Unnatural C-1 homologues of pancratistatin – Synthesis and promising biological activities

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Abstract: Several C-1 homologues of pancratistatin and 7-deoxypancratistatin were synthesized by a phenanthrene–phenathridone oxidative recyclization strategy. The key steps involved the enzymatic dihydroxylation of bromobenzene, addition of an aryl alane to an epoxyaziridine, an intramolecular aziridine opening on silica gel in solid phase, and the above-mentioned recylization strategy. Experimental and spectral data are reported for all new compounds. All synthesized C-1 homologues of pancratistatin and 7-deoxypancratistatin were evaluated for antiproliferative activity in a panel of human cancer cell lines. As expected, the 7-hydroxy compounds were found to be more potent and the activity of the C-1 benzoxymethyl analogue exceeded that of narciclasine, which was used as a positive control.

Key words: C-1 homologues of pancratistatin, amaryllidaceae alkaloids, anticancer activities, intramolecular aziridine opening, solid-phase silica catalysis.

Résumé : On a réalisé la synthèse de plusieurs homologues en C-1 de la pancratistatine et de la 7-désoxypencratistatine en faisant appel à une stratégie de recyclisation oxydante phenantrène–phénathridone. Les étapes clés impliquent la dihydroxylation du bromobenzène, l'addition d'une arylalane à une époxyaziridine, une ouverture intramoléculaire d'aziridine sur gel de silice en phase solide et la stratégie de recyclisation mentionnée plus haut. Les données expérimentales et spectrales sont rapportées pour tous les nouveaux produits. Tous les homologues en C-1 de la pancratistatine et de la 7-désoxypencratistatine ont été évalués pour leur activité à contrer la prolifération dans un éventail de lignées de cellules cancéreuses humaines. Tel que prévu, les composés 7-hydroxy sont les plus puissants alors que l'activité de l'analogue C-1 benzoxyméthyle est supérieure à celle de la narciclasine qui a été utilisée comme contrôle positif.

Mots-clés : analogues en C-1 de la pancratistatine, alcaloïdes des amaryllidacées, activité anticancéreuse, ouverture intramoléculaire d'aziridine, catalyse par le gel de silice en phase solide.

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Introduction

Pancratistatin (1), narciclasine (2), and other Amaryllidaceae constituents, Fig. 1, continue to attract attention on account of their promising anticancer properties. Since the isolation of pancratistatin in 1984 by Pettit et al.¹ and its preliminary evaluation as a potent antitumor agent, many creative syntheses of 1 and its congeners have been reported.² The activity concerning the total synthesis of these constituents continues unabated to the present day.³ More recently, however, the studies of these natural products have shifted toward the investigation of unnatural derivatives that would offer the potential of better bioavailability than the rather insoluble natural products. In the last two decades, many reports have appeared describing the synthesis and evaluation of truncated derivatives from the groups of Pettit,⁴ McNulty,⁵ Kornienko,⁶ Chapleur,⁷ Alonso,⁸ Marion,⁹ Banwell,¹⁰ and Gonzalez.¹¹ Truncated derivatives of the core of these constituents as well as various heteroatom analogues have been reported and some of these compounds displayed various levels of biological activity, usually of lower potency than the natural products. The only exceptions were compounds that contained a large lipophilic fragment in position C-1. The first example of such an analogue was the benzoate ester of the C-1 hydroxyl of pancratistatin, prepared and tested by Pettit et al.,^{4c} which displayed nanomolar activity. Later, a library of C-1 nitrogenated derivatives prepared by Marion et al.⁹ showed the highest activity with substituents such as benzamide.

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Dedicated to Professor Derrick Clive in recognition of his outstanding contributions to the art and craft of organic synthesis.

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Fig. 1. Pancratistatin, other Amaryllidaceae congeners, and C-1 homologues.



In our group, we have been investigating various truncated derivatives of pancratistatin, and 7-deoxypancratistatin,¹² as well as derivatives with variations of functionality at the aromatic core,¹³ including an indole mimic of 7-deoxypancratistatin.¹⁴ More recently, and inspired by Pettit's report of the C-1 benzoate ester,4c we investigated carbon homologues of 7-deoxypancratistatin at position C-1 and found that the hydroxymethyl and acetoxymethyl derivatives (5 and 6, respectively) were reasonably active.^{3a} 7-Deoxypancratistatin is far less active than pancratistatin as it lacks the 7-OH functionality and we reasoned that the C-1 homologues of 1 might approach the activity of the natural products. Indeed, this was shown to be the case and homologues 7 and 8 showed nanomolar activities in preliminary screening.¹⁵ In this paper, we report the full details of the synthesis of these compounds, the synthesis of the C-1 benzoxymethyl analogue, as well as the complete details of the biological evaluation in several cell lines.

