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NOVEL BIOACTIVE PHENANTHRENE DERIVATIVES FROM *DOMOHINEA PERRIERI*

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Abstract: Four novel phenanthrene derivatives were isolated from *Domohinea perrieri* Leandri (Euphorbiaceae) collected in Madagascar, and have been structurally assigned as 3,6-dihydroxy-1,7-dimethyl-9-methoxyphenanthrene (**1**), 3,6-dihydroxy-1-hydroxymethyl-9-methoxy-7-methylphenanthrene (**2**), 3,6-dihydroxy-7-hydroxymethyl-9-methoxy-1-methylphenanthrene (**3**), and 3,6-dihydroxy-1,7-dihydroxymethyl-9-methoxyphenanthrene (**4**), respectively. A further constituent was the novel hexahydrophenanthrene derivative, domohinone (**5**), whose structure was confirmed by single-crystal X-ray crystallography. Compounds **1-5** were evaluated for cytotoxic and bleomycin-mediated DNA strand-scission activities.

INTRODUCTION

Domohinea (Euphorbiaceae) is a monotypic genus whose sole representative, *D. perrieri* Leandri grows in Madagascar.[1] There appears to have been no previous scientific investigation on this species. As part of our search for novel anticancer agents from plants, an ethyl acetate extract of the stem bark of *D. perrieri* was found to exhibit significant cytotoxicity against several human cancer cell lines. Bioassay-guided fractionation of this extract, monitored by growth inhibition of the Lu1 (lung cancer) cell line, led to the isolation and characterization of five novel natural products, represented by four phenanthrene derivatives and one hexahydrophenanthrene derivative, namely, 3,6-dihydroxy-1,7-dimethyl-9-methoxyphenanthrene (**1**), 3,6-dihydroxy-1-hydroxymethyl-9-methoxy-7-methylphenanthrene (**2**), 3,6-dihydroxy-7-hydroxymethyl-9-methoxy-1-methylphenanthrene (**3**), 3,6-dihydroxy-1,7-dihydroxymethyl-9-methoxyphenanthrene (**4**), and domohinone (**5**), respectively (Figure 1). In this communication, we wish to report the isolation and structure elucidation of compounds **1-5**, as well as the results of their biological evaluation in terms of cytotoxicity against a panel of human cancer cell lines and activity in a DNA strand-scission assay.

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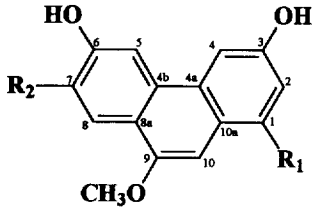
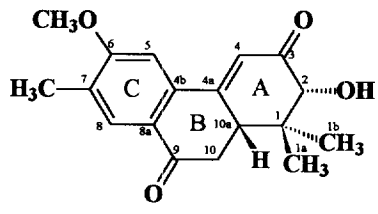
RESULTS AND DISCUSSION

The dried and ground stem bark of *D. perrieri* was extracted with ethanol and shipped from its country of origin in the form of a dried ethanol extract. This residue was then partitioned between hexane and 90% methanol in water, with the more polar layer then partitioned with ethyl acetate. The dried residue from the ethyl acetate phase was subjected to a series of activity-guided chromatographic fractionation steps to afford compounds **1-5**.



1 R_1=CH_3, R_2=CH_3
2 R_1=CH_2OH, R_2=CH_3
3 R_1=CH_3, R_2=CH_2OH
4 R_1=CH_2OH, R_2=CH_2OH



5

Figure 1. Structures of compounds **1-5**.

