H-1), 7.82 (1H, d, J=8.3, H-8), 7.6-7.4 (8H, m, Ar-H), 7.36 (1H, s, H-9), 7.23 (1H, d, J=7.7, H-3), 5.38 (2H, s, CH₂Bn), 4.03 (4H, br t, CH₂OCH₂), 3.18 (4H, br s, CH₂NCH₂); δC (CDCl₃) 158.1 (C-4), 147.0 (C-10), 136.8, 132.8, 131.3, 127.9, 122.5 (C), 128.6, 128.5, 128.0, 127.6, 127.5, 126.2, 126.0, 125.2, 116.9, 115.6, 110.1 (CH), 71.2 (CH₂Bn), 67.4 (CH₂OCH₂), 53.3 (CH₂NCH₂); m/z (%) 369 (M⁺, 59), 278 (100), 91 (37); HRMS calcd. for C₂₅H₂₃NO₂ (M⁺) m/z 369.1729, found 369.1729.

Synthesis of 10-phenanthrols 2a-c

A solution of the amines 9a-c (8.5 mmol) in THF (90 ml) and 1M HC1 (70 ml) was heated at 60 °C for 12 h. After cooling, TBME was added and the organic layer washed with H₂O, dried over anhydrous MgSO₄ and concentrated in vacuo. The phenanthrols obtained were manipulated under an N₂ atmosphere to prevent oxidation to the quinone.

3,4-Dimethoxy-10-phenanthrol 2a: Yield: 87%; amorphous reddish solid; 160-163 °C (CHCl₃); ν (KBr) cm⁻¹ 3379 (OH); δH (CDCl₃) 9.51 (1H, m, H-5), 8.11 (1H, d, J=9.0, H-1), 7.34 (1H, d, J=9.0, H-2), 7.63 (1H, m, H-8), 7.52-7.40 (2H, m, H-6, -7), 6.85 (1H, s, H-9), 4.03 (3H, s, OMe), 3.93 (3H, s, OMe); δC (CDCl₃) 151.9, 149.5, 146.8 (C-3, C-4, C-10), 133.9, 126.0, 125.8, 121.8 (C), 127.6, 126.7, 126.4, 124.0, 118.9, 112.3, 104.8 (CH), 59.7 (OMe), 56.3 (OMe); m/z (%) 254 (M⁺, 100), 239 (50); HRMS calcd. for C₁₆H₁₄O₃ (M⁺) m/z 254.0943, found 254.0942.

4-Methoxy-10-phenanthrol 2b: Yield: 74%; amorphous reddish solid; m.p. 153-155 °C (CHCl₃); ν (KBr) cm⁻¹ 3361 (OH); δH (CDCl₃) 9.52 (1H, m, H-5), 8.01 (1H, dd, J=7.8 and 0.8, H-1), 7.63 (1H, m, H-8), 7.53 (1H, t, J=7.8, H-2), 7.46-7.40 (2H, m, H-6, -7), 7.23 (1H, s, OH), 7.16 (1H, dd, J=7.8 and 0.8, H-3), 7.05 (1H, s, H-9), 4.09 (3H, s, OMe); δC (CDCl₃) 158.8, 148.9 (C-4, C-10), 132.9, 127.9, 126.7, 121.9 (C), 128.5, 126.5, 126.4, 126.2, 124.1, 114.7, 109.1, 107.3 (CH), 55.7 (OMe); m/z (%) 224 (M⁺, 100), 209 (20), 181 (40); HRMS calcd. for C₁₅H₁₂O₂ (M⁺) m/z 224.0837, found 224.0838.