Results and discussion

The strategy for the synthesis of C-1 homologues followed the previously disclosed approach in which a C-1 aldehyde possessing the full pancratistatin core is generated by the oxidative cleavage–recyclization in a phenanthrene–phenanthridol transformation.¹⁶ To adjust this protocol to the synthesis of the pancratistatin core, the hydroxy aldehyde **10**, prepared from piperonal by a known method,¹⁷ was chosen as the starting material.

As shown in Scheme 1, compound 10 was methylated to 11^{18} and converted via the Corey–Fuchs reaction to acetylene 13.

The alane generated from 13 was reacted with epoxyaziridine 14,¹⁹ which was prepared in five steps from bromobenzene via enzymatic dihydroxylation, the Yamada aziridination protocol, dehalogenation, and epoxidation. Addition of the epoxyaziridine 14 to the alane gave acetylene 15, which was subjected to reduction conditions. The best results for selective alkyne–alkene reduction were obtained using a short time for the hydrogenation (1–1.5 h) under Lindlar's conditions with 1 atm (1 atm = 101.325 kPa) of hydrogen to provide the *cis*-olefin 16. Different catalysts and conditions led to significant over-reduction of **15**. The intramolecular aziridine opening was accomplished by heating the *tert*-butyldimethylsilyl triflate (TBS)-protected ether **17** adsorbed on silica gel according to a solid-phase protocol developed in our laboratory for an aziridine opening of this type.^{14–16} It is worth mentioning that the addition of 10% *w/w* quinoline to the substrate during the solid-phase cyclization helps to suppress the decomposition of material and improves the yield of this reaction from 45% to 74%, probably because of the decreased acidity of the silica gel.

The product of the silica-catalyzed cyclization, phenanthrene 18, was subjected to several different sets of oxidative protocols. The originally disclosed conditions,¹⁶ which included OsO₄/NaIO₄-promoted oxidation-recyclization, did not lead to the desired product. This was probably the result of an increased steric hindrance by the methoxy group in the environment of the double bond (this interaction was absent in the cyclization of the precursor to 7-deoxypancratistatin). Instead, a different protocol was developed, which included selective ozonolysis of the double bond in the presence of azo dye Sudan Red 7B to help prevent oxidation of the electronrich aromatic ring. Reductive workup of the ozonide with sodium borohydride led to a mixture of diol 19 and hemiaminal 20. The former compound was subjected to selective oxidation of the benzylic alcohol by MnO₂, generating the intermediate aldehyde, which, after in situ cyclization, produced compound 20.

The primary alcohol in **20** was selectively acetylated by stirring with acetic anhydride in pyridine–dichloromethane. Several different oxidation conditions were attempted to transform the hemiaminal moiety to the phenathridone amide, including pyridinium dichromate (PDC), pyridinium chlorochromate (PCC), *o*-iodoxybenzoic acid (IBX), Dess–Martin, and tetrapropylammonium perruthenate/*N*-methylmorpholine-*N*-oxide (TPAP/NMO). Of these, only the latter conditions led to the desired product; all other conditions led to the decomposition of the starting material. Detosylation with Na/naphthalene in 1,2-dimethoxyethane (DME) provided compound **23** with traces of alcohol **24** (Scheme 2).

The O-demethylation of **23** was performed by freshly fused LiCl in dry dimethylformamide (DMF).²⁰ Alternative

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Scheme 2.



protocols of demethylation such as BBr_3^{21} or sodium dodecathiolate²² did not lead to product. In the first case, overdeprotection of the acetonide and the methylenedioxo groups was observed. In the second case, the reaction did not proceed at all. Desilylation led to compound **25**, which can be selectively deprotected to provide either acetoxymethyl derivative **8** or hydroxymethyl analogue **7** (Scheme 3).