The molecular formula of compound **1** was established by HREIMS ($[M]^+$ *m/z* 268.1093) as $C_{17}H_{16}O_3$. Its UV absorption maxima at 252, 288, and 316 nm resembled those of phenanthrene derivatives; and its IR spectrum displayed hydroxyl (3407 cm^{-1}) and olefinic (1645 cm^{-1}) absorption bands. The ^{13}C-NMR spectrum (Table 1) of **1** indicated 14 aromatic carbons, of which five had protons attached and nine were quaternary. There were three methyl (δ 16.5, 19.8, and 55.0) resonances, with the latter signal indicating the presence of an aromatic methoxyl group. The ^{1}H-NMR spectrum (Table 2) of **1** showed two phenolic hydroxyl groups and an aromatic methoxyl functionality at δ 9.43, 9.93, and 3.99, respectively. Two aromatic signals were *meta*-coupled, while three others appeared as singlets, with two aromatic methyl signals at δ 2.33 and 2.57. A phenanthrene skeleton was therefore deduced for compound **1**, and its spectral parameters were consistent with those of similar phenanthrenes previously reported.[2,3] The complete assignments of the ^{1}H- and ^{13}C-NMR spectra of **1** were carried out by APT, one-bond ^{1}H-^{13}C correlation (HETCOR) and long-range ^{1}H-^{13}C correlation (FLOCK)[4] (Figure 2) NMR experiments. The relative positions of the substituents on the phenanthrene nucleus were determined by FLOCK and NOEDS (Figure 3) NMR data. Therefore, the structure of the novel isolate **1** was elucidated as 3,6-dihydroxy-1,7-dimethyl-9-methoxyphenanthrene.

Compound **2** showed a molecular ion at *m/z* 284.1047 in its HREIMS, analyzing for $C_{17}H_{16}O_4$ (calculated *m/z* 284.1049), and was observed to be closely related to **1** by comparison of their physical and spectral data. The primary difference between these isolates was that the methyl group at C-1 (δ_C 19.8; δ_H 2.57) of **1** was replaced by a hydroxymethyl group (δ_C 61.4; δ_H 4.88) in **2**. A FLOCK[4] experiment showed a three-bond correlation between the methylene proton signal at δ 4.88 and the carbon signals at δ 115.7 (C-2) and δ

Table 1. ^{13}C-NMR data of compounds **1-4**.[a]

^{13}C#	1	2	3	4
1	134.7	138.8	134.7	138.8
2	118.3	115.7	118.4	115.8
3	153.5	153.6	153.5	153.6
4	104.0	104.7	104.1	104.7
4a	126.6	126.6	126.5	126.6
4b	130.3	130.2	130.5	130.4
5	105.9	105.9	105.8	105.8
6	155.3	155.2	153.8	153.8
7	125.7	125.7	130.3	130.3
8	123.6	123.6	120.4	120.5
8a	119.2	119.1	119.1	119.1
9	150.7	150.6	151.0	151.0
10	95.6	95.1	95.6	95.1
10a	124.5	123.0	124.6	123.1
CH_3/CH_2-1	19.8	61.4	19.6	61.4
CH_3/CH_2-7	16.5	16.5	58.5	58.5
OCH_3-9	55.0	55.1	55.1	55.1

[a] In DMSO-d_6 at 75 MHz for ^{13}C. Chemical shifts are given in ppm using TMS as an internal reference.

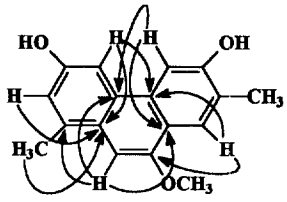


Figure 2. Key long-range ^{1}H-^{13}C-NMR (FLOCK) correlations for compound **1**.

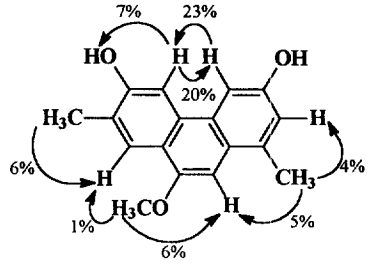


Figure 3. Key NOEDS interactions of compound **1**.

123.0 (C-10a). When the proton signal at δ 4.88 was irradiated in a NOEDS experiment, enhancements of the signals at δ 7.17 (H-2) and δ 6.88 (H-10) were observed. Therefore, the structure 3,6-dihydroxy-1-hydroxymethyl-9-methoxy-7-methylphenanthrene was proposed for **2**.