4-Benzoyloxy-10-phenanthrol 2c: Yield: 67%; amorphous reddish solid; m.p. 124-127 °C (CHCl₃); ν (KBr) cm⁻¹ 3375 (OH); δH (CDCl₃ + CD₃OD) 9.59 (1H, d, J=8.4, H-5), 8.02 (1H, d, J=8.4, H-1), 7.65-7.19 (10H, m, Ar-H), 7.02 (1H, s, H-9), 5.33 (2H, s, CH₂Bn); δC (CDCl₃ + CD₃OD) 157.6, 150.1 (C-4, C-10), 136.8, 133.5, 128.6, 122.0 (C), 128.5, 127.9, 127.6, 126.1, 125.9, 123.4, 115.4, 110.5, 106.5 (CH), 71.1 (CH₂Bn); m/z (%) 300 (M⁺, 34), 209 (9), 91 (100); HRMS calcd. for C₂₁H₁₆O₂ (M⁺) m/z 300.1150, found 300.1156.

Synthesis of phenanthrylamines 11a-c and 12b

A stirred mixture of phenanthrols 2a-c (3.6 mmol), 40% aq. sodium bisulfite (8.6 ml) and 40% aq. methylamine (36 mmol) was heated at 150 °C in a sealed tube for 18 h. The reaction mixture was cooled and extracted with ether, the organic layer being washed (1M NaOH and H₂O), dried over anhydrous CaCl₂ and evaporated in vacuo.
10-N-Methylamino-3,4-dimethoxy phenanthrene 11a: Yield: 62%; amorphous yellow solid; \( \nu \) (KBr) cm\(^{-1} \) 3445 (NH); \( \delta_h \) (CDCl\(_3\)) 9.47 (1H, dd, \( J=7.8 \) and 1.5, H-5), 7.65 (1H, dd, \( J=7.8 \) and 1.5, H-8), 7.60-7.24 (4H, m, Ar-H), 6.64 (1H, s, H-9), 3.99 (3H, s, OMe), 3.91 (3H, s, OMe), 3.04 (3H, s, NMe); \( \delta_c \) (CDCl\(_3\)) 151.5, 147.6, 141.9 (C-3, C-4, C-10), 134.8, 125.7, 124.7, 121.8 (C), 127.7, 126.8, 126.4, 122.9, 116.2, 112.0, 101.3 (CH), 59.7 (OMe), 56.3 (OMe), 31.1 (NMe); \( m/z \) (%) 267 (M\(^+\), 100), 252 (37), 237 (17), 209 (34); HRMS calcd. for C\(_{17}\)H\(_{14}\)NO\(_2\) (M\(^+\)) \( m/z \) 267.1259, found 267.1259.

10-N-Methylamino-4-methoxy phenanthrene 11b: Yield: 88%; brownish syrup; \( \nu \) (film) cm\(^{-1} \) 3447 (NH); \( \delta_h \) (CDCl\(_3\)) 9.59 (1H, dd, \( J=8.4 \) and 1.3, H-5), 7.79 (1H, dd, \( J=8.4 \) and 1.7, H-8), 7.53 (4H, m, Ar-H), 7.15 (1H, dd, \( J=7.8 \) and 1.7, H-3), 6.85 (1H, s, H-9), 4.09 (3H, s, OMe), 3.04 (3H, s, NMe); \( \delta_c \) (CDCl\(_3\)) 159.3 (C-4), 141.8 (C-10), 134.1, 128.0, 125.1, 121.4 (C), 128.3, 126.3, 126.2, 126.1, 122.7, 112.6, 108.5, 103.3 (CH), 55.5 (OMe), 31.0 (NMe); \( m/z \) (%) 237 (M\(^+\), 100%), 222 (22), 207 (14); HRMS calcd. for C\(_{16}\)H\(_{15}\)NO (M\(^+\)) \( m/z \) 237.1154, found 237.1152.

10-N-Methylamino-4-benzyloxy phenanthrene 11c: Yield: 62%, brown syrup; \( \nu \) (film) cm\(^{-1} \) 3420 (NH); \( \delta_h \) (CDCl\(_3\)) 9.76 (1H, d, \( J=8.5 \), H-5), 7.89 (1H, d, \( J=8.5 \), H-8), 7.54-7.30 (10H, m, Ar-H), 7.00 (1H, s, H-9), 5.53 (2H, s, CH\(_2\)Bn), 3.24 (3H, s, NMe); \( \delta_c \) (CDCl\(_3\)) 158.4 (C-4), 141.8 (C-10), 136.8, 134.1, 128.2, 125.1, 121.8 (C), 128.6, 128.5, 128.0, 127.6, 126.4, 126.2, 122.8, 113.0, 110.3, 103.5 (CH), 71.2 (CH\(_2\)Bn), 31.3 (NMe); \( m/z \) (%) 313 (M\(^+\), 37), 222 (49), 91 (100); HRMS calcd. for C\(_{22}\)H\(_{19}\)NO (M\(^+\)) \( m/z \) 313.1467, found 313.1466.