To provide access to the benzoate derivative **9**, a point of diversion in synthesis was chosen to be compound **23**, which possesses all the required functionality and is prone to basic hydrolysis. Benzoylation of alcohol **24** provided access to compound **27**, which was subjected to the deprotection protocol to furnish the C-1 benzoxymethyl derivative **9** (Scheme 4).

Evaluation of biological activities

The hydroxymethyl, acetoxymethyl, and benzoxymethyl analogues (7, 8, and 9, respectively), together with their 7-deoxy counterparts (5 and 6), were evaluated in a small panel of cancer cell lines diversely representing several types of human malignancy (Table 1). As expected, the 7-hydroxy compounds were found to be more potent, again underscoring the beneficial effect of the 7-hydroxy substitu-

ent. For example, both 7 and 8 were about 10 times more potent than their 7-deoxy analogues (5 and 6) against the prostate DU-145 cells, and this 10-fold difference in activity is similar to that between 1 and 3.^{2a,13b} In addition, the double-digit nanomolar potency of the acetoxymethyl analogue (8) is noteworthy and it approaches that of narciclasine, which was used as a reference compound. The most encouraging activity was found, however, with the benzoxymethyl compound (9). It is three to five times more potent than narciclasine against the pancreatic BXPC-3 and prostate DU-145 cancer cells, while showing a similar magnitude of effect toward the other two cell lines used. This finding is consistent with the previous observations of the beneficial effects imparted by large hydrophobic C-1 substituents as was reported by Pettit et al.4c for the C1-benzoate derivative of pancratistatin. The benzoate moiety could be part of the cytotoxic pharmacophore or it could merely assist the parent hydroxyl compound in cell penetration and then undergo intracellular hydrolytic removal to its hydroxy analogue (7). Further studies are required to provide a satisfactory answer to this important question.





As pancratistatin was previously found to be effective in selectively producing cytotoxicity in an array of cancers, we additionally evaluated the activity of **5–9** in the colorectal cancer cell line HCT 116 and in the osteosarcoma cell line Saos-2 with the water soluble tetrazolium salt (WST-1)-based colorimetric assay for cell viability. After 48 h of treatment, all of these pancratistatin analogues decreased cell viability in a dose-dependent manner. The benzoate ester analogue (**9**) demonstrated the greatest anticancer activity at lower concentrations and had half-maximal inhibitory concentration (IC₅₀) values below 0.05 μ mol/L after 48 h in both HCT 116 and Saos-2 cells (Figs. 2A and 2B, respectively); however, its effectiveness plateaus at 0.1 μ mol/L, after which **7** becomes more effective against HCT 116 cells and **8** becomes more effective against Saos-2 cells.

The effect of pancratistatin analogues on cellular viability of colorectal cancer and osteosarcoma cells was determined by the WST-1-based colorimetric assay. HCT 116 and Saos-2 cells (Figs. 2A and 2B, respectively) were treated with pancratistatin analogues for 48 h and the WST-1 reagent was used to quantify cell viability. Absorbance was read at 450 nm and expressed as a percentage of the control (Me₂SO). Values are expressed as the mean \pm SD from quadruplicates of three independent experiments.

Conclusions

Three new C-1 homologues of pancratistatin have been synthesized and tested in various cancer cell lines. The results were compared with the two previously tested C-1 homologues of 7-deoxypancratistatin. The newly synthesized C-1 homologues manifested nanomolar antiproliferative activities against a panel of human cancer cell lines similar to the parent natural products. The benzoxymethyl compound (9), however, exhibited the most encouraging activity, which was three to five times more potent than that of narciclasine against the pancreatic BXPC-3 and prostate DU-145 cancer cells. The various C-1 analogues screened in this study have very high cytoxicity at low concentrations in a variety of aggressive cancer cells. We observed remarkable enhancement of cytotoxicity in compounds containing the hydroxyl group at C-7 and the benzoate ester at C-1 in human colorectal cancer and osteosarcoma. These novel analogues may hold the potential to be very effective anticancer therapeutic agents.





Note: The half-maximal inhibitory concentration (IC_{50}) is the concentration required to reduce the viability of cells by 50%, after 48 h of treatment with indicated compounds, relative to the dimethyl sulfoxide (DMSO) control; ±standard deviation (SD) from two independent experiments, each performed in four replicates, determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.