The HRFABMS of compound **3** showed a pseudomolecular ion peak at *m/z* 285.1130, indicating the molecular formula to be $C_{17}H_{16}O_4$. Inspection of its spectral data revealed that **3** was closely related to **2**, except that a C-7 hydroxymethyl group (δ_C 58.5; δ_H 4.66) was apparent in **3**, rather than a C-1 hydroxymethyl group (δ_C 61.4; δ_H 4.88) in **2**, while a C-1 methyl group (δ_C 19.6; δ_H 2.58) was present in **3** instead of the C-7 methyl group (δ_C 16.5; δ_H 2.33) of **2**. The FLOCK[4] NMR spectrum of **3** confirmed a three-bond correlation between the ^{1}H-NMR signal at δ 4.66 and the ^{13}C-NMR signals at δ 130.3 (C-7) and δ 120.4 (C-8). In a NOEDS experiment, irradiation of the methylene proton signal at δ 4.66

Table 2. ^{1}H-NMR data of compounds **1-4**.[a]

^{1}H#	1	2	3	4
2	6.94 d (1.6)	7.17 d (2.3)	6.94 d (1.9)	7.20 s
3	9.43 br s (OH)	9.47 br s (OH)	9.43 br s (OH)	9.48 br s (OH)
4	7.54 d (2.0)	7.57 d (2.3)	7.54 d (2.0)	7.59 d (1.7)
5	7.81 s	7.80 s	7.78 (s)	7.80 s
6	9.93 br s (OH)	9.90 br s (OH)	9.93 br s (OH)	9.95 br s (OH)
8	7.93 s	7.92 s	8.20 s	8.22 s
10	6.85 s	6.88 s	6.86 s	6.92 s
CH_3/CH_2-1	2.57 s	4.88 d (5.1)	2.58 s	4.91 d (5.5)
CH_3/CH_2-7	2.33 s	2.33 s	4.66 s	4.68 d (5.5)
OCH_3-9	3.99 s	3.97 s	4.01 s	4.01 s

[a] In DMSO-d_6 at 300 MHz for ^{1}H. Chemical shifts are given in ppm using TMS as an internal reference. ^{1}H-NMR coupling constants (Hz) are in parentheses.

enhanced the resonance of H-8 (δ 8.20) by 12%. Therefore, on the basis of the evidence obtained, the structure of **3** was assigned as 3,6-dihydroxy-7-hydroxymethyl-9-methoxy-1-methylphenanthrene.

The molecular formula, $C_{17}H_{16}O_5$, was derived from the HREIMS data (*m/z* 300.1000) of compound **4**. Analysis of its other spectral measurements indicated that this isolate differed from compounds **1-3** only in the number of hydroxyl groups present. The ^{1}H- and ^{13}C-NMR spectra of **4** provided evidence that hydroxymethyl groups were affixed to both C-1 (δ_C 61.4; δ_H 4.91) and C-7 (δ_C 58.5; δ_H 4.68). Accordingly, the structure of this novel compound was proposed as 3,6-dihydroxy-1,7-dihydroxymethyl-9-methoxyphenanthrene. Detailed interpretation of the ^{1}H- and ^{13}C-NMR, APT, HETCOR, and FLOCK[4] NMR data fully supported the proposed structure for compound **4** (Tables 1 and 2).

Compound **5** was obtained as pale-yellow crystals, with a molecular formula of $C_{18}H_{20}O_4$ being derived from its HREIMS data ($[M]^+$ *m/z* 300.1350). The UV spectrum showed absorption maxima at 317, 289, and 271 nm and suggested that this compound was highly conjugated. The IR spectrum showed a band at 1673 cm^{-1} which indicated the presence of α,β-unsaturated carbonyl absorption. The ^{13}C-NMR spectrum of **5** showed 18 signals, and the APT and HMQC (Table 3) NMR data confirmed the presence of two carbonyls, eight aromatic/olefinic carbons, three CH_3 groups, one methoxyl carbon, an aliphatic CH, an aliphatic CH_2, an oxygenated CH, and an aliphatic quaternary carbon. The ^{1}H-NMR (Table 3) spectrum confirmed the presence of three aromatic protons (δ 6.85, 7.10, and 7.89) and an aromatic methoxyl group (δ 3.95). Further analysis of the ^{1}H-NMR spectrum, which was confirmed by COSY and HMQC (Table 3) experiments, revealed a spin system between the H-10$_{ax}$ (δ 2.66), H-10$_{eq}$ (δ 2.90), and H-10a (δ 3.19) protons. A large coupling constant observed between the signals at δ 2.66 and δ 2.90 (J=15.6