10-Amino-4-methoxy phenanthrene 12b: Yield: 96%; brown syrup; \( \delta_h \) (CDCl\(_3\)) 9.59 (1H, m, H-5), 7.67 (1H, m, H-8), 7.55-7.47 (4H, m, Ar-H), 7.16 (1H, m, H-3), 6.97 (1H, s, H-9), 4.08 (3H, s, OMe), 4.05 (2H, br s, NH\(_2\)); \( \delta_c \) (CDCl\(_3\)) 159.2 (C-4), 139.4 (C-10), 133.5, 127.9, 121.5 (C), 128.4, 126.2, 126.1, 126.0, 123.2, 113.7, 109.0, 108.6 (CH), 55.6 (OMe); \( m/z \) (%) 223 (M\(^+\), 100%), 208 (18), 180 (41); HRMS calcd. for C\(_{18}\)H\(_{14}\)NO (M\(^+\)) \( m/z \) 223.0997, found 223.0998.

Synthesis of 10-phenanthryl chloroacetamides 13a-c and 14b

An ice cooled mixture of 10% aq. NaOH (1.6 ml) and a dichloromethane solution (6 ml) of the amines 11a-c and 12b (2.5 mmol) was stirred while chloroacetyl chloride (3 mmol) in dichloromethane (4 ml) was added dropwise. The mixture was stirred for a further hour at RT and diluted with CH\(_2\)Cl\(_2\). The organic layer was washed with H\(_2\)O, dried over anhydrous MgSO\(_4\) and concentrated in vacuo.

Chloroacetamide 13a was purified by TLC (SiO\(_2\), 20:0.2 CH\(_2\)Cl\(_2\)-MeOH as eluent).

10-N-Methyl-chloroacetamido-3,4-dimethoxy phenanthrene 13a: Yield: 59%; white solid; m.p. 128-130 °C (CH\(_2\)Cl\(_2\)); \( \nu \) (KBr) cm\(^{-1} \) 1676 (C=O); \( \lambda \) (CHCl\(_3\)) nm (log \( e \)) 362 (2.97), 344 (3.11), 306 (3.75), 280 (3.90), 260 (4.31); \( \delta_h \) (CDCl\(_3\)) 9.64 (1H, dd, \( J=7.5 \) and 1.9, H-5), 7.82 (1H, dd, \( J=7.5 \) and 1.9, H-8), 7.74-7.55 (3H, m, Ar-H), 7.54 (1H, s, H-9), 7.38 (1H, d, 1H, \( J=9.0 \), H-1), 4.03 (3H, s, OMe), 3.92 (1H, d, \( J=13.4 \), HCH), 3.74 (1H, d, \( J=13.4 \), HCH), 3.97 (3H, s, OMe), 3.40 (3H, s, NMe); \( \delta_c \) (CDCl\(_3\)) 167.1 (CO), 152.5, 147.6 (C-3, C-4), 136.8 (C-10), 132.0, 129.7 126.3, 124.0 (C), 128.7, 127.9, 127.3, 125.2, 118.9, 113.6 (CH), 59.8 (OMe), 56.4 (OMe), 41.7 (CH\(_2\)), 37.6 (NMe); \( m/z \) (%) 345 (M\(^+\), 31), 343 (M\(^+\), 90), 254 (100);
HRMS calcd. for $C_{19}H_{11}NO_3^{35}Cl$ (M$^+$) $m/z$ 343.0975, found 343.0970.