Fig. 2. Pancratistatin analogues cause cytotoxicity in cancer cells in a dose-dependent manner.

Experimental section

General

Reactions were carried out under inert atmosphere in ovendried glassware unless stated otherwise. LiCl was fused under vacuum immediately before use. Solvents were distilled: CH₂Cl₂, DMF, *i*-Pr₂NEt, and pyridine from CaH₂, MeOH from magnesium methoxide, tetrahydrofuran (THF) and DME from Na/benzophenone, toluene from Na, and quinoline from Zn. Qualitative thin-layer chromatography (TLC) was done with precoated silica gel aluminum sheets (EMD silica gel 60 F_{254}) with detection by UV or by spraying with a cerium ammonium molybdate (CAM) solution (5 g of (NH₄)₆Mo₇O₂₄·4H₂O, 1 g of Ce(SO₄)₂, and 100 mL of 10% H₂SO₄) or a 0.5% aqueous KMnO₄ solution followed by heating. Melting points are uncorrected. Flash chromatography was performed using silica gel SiliaFlash P60 from Silicycle (40-66 µm). Optical rotation was measured in a 1 dm cell at 20–25 °C and 589 nm with a concentration (c) in g/100 mL. IR spectra were recorded in KBr pellets or as a thin film. ¹H and ¹³C NMR spectra were recorded at 300 or 600 MHz and 75 or 150 MHz, respectively, and were calibrated on the solvent residual peak or tetramethylsilane (TMS) signal (CDCl₃, 7.28 ppm; DMSO-d₆, 2.51 ppm); the chemical shifts are reported in ppm. Copies of ¹H and ¹³C NMR spectra for compounds 7-9, 12, 13, and 15-29) are available in the Supplementary data.

4-Methoxy-1,3-benzodioxole-5-carbaldehyde (11)

Dimethyl sulfate (22.7 mL, 240 mmol) was added to a mixture of K₂CO₃ (55.2 g, 400 mmol) and phenol 10 (made from piperonal by literature procedure;¹⁷ 33.2 g, 200 mmol) in acetone (260 mL). The reaction was stirred under reflux until consumption of the starting material was observed (TLC, approximately 4 h). Then the reaction mixture was cooled and inorganic salts were removed by filtration and rinsed with acetone (2 \times 100 mL). The solution was evaporated, redissolved in CH₂Cl₂, and washed sequentially with a 10% solution of NaOH, water, and saturated solution of NaCl. The organic solution was then dried over anhydrous Na₂SO₄ and evaporated to obtain compound 11 (30.2 g, 84%) as pale brown crystals, which was used without further purification; mp 102–104 °C (EtOH) (lit.¹⁸ mp 103–105 °C (EtOH)). $R_f =$ 0.65 (hexanes/EtOAc, 9:1). ¹H NMR (CDCl₃, 300 MHz) δ: 10.24 (s, 1H), 7.49 (d, J = 8.3 Hz, 1H), 6.62 (d, J =8.3 Hz, 1H), 6.05 (s, 2H), 4.14 (s, 3H).

5-(2,2-Dibromovinyl)-4-methoxy-1,3-benzodioxole (12)

Triphenylphosphine (64.0 g, 244 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a stirring solution of CBr_4 (40.5 g, 122 mmol) in CH_2Cl_2 (150 mL) at 0 °C (ice bath). After 15 min of stirring, a solution of aldehyde (**11**; 11.0 g,