Table 3. ^{1}H, ^{13}C, HMQC, HMBC-NMR spectral data of **5**.[a]

^{13}C#	Shift	Connecting ^{1}H (HMQC)	Long-range correlations (HMBC)
1	41.5		H-2, H-10$_{ax}$, CH_3-11, CH_3-12
2	79.7	4.11 s	H-4, CH_3-11, CH_3-12
3	199.1		H-4
4	119.1	6.85 d (2.7)	H-10a
4a	153.9		H-5, H-10$_{ax}$, H-10$_{eq}$, CH_3-10a
4b	136.2		H-4, H-8
5	105.2	7.10 s	H-8
6	162.5		H-5, H-8, CH_3-7, CH_3O-6,
7	132.1		H-8, CH_3-7
8	129.4	7.89 s	CH_3-7
8a	125.6		H-5, H-10$_{eq}$
9	195.6		H-8, H-10$_{ax}$, H-10$_{eq}$
10	37.7	2.66 dd, (15.6, 13.6) 2.90 dd (15.7, 5.0)	H-10a
10a	46.4	3.19 ddd (13.5, 5.0, 2.8)	H-4, H-10$_{ax}$, H-10$_{eq}$, CH_3-11, CH_3-12
CH_3-1a	24.2	1.29 s	H-2, CH_3-1
CH_3-1b	13.8	0.86 s	H-2, H-10a, CH_3-7
OCH_3-6	55.7	3.95 s	
CH_3-7	16.3	2.28 s	H-8

[a] In $CDCl_3$ at 300 MHz (^{1}H), 75 MHz (^{13}C), and 500 MHz (HMQC and HMBC). Chemical shifts are given in ppm using TMS as an internal reference. ^{1}H-NMR coupling constants (Hz) are in parentheses.

Hz) showed geminal proton coupling. The H-10a signal exhibited diagnostic couplings with H-10_{ax} (J=13.5 Hz, axial-axial) and with H-10_{eq} (J=5.0 Hz, axial-equatorial), suggesting an axial orientation. A small coupling constant between H-10a and H-4 (J=2.8 Hz) inferred that these protons were long-range coupled. The ^{1}H-NMR singlet at δ 4.11 and the ^{13}C-NMR signal at δ 79.7 indicated the presence of an isolated secondary hydroxyl group in **5**. This hydroxyl group could be positioned on a cyclohexenone ring (ring A) between a carbonyl (δ 199.1) and a quaternary carbon (δ 41.5) bearing a *gem*-dimethyl group. The general structure of compound **5** could be proposed as that of a hexahydrophenanthrene with two of the rings (rings A and B) only partially aromatized. It was apparent that a methyl group (δ_H 2.28 and δ_C 16.3) and a methoxyl group (δ_H 3.95 and δ_C 55.7) were affixed to the aromatic ring (ring C), and that a second keto group (δ_C 195.6) was present in the remaining non-aromatic ring (ring B). In an attempt to determine the stereochemistry of the two chiral centers at C-2 and C-10a, a NOEDS experiment was performed. NOE enhancements of 13% and 9% resulting from the irradiation of H-2 and H-10a indicated that they were both on the same side of the molecule. Since data obtained from the HMBC NMR spectrum were not additionally informative, the unambiguous structure and stereochemistry of the novel isolate **5** was finally determined by single-crystal X-ray crystallography (Figure 3) as 1,2,3,9,10,10a(*S*)-hexahydro-2*R*-hydroxy-6-methoxy-1,1,7-trimethylphenanthrene-3,9-dione, to which we have accorded the trivial name, domohinone. Two crystallographically independent molecules occurred in each asymmetric unit and were found to assume the same conformation. The molecule of **5** was found to deviate from a planar structure due to the slightly puckered A and B rings.

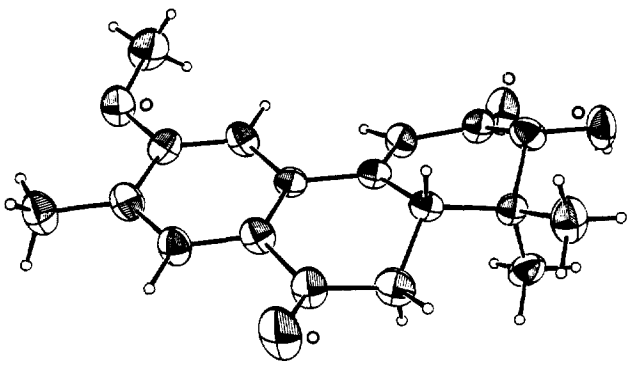


Figure 4. ORTEP diagram of domohinone (**5**) with ellipsoids drawn at 40% level. Only oxygen atoms are labeled.