**10-N-Methyl-chloroacetamido-4-methoxy phenanthrene 13b:** Yield: 98%; brown solid; m.p. 216-218 °C (MeOH); $\nu$ (KBr) cm$^{-1}$ 1677 (C=O); $\lambda$ (EtOH) nm (log $\varepsilon$) 356 (3.08), 340 (3.13), 276 (4.21), 246 (4.28); $\delta_H$ (CDCl$_3$) 9.67 (1H, d, $J=8.5$, H-5), 7.87 (1H, dd, $J=7.6$ and 1.8, H-8), 7.8-7.6 (2H, m, H-6, -7), 7.73 (1H, s, H-9), 7.62 (1H, t, $J=8.1$, H-2), 7.44 (1H, d, $J=8.1$, H-1), 7.25 (1H, d, $J=8.1$, H-3), 4.16 (3H, s, OMe), 3.90 (1H, d, $J=13.3$, HCH), 3.74 (1H, d, $J=13.3$, HCHF), 3.42 (3H, s, NMe); $\delta_C$ (CDCl$_3$) 167.2 (CO), 159.4 (C-4), 136.7 (C-10), 131.4, 130.8, 130.4, 122.2 (C), 128.7, 128.6, 128.0, 127.9, 127.8, 126.7, 115.0, 109.5 (CH), 55.9 (OMe), 41.8 (CH$_2$), 37.6 (NMe); $m/z$ (%) 315 (M$^+$, 7), 313 (M$^+$, 36), 224 (88), 195 (29), 165 (22), 152 (37), 90 (100); HRMS calcd. for $C_{18}H_{16}NO_3^{35}Cl$ (M$^+$) $m/z$ 313.0870, found 313.0871.

**10-N-Methyl-chloroacetamido-4-benzyloxy phenanthrene 13c:** Yield: 90%; solid foam; m.p. 74-75 °C; $\nu$ (KBr) cm$^{-1}$ 1674 (C=O); $\lambda$ (CHCl$_3$) nm (log $\varepsilon$) 374 (3.03), 358 (3.11), 278 (3.91), 256 (4.05); $\delta_H$ (CDCl$_3$) 9.73 (1H, m, H-5), 7.86 (1H, m, H-8), 7.74 (1H, s, H-9), 7.7-7.4 (9H, m, Ar-H), 7.31 (1H, d, $J=7.9$, H-3), 5.42 (2H, s, CH$_2$Bn), 3.90 (1H, d, $J=13.4$, HCHCl), 3.73 (1H, d, $J=13.4$, HCHCl), 3.42 (3H, s, NMe); $\delta_C$ (CDCl$_3$) 167.2 (CO), 158.4 (C-4), 136.7 (C-10), 136.3, 131.5, 130.9, 122.4 (C), 128.9, 128.8, 128.6, 128.3, 128.0, 127.9, 127.9, 127.7, 127.6, 126.7, 115.3, 111.0 (CH), 71.4 (CH$_2$Bn), 41.8 (CH$_2$Cl), 37.6 (NMe); $m/z$ (%) 391 (M$^+$, 5), 389 (M$^+$, 15), 340 (1), 300 (17), 165 (13), 152 (7), 91 (100); HRMS calcd. for $C_{24}H_{20}NO_2^{35}Cl$ (M$^+$) $m/z$ 389.1183, found 389.1172.

**10-Chloroacetamido-4-methoxyphenanthrene 14b:** Yield: 98%; white solid; m.p. 232 °C (CHCl$_3$); $\nu$ (KBr) cm$^{-1}$ 3260 (NH), 1668 (C=O); $\lambda$ (EtOH) nm (log $\varepsilon$) 358 (3.17), 340 (3.21), 304 (3.93), 276 (4.23), 242 (4.27), 214 (4.17); $\delta_H$ (CDCl$_3$ + CD$_3$OD) 9.50 (1H, m, H-5), 7.92 (1H, s, H-9), 7.72 (1H, m, H-8), 7.53-7.39 (4H, m, Ar-H), 7.12 (1H, m, H-3), 4.21 (2H, s, CH$_2$), 4.00 (3H, s, OMe); $\delta_C$ (CDCl$_3$ + CD$_3$OD) 166.1 (CO), 158.9 (C-4), 131.4 (C-10), 129.8, 128.9, 128.9, 121.5 (C), 128.1, 128.0 126.7, 126.3, 126.0, 123.9, 113.9, 108.7 (CH), 55.4 (OMe), 42.6 (CH$_2$); $m/z$ (%) 301 (M$^+$, 30) 299 (M$^+$, 100), 224 (26), 223 (24), 195 (43); HRMS calcd. for $C_{17}H_{14}NO_2^{35}Cl$ (M$^+$) $m/z$ 299.0713, found 299.0708.