61.0 mmol) in CH₂Cl₂ (50 mL) was added dropwise. Upon completion, the reaction was reduced in volume to 100 mL and slowly poured into vigorously stirred hexanes (1400 mL). The mixture was then filtered through a short plug of silica, washed with a mixture of hexanes/EtOAc (10:1, 200 mL), and evaporated. Subjection of this material to flash column chromatography (eluent hexanes/EtOAc, 9:1) and concentration of the relevant fractions gave 12 (16.11 g, 78.6%) as a white solid; mp 38-40 °C (pentane). $R_f = 0.9$ (hexanes/EtOAc, 9:1). IR (KBr, cm⁻¹) v: 3448, 2981, 2948, 2934, 2900, 2876, 2838, 2770, 1625, 1605, 1471, 1427, 1384, 1350, 1265, 1213, 1126, 1072, 1045, 979, 960, 939, 929, 848, 829, 788, 767, 729, 644. ¹H NMR $(CDCl_3, 300 \text{ MHz}) \delta$: 7.49 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 6.57 (d, J = 8.3 Hz, 1H), 5.96 (s, 2H), 4.02 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) & 149.7, 141.1, 136.1, 132.3, 122.5, 121.4, 102.3, 101.2, 89.0, 59.9. MS (+EI) m/z (%): 338 ([$^{81}Br + {}^{81}Br, M$]+, 49), 336 ([$^{81}Br + {}^{79}Br, M$]+, 100), 334 ([⁷⁹Br + ⁷⁹Br, M⁺], 51), 242 (55), 240 (57), 176 (53), 175 (42), 131 (29). HR-MS (+EI) calcd for C₁₀H₈Br₂O₃: 333.8820; found: 333.8845. Anal. calcd for C₁₀H₈Br₂O₃: C 35.75, H 2.40; found: C 35.99, H 2.41.

5-Ethynyl-4-methoxy-1,3-benzodioxole (13)

To a solution of 12 (19.38 g, 57.68 mmol) in THF (350 mL) was added a solution of n-BuLi (52.9 mL, 2.5 mol/L, and 130 mmol) at -78 °C. After 20 min of stirring at -78 °C, the reaction mixture was warmed to room temperature over a period of 2 h. A saturated solution of NH₄Cl (40 mL) was poured into the reaction mixture, which was later extracted by CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The crude compound was purified by flash chromatography (hexanes/EtOAc 2:1) and white crystals of acetylene 13 were obtained (8.5 g, 81.7%); mp 77–78 °C (pentane). $R_f =$ 0.9 (hexanes/EtOAc, 2:1). IR (KBr, cm⁻¹) v: 3278, 3254, 3000, 2945, 2901, 2846, 2794, 2097 (weak), 1620, 1600, 1469, 1433, 1336, 1267, 1229, 1077, 1043, 979, 950, 930, 797. ¹H NMR (CDCl₃, 300 MHz) δ : 7.01 (d, J = 7.9 Hz, 1H), 6.50 (d, J = 8.29 Hz, 1H), 5.98 (s, 2H), 4.11 (s, 3H), 3.20 (s, 1H). ¹³C NMR (CDCl₃, 75 MHz) δ: 150.1, 144.8, 136.2, 128.0, 108.1, 102.9, 101.3, 79.9, 79.4, 60.0. MS (+EI) m/z (%): 176 ([M]+, 100), 175 (29), 131 (16), 53 (18). HR-MS (+EI) calcd for $C_{10}H_8O_3$: 176.0473; found: 176.0475. Anal. calcd for $C_{10}H_8O_3$: C 68.18, H 4.58; found: C 68.27, H 4.55.

(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)-6-[(4-Methoxy-1,3-benzodioxol-5-yl)ethynyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl] hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (15)

To a solution of alkyne **13** (1.580 g, 8.95 mmol) in toluene (30 mL) at -50 °C, *n*-BuLi (3.80 mL, 2.35 mol/L, and 8.95 mmol) was added dropwise. After 15 min of stirring, Me₂AlCl (9.0 mL, 1.0 mol/L, and 8.95 mmol) was added dropwise. The reaction mixture was warmed to 0 °C within 1 h and stirred for an additional 40 min at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 40 min. The reaction mixture was cooled to -30 °C and a solution of epoxide **14** (1.510 g, 4.47 mmol) in toluene