Compounds **1-4** are phenanthrenoids, which are somewhat typical of species in the orchid family (Orchidaceae).[2,3,5-7] However, examples of this class of compound have been isolated previously from two species of the Euphorbiaceae, namely, *Micranotropsis scleroxylon*[8] and *Sagotia racemosa*.[9] Both of these species are in the same subfamily, Crotonoideae, as *D. perrieri*. On the basis of its co-occurrence with the phenanthrene derivatives **1-4**, domohinone (**5**) may be proposed as a hexahydrophenanthrene derivative. Curiously, it also bears a close structural resemblance to the C_{17} mevalonate-derived compound, podocarpic acid,[10] although in the case of **5** the C-20 methyl group is absent.

Compounds **1-5** were initially screened for *in vitro* cytotoxicity against a number of human cancer cell lines using a standard protocol.[11] Compounds **1** and **2** were found to demonstrate significant cytotoxic responses against several cell lines with some cell-type selectivity (Table 4). For example, **1** showed greater cytotoxic activity with drug-resistant KB cells (with and without vinblastine) as compared to other cell lines in which it was evaluated. The cytotoxic activity of **2** against the HT (fibrosarcoma) and U373 (glioma) cell lines was greater than for the other tumor systems. Compounds **3-5** were not significantly active in any of the cell lines tested (data not shown).

Table 4. *In vitro* cytotoxic activity mediated by compounds **1** and **2** with human cancer cell lines.[a]

Compound	Human cancer cell line[a]											
	A-431	BC1	Col2	HT	KB	KB-V (+VLB)	KB-V (-VLB)	Mel2	LNCaP	Lu1	U373	ZR-75-1
1	11.3	6.9	13.1	5.5	15.0	0.8	3.0	10.0	19.0	6.1	>20	11.2
2	9.4	13.4	>20	3.1	6.4	3.6	5.9	9.2	10.3	19.9	3.2	10.5

[a] ED_{50} values given in μg/ml. Key: A-431 = human epidermoid carcinoma; BC1 = human breast cancer; Col2 = human colon cancer; HT = human fibrosarcoma; KB = human oral epidermoid; KB-V(+VLB) = drug-resistant KB + vinblastine (1 μg/ml); KB-V(-VLB) = drug-resistant KB (no vinblastine); LNCaP = human prostate cancer; Lu1 = human lung cancer; Mel2 = human melanoma; U373 = human glioma; ZR-75-1 = hormone-dependent human breast cancer.

Compounds **1-5** were then subjected to a DNA strand-scission assay and the results are summarized in Table 5. Test compound DNA strand-scission activity is investigated relative to bleomycin activity.[12] Compounds **1-3** were the most active in the DNA strand-scission assay, having about 800-fold less potency than bleomycin; compounds **4-5** were less active.

Table 5. Evaluation of DNA nicking activity by compounds **1-5**.[a]

	1	**2**	**3**	**4**	**5**
Bleomycin	1.21	1.08	1.10	0.60	0.91

[a] A value of 1.0 indicates 2 μg of test compound is as potent as 2 ng bleomycin.

Two phenanthrene derivatives have been reported as active against cultured human lung carcinoma, ovary adenocarcinoma, and promyelocytic leukemia cell lines *in vitro*, and one of them exhibited *in vivo* antitumor activity with ICR mice implanted intraperitoneally with sarcoma 180.[13] While compounds **1** and **2** were significantly cytotoxic against several human cancer cell lines tested, compounds **3** and **4**, which have a hydroxymethyl group at C-7, were not active, thereby suggesting that a free CH_3 group at position 7 is important for such activity among these compounds. Compound **1** was most active in a DNA strand-scission assay, followed by **3**, **2**, and **5**, with compound **4** being less active. This effect is probably not responsible for the cytotoxic effects **1** and **2**, due to lack of a strong correlation of the activities and the relatively low potency of the compounds in terms of DNA strand-scission potential. It is interesting to note, however, that none of the isolates **1-5** bears a catechol or a resorcinol unit, functional groups generally considered important for activity in this assay among plant secondary metabolites.[14-16]

EXPERIMENTAL

General procedures: Mps: uncorr.; UV: MeOH; IR: film; ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-300 (300 MHz) or a GE 500 Omega (500 MHz) instrument with TMS as int. standard; low- and high-resolution mass spectra were obtained on a Finnigan MAT-90 instrument.