**Irradiation of N-methyl-10-phenanthryl chloroacetamides 13a-c. Synthesis of 4,5-dioxoaporphines 1a-c**

A non-degassed solution of the chloroacetamide 15a-c (0.2 mmol) in 30% aqueous methanol or 30% aqueous acetonitrile (50 ml) was irradiated until a deep yellow colour developed (4h approximately). The solvent was evaporated and the residue purified by preparative TLC (SiO$_2$, 20:1 CH$_2$Cl$_2$:MeOH as eluent).

**Cepharadione-B 1a:** Yield: 31%; orange crystals; m.p. 257-260 °C (EtOH) (lit.$^{24}$ 266-268).

**2-Demethoxy-cepharadione-B 1b:** Yield: 60%; amorphous orange powder; m.p. 255-257 °C (EtOH); $\nu$ (KBr) cm$^{-1}$ 1653 (C=O); $\lambda$ (EtOH) nm (log $\varepsilon$) 398 (2.57), 308 (2.74), 276 (2.92), 240 (3.18), 214 (3.30); $\delta_H$ (DMSO-$d_6$) 9.40 (1H, m, H-11), 8.45 (1H, d, $J=8.7$, H-3), 8.08 (1H, m, H-8), 7.91 (1H, s, H-7), 7.75-7.60 (2H, m, H-9, -10), 7.60 (1H, d, $J=8.7$, H-2), 4.24 (3H, s, OMe), 3.68 (3H, s, NMe); $\delta_C$ (DMSO-$d_6$) 174.7 (C-4), 164.6, 156.0 (C-1, C-5), 131.9, 131.8 (C-6a, C-7a), 126.2, 124.2, 120.8, 118.5 (C), 130.9, 129.1,
127.6, 127.2, 127.1 (CH), 115.8 (C-7), 111.3 (C-2), 56.8 (OMe), 30.4 (NMe); m/z (%) 291 (M⁺, 100), 263 (98), 248 (31), 220 (44); HRMS calcd. for C₁₈H₁₃NO₃ (M⁺) m/z 291.0895, found 291.0895.

1-Benzylxoy-2-demethoxy-cep~ne-B 1c: Yield: 57%; yellow needles; 279-280 °C (CHCl₃); ν (KBr) cm⁻¹ 1655 (C=O); λ (EtOH) nm (log ε) 400 (2.70), 308 (2.90), 276 (3.10), 248 (3.32); δ H (CDCl₃) 9.54 (1H, d, J=7.9, H-11), 8.66 (1H, d, J=8.6, H-3), 7.92 (1H, d, J=7.9, H-8), 7.66 (1H, s, H-7), 7.6-7.3 (8H, m, Ar-H), 5.54 (2H, s, CH₂), 3.86 (3H, s, NMe); δ C (CDCl₃) 175.2 (C-4), 164.1, 156.7 (C-1, C-5), 135.0 (C-1'), 131.9, 131.8 (C-6a, C-7a), 127.1, 124.9, 121.2, 119.8 (C), 131.5, 129.0, 129.0, 128.8, 128.3, 128.0, 128.0, 127.4, 127.4 (CH), 115.8 (C-7), 111.7 (C-2), 71.9 (CH₂), 30.6 (NMe); m/z (%) 367 (M⁺, 10), 391 (1), 91 (100); HRMS calcd. for C₂₄H₁₆NO₃ (M⁺) m/z 367.1208, found 367.1209.