(20 mL) was added dropwise. The reaction mixture was stirred for 1 h and was allowed to warm to room temperature overnight. The reaction mixture was cooled to 0 °C by ice bath and quenched with 1 N HCl (1 mL), followed by ice-cold water (1 mL) and 1 N HCl (2 mL). The reaction mixture was filtered through a plug of Celite and extracted with EtOAc (3 \times 20 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The crude product was purified by column chromatography (gradient hexanes/EtOAc 5:1 to 3:1) to give the product as colourless oil (1.02 g, 44%). Repetition of this procedure on a 4.12 g scale of 13 provided 15 in a 38% yield. $[\alpha]_{D}^{24}$ +78.4 (c 1.0, CHCl₃). $R_f = 0.3$ (hexanes/EtOAc, 2:1). IR (film, cm⁻¹) v: 3482, 3093, 2986, 2935, 2900, 1620, 1599, 1470, 1434, 1404, 1383, 1332, 1307, 1260, 1225, 1186, 1183, 1071, 985. ¹H NMR (CDCl₃, 300 MHz) δ : 7.83 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 6.88 (d, J = 8.1 Hz, 1H), 6.47 (d, J = 8.3 Hz, 1H), 5.96 (s, 2H), 4.48 (d, J =6.2 Hz, 1H), 4.19 (t, J = 5.7 Hz, 1H), 4.06 (s, 3H), 3.98– 3.95 (m, 1H), 3.44-3.41 (m, 1H), 3.26 (d, J = 6.8 Hz, 1H), 3.24 (dd, J = 5.0, 2.1 Hz, 1 H), 3.08 (d, J = 8.7 Hz, 1 H),2.48 (s, 3H), 1.51 (s, 3H), 1.34 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) & 149.7, 145.4, 144.4, 136.3, 134.1, 130.1, 127.9, 127.2, 110.1, 108.8, 102.8, 101.3, 87.6, 80.5, 75.5, 70.3, 69.0, 60.0, 42.3, 40.2, 31.9, 27.3, 25.1, 21.7. MS (+FAB) m/z (%): 514 ([M + H]⁺, 24), 513 ([M]⁺, 13), 258 (12), 238 (11), 230 (14), 179 (26), 155 (28), 149 (23), 43 (100). HR-MS (+FAB) calcd. for $C_{26}H_{28}NO_8S^+$ [M + 1]+: 514.1457; found: 514.1502.

(3aS,4R,5R,6R,7S,7aR)-6-[(Z)-2-(4-Methoxy-1,3benzodioxol-5-yl)vinyl]-2,2-dimethyl-8-[(4-methylphenyl) sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (16)

To a solution of compound 15 (427 mg, 0.83 mmol) in MeOH (20 mL) was added quinoline (11 mg, 0.09 mmol). The solution was charged with a Lindlar catalyst (5%, 100 mg) and allowed to stir under H₂ (1 atm) for 45 min. After consumption of the starting material was observed (NMR), the reaction mixture was filtered through a pad of Celite, washed with CH₃OH (3 \times 30 mL), evaporated, and used without further purification. An analytical sample was purified by column chromatography (hexanes/EtOAc, 3:1) to provide **16** as a waxy solid. $[\alpha]_{D}^{20}$ +2.7 (*c* 1.78, CHCl₃). R_{f} = 0.4 (hexanes/ EtOAc, 2:1). IR (film, cm⁻¹) v: 3482, 2987, 2934, 2900, 1621, 1598, 1470, 1434, 1404, 1382, 1332, 1260, 1218, 1162, 1070, 1043. ¹H NMR (CDCl₃, 300 MHz) δ: 7.74 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 6.60 (d, J = 11.4 Hz, 1H), 6.55 (d, J = 8.1 Hz, 1H), 6.46 (d, J = 10.1 Hz)8.0 Hz, 1H), 5.98 (d, J = 1.2 Hz, 1H), 5.96 (d, J = 1.2 Hz, 1H), 5.73 (t, J = 11.2 Hz, 1H), 4.43 (d, J = 6.2 Hz, 1H), 4.16-4.13 (m, 1H), 3.96 (s, 3H), 3.72-3.68 (m, 1H), 3.18 (d, J = 6.4 Hz, 1H), 3.15–3.10 (m, 1H), 3.08 (d, J = 6.4 Hz, 1H), 2.84 (d, J = 9.2 Hz, 2H), 2.43 (s, 3H), 1.49 (s, 3H), 1.30 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) & 148.9, 145.2, 141.3, 136.6, 134.3, 130.0, 128.1, 127.9, 127.8, 122.5, 122.2, 109.7, 102.6, 101.0, 75.8, 70.1, 69.3, 59.7, 43.1, 40.5, 37.8, 27.1, 24.7, 21.7. MS (+FAB) m/z (%) [M]+: 517 (16), 516 (42), 515 (29), 514 (15), 386 (25), 285 (14), 284 (15), 269 (15), 203 (30), 165 (61), 91 (100). HR-MS

(+FAB) calcd for $C_{26}H_{31}NO_8S$ [M + 1]⁺: 516.1692; found: 516.1666. Anal. calcd for $C_{26}H_{29}NO_8S$: C 60.57, H 5.67; found: C 60.33, H 5.55.