Collection and extraction of *Domohinea perrieri*. The stem bark of *Domohinea perrieri* Leandri (Euphorbiaceae) was collected in the "Reserves Naturelles" in the east central part of Madagascar in April, 1995 and identified by one of us (P. R.). A voucher specimen (M-90027) has been deposited at the Institut Malgache de Recherches Appliquèes, Antananarivo, Madagascar.

The stem bark of *D. perrieri* (3 kg) was air-dried, powdered, and extracted exhaustively by repeated maceration with ethanol. The combined ethanol extracts were evaporated to dryness under reduced pressure below 40°C. The residue was resuspended in H_2O-MeOH (9:1) (3 L), and defatted with hexane (1.2 L). The H_2O-MeOH layer was then partitioned with EtOAc (2 L) to give, on drying *in vacuo*, an EtOAc-soluble residue (8.05 g). The dried EtOAc extract (IC_{50} value of 4.4 μg/ml against Lu1 cells) was absorbed onto silica gel and fractionated by column chromatography, eluting with $CHCl_3$, $CHCl_3$-MeOH (1, 2, 4, 5, 6, 8, 10, 20, 50%), and washed with MeOH. The further chromatography of combined fractions 12 and 13 (IC_{50} 5.4 and 5.0 μg/ml; 0.183 g; eluted with 5% MeOH in $CHCl_3$), and fraction 15 (IC_{50} 11.5 μg/ml; 0.329 g; eluted with 6% MeOH in $CHCl_3$) led to the purification of two cytotoxic compounds, **1** (25 mg, yield 0.00083% w/w) and **2** (11 mg, 0.00037%). Workup of the inactive combined fractions 16 and 17 (0.257 g; eluted with 8% MeOH in $CHCl_3$), fraction 18 (0.130 g; eluted with 10% MeOH in $CHCl_3$), and fraction 7 (0.658 g; eluted with 4% MeOH in $CHCl_3$), led to the isolation of the non-cytotoxic isolates **3** (9 mg, 0.00028%), **4** (12 mg, 0.00038%), and **5** (17 mg, 0.00057%), respectively.

3,6-Dihydroxy-1,7-dimethyl-9-methoxyphenanthrene (**1**). Amorphous powder; mp 172°C; UV (MeOH) λ_{max} (log ε) 217 (4.04), 252 (4.10), 288 (3.79), 316 (3.43) nm; IR (AgCl) ν_{max} 3407 (br), 2160, 1645, 1260, 1155, 683 cm^{-1}; ^{1}H-, and ^{13}C- NMR data, see Tables 1 and 2, respectively; long-range ^{1}H-^{13}C correlation (FLOCK)[4] NMR, see Figure 2; NOEDS NMR, see Figure 3; EIMS *m/z* (rel. int. %): $[M]^+$ 268 (100), 267 (13), 253 (17), 226 (12), 225 (69), 134 (10); HREIMS *m/z* $[M]^+$ 268.1093 ($C_{17}H_{16}O_3$ requires 268.1099).

3,6-Dihydroxy-1-hydroxymethyl-9-methoxy-7-methylphenanthrene (**2**). Amorphous powder; mp 184°C; UV (MeOH) λ_{max} (log ε) 218 (4.39), 253 (4.56), 291 (4.27), 315 (3.92) nm; IR (AgCl) ν_{max} 3396 (br), 2359, 2133, 1662, 1445, 1031, 701 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2, respectively; EIMS *m/z* (rel. int. %): $[M]^+$ 284 (100), 241 (29), 151 (20), 149 (31), 137 (25), 133 (30), 123 (40), 119 (46), 111 (75), 109 (54); HREIMS *m/z* $[M]^+$ 284.1047 ($C_{17}H_{16}O_4$ requires 284.1049).