Irradiation of 14b

A solution of the chloroacetamide 14b (0.055 g, 0.18 mmol) in acetonitrile (65 ml) and water (15ml) was irradiated for 3 h. After that time, the reaction acquired a pale brownish colour and the solution was concentrated to dryness to give 15b.

10-Hydroxycetamido-4-methoxyphenanthrene 15b: Yield: 95%; amorphous brownish solid; ν (KBr) cm⁻¹ 3329 (NH, OH), 1699 (C=O); λ (CHCl₃) nm (log ε) 360 (3.20), 344 (3.26), 306 (3.79), 278 (4.07), 256 (4.25); δ H (CDCl₃+CD₃OD) 9.54 (1H, m, H-5), 8.21 (1H, s, H-9), 7.80 (1H, m, H-8), 7.58-7.47 (4H, m, Ar-H), 7.14 (1H, dd, J=6.9 and 2.2, H-3), 4.24 (2H, s, CH₂) 4.07 (3H, s, OMe); δ C (CDCl₃+CD₃OD) 171.2 (CO), 159.2 (C-4), 132.0 (C-10), 129.3, 128.9, 127.7 (C), 128.3, 128.3, 126.9, 126.2, 126.2, 121.7, 113.4, 108.7 (CH), 62.5 (CH₂), 55.7 (OMe); m/z (%) 281 (M⁺, 100), 223 (75), 152 (35); HRMS calcd. for C₁₉H₁₄NO₃ (M⁺) m/z 281.0525, found 281.0515.

Reaction of 4d with dibromomethane

Under an N₂ atmosphere, a THF (6 ml) solution of lithium 2,2,6,6-tetramethyl piperidide (LTMP) was added dropwise to a stirred solution of dibromomethane (4.4 mmol) in THF (6 ml), at -94 °C (hexane/ liq. N₂). After 5 min, a solution of 4d (2 mmol) in THF (5 ml) was added, followed by 1.6M n-butyllithium in hexane (10 mmol). The reaction mixture was allowed to warm up to RT, and after 15 min, poured into an ice-cooled solution of acetyl chloride (5 ml) in ethanol (25 ml). The mixture was diluted with ether and washed with 10% H₂SO₄, 5% aq. NaHCO₃, and saturated brine. The ether layer was dried over anhydrous CaCl₂ and evaporated to dryness. The residue was purified by column chromatography (SiO₂, 20:2 hexane-EtOAc) to afford 16.

6,7-Dihydro-dibenz[b,d]oxepin-6-one 16: Yield: 63%; colourless needles; m.p. 79-80 °C (MeOH); ν (KBr) cm⁻¹ 1685 (C=O); λ (CHCl₃) nm (log ε) 310 (3.50), 248 (3.96); δ H (CDCl₃) 7.90 (1H, dd, J=8.0, 1.5, Ar-H), 7.71-7.19 (7H, m, Ar-H), 4.83 (2H, s, CH₂); δ C (CDCl₃) 203.7 (CO), 156.7 (C-2'), 136.9, 136.0, 133.2 (C), 133.5, 130.2, 130.2, 129.4, 129.3, 127.9, 125.9, 121.3 (CH), 82.7 (CH₂); m/z (%) 210 (M⁺, 96), 181 (100), 165 (37), 152 (78); HRMS calcd. for C₁₄H₁₀O₂ (M⁺) m/z 210.0681, found 210.0680.
Cytotoxicity testing

HT-29 and MDA-MB-231 cells were obtained from the American Type Culture Collection; P-388 and Schabel cells were kindly provided by Pharmamar (Madrid, Spain). The effects of the alkaloids on cellular proliferation were assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Each cell line was initiated at an appropriate concentration in 96-well culture plates. After 12 h incubation, the medium culture was discarded and the test compounds were added in serial dilutions to the wells. After exposure to the alkaloids for 2 days, MTT was added and the culture allowed to stand at 37°C for 3h. Formazan crystals were redissolved (0.04 M HCl in isopropanol) and plate absorbance measured spectrophotometrically (570 nm) on a Antos 2000 ELISA plate reader.

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REFERENCES


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