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(3aS,4R,5R,6R,7S,7aR)-7-{*Tert*-butyl[dimethylsylil]oxy}-6-[(Z)-2-(4-methoxy-1,3-benzodioxol-5-yl)vinyl]-2,2dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5epimino-1,3-benzodioxol (17)

To a solution of alcohol 16 (1.07 g, 2.07 mmol) in 30 mL of CH₂Cl₂ was added Et₃N (0.58 mL, 4.15 mmol) at 0 °C and *tert*-butyldimethylsilyl triflate (0.53 mL, 2.29 mmol) was added dropwise. After the complete consumption of the starting material was observed (TLC), the reaction mixture was quenched by water (10.0 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with 10% citric acid (5 mL), brine (5 mL), dried over Na₂SO₄, and concentrated to afford 17 as pale yellow oil (1.26 g, 97%). The compound was used without further purification. An analytical sample was purified by silica gel chromatography (hexanes/EtOAc, 4:1). $\left[\alpha\right]_{D}^{24}$ -18.5 (c 2.0, CHCl₃). $R_f = 0.85$ (hexanes/EtOAc, 2:1). IR (KBr, cm⁻¹) v: 3446, 2986, 2954, 2931, 2887, 2856, 1622, 1600, 1470, 1435, 1382, 1332, 1257, 1218, 1163, 1071, 1043. ¹H NMR (CDCl₃, 300 MHz) δ : 7.78 (d, J = 8.2 Hz, 2H), 7.30 (d, J =8.2 Hz, 2H), 6.67 (d, J = 8.0 Hz, 1H), 6.60 (d, J = 11.5 Hz, 1H), 6.39 (d, J = 8.0 Hz, 1H), 5.96–5.93 (m, 2H), 5.65 (t, J = 11.5 Hz, 1H), 4.39 (d, J = 5.9 Hz, 1H), 3.98 (s, 3H), 3.89 (t, J = 6.03 Hz, 1H), 3.67 (t, J = 6.3 Hz, 1H), 3.12 (d, J = 6.6 Hz, 1H), 2.98–2.91 (m, 2H), 2.45 (s, 3H), 1.52 (s, 3H), 1.34 (s, 3H), 0.78 (s, 9H), 0.00 (s, 3H), -0.08 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ: 148.7, 144.4, 141.4, 136.3, 135.0, 129.7, 129.6, 127.8, 127.6, 122.9, 122.4, 109.3, 102.3, 100.8, 71.8, 71.4, 59.6, 43.4, 39.7, 39.2, 27.7, 25.7, 25.5, 21.6, 18.0, -4.6, -4.8. MS (+FAB) m/z (%) [M]+: 628 (8), 514 (13), 343 (17), 256 (10), 228 (10), 215 (19), 165 (36), 73(100). HR-MS (+FAB) calcd for C₃₂H₄₄NO₈SSi $[M + 1]^+$: 630.2557; found: 630.2492. Anal. calcd for C₃₂H₄₃NO₈SSi: C 61.02, H 6.88; found: C 61.28, H 7.02.

N-((1*R*,2*S*,3*S*,4*S*,4*aR*,11b*R*)-4-{[*Tert*-butyl(dimethyl)silyl] oxy}-3,3-dimethoxy-7-methoxy-1,2,3,4,4a,11b-hexahydrophenanthro[2,3-*d*][1,3]dioxol-1-yl) benzenesulfonamide (18)

Olefin 17 (100 mg, 0.561 mmol), quinoline (15 mg, 0.12 mmol), and silica gel (500 mg), which has been activated in advance by heating under vacuum for 24 h at 150 °C, were suspended in CH₂Cl₂ (10 mL). The solvent was removed in vacuo and the flask containing silica gel supporting the absorbed reactants was heated at 120 °C under a nitrogen atmosphere and stirred for 36 h. The reaction mixture was then separated by column chromatography (hexanes/EtOAc, 4:1) to give 74 mg (74%) of olefin 18 as a clear and colourless oil. $[\alpha]_D^{24}$ -25.1 (c 1.0, CHCl₃). $R_f =$ 0.45 (hexanes/EtOAc, 2:1). IR (KBr, cm⁻