3,6-Dihydroxy-7-hydroxymethyl-9-methoxy-1-methylphenanthrene (**3**). Amorphous powder; mp 186°C; UV (MeOH) λ_{max} (log ε) 217 (4.26), 254 (4.45), 289 (4.12), 315 (3.78) nm; IR (AgCl) ν_{max} 3407 (br), 1645, 1260, 1155, 683 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2, respectively; EIMS *m/z* (rel. int. %): $[M]^+$ 282 (100), 266 (30) (M^+–H_2O); FABMS *m/z* $[M+1]^+$ 285 (28), 284 (70), 268 (100); HRFABMS *m/z* $[M+1]^+$ 285.1130 ($C_{17}H_{17}O_4$ requires 285.1127).

3,6-Dihydroxy-1,7-dihydroxymethyl-9-methoxyphenanthrene (**4**). Amorphous powder; mp 190°C; UV (MeOH) λ_{max} (log ε) 217 (4.25), 255 (4.46), 292 (4.10), 316 (3.77) nm; IR (AgCl) ν_{max} 3405 (br), 1640, 1261, 683 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2, respectively; EIMS *m/z* (rel. int. %): $[M]^+$ 300 (77), 298 (16), 283 (28), 282 (100), 239 (30), 165 (15); HREIMS *m/z* $[M+1]^+$ 300.1000 ($C_{17}H_{16}O_5$ requires 300.0998).

Domohinone (**5**). Pale yellow crystals; mp 166-168°C; $[\alpha]^{25}_D$ -47° (*c*=0.2); UV λ_{max} (log ε) (MeOH) 203 (3.87), 271 (4.08), 289 (4.12), 317 (3.87) nm; IR (AgCl) ν_{max} 3387 (br), 2954, 2842, 2530, 1673, 1449, 1296, 704 cm^{-1}; ^{1}H-, ^{13}C-, and HETCOR NMR data, see Table 3; EIMS *m/z* (rel. int. %): $[M]^+$ 300 (43), 229 (32), 201 (29), 200 (100), 149 (27); HREIMS *m/z* $[M]^+$ 300.1350 ($C_{18}H_{20}O_4$ requires 300.1362).

X-ray crystal structure analysis of compound 5: A pale-yellow needle of approximate size 0.05 ×0.07×0.50 mm, grown from EtOAc/isopropyl acetate, was used for X-ray diffraction experiments carried out at room temperature (295°K) on an Enraf-Nonius CAD4 diffractometer using graphite monochromated CuKα

radiation (λ=1.5418 Å). The crystal is monoclinic, the space group $P2_1$, the cell constants a=8.049(1), b=16.319(3), c=12.102(2) Å, β=105.74(1)°, V=1530.1(4) Å^3 with 4 molecules in the unit cell; the calculated crystal density Dx=1.305 g/cm^3; and the absorption coefficient μ=0.71 mm^{-1}. The structure was solved by direct methods and refined by full-matrix least-squares.[17] Final agreement factors were $R(F)$=0.051; $wR(F)$=0.057, where $w=1/(\sigma^2+0.02F^2)$, S=2.094 for 1767 reflections with $I \geq 3\sigma$ (I) and 396 variables. No recognizable features ($-0.095 \leq \Delta\rho \leq 0.228$e/Å^3) were observed in the final difference electron density map.

Evaluation of cytotoxic potential: Compounds **1-5** were tested for cytotoxicity against a panel of human cancer cell lines using established protocols;[11] the results are listed in Table 4. ED_{50} values of < 5 μg/ml were regarded as significantly active.

Bleomycin-mediated DNA strand-scission assay: A modification of a previously reported method[12] was used for compounds **1-5**, as previously described.[15,16] The relative activity of the test compounds is reported as units of bleomycin activity (defined as the % reduction in supercoiled $DNA_{test\ compound}$/% reduction in supercoiled $DNA_{bleomycin}$) in Table 5. Thus, 2.0 μg of test substance was calculated to have 1.0 unit of bleomycin activity if it relaxed as much supercoiled DNA as 2 ng/ml bleomycin.

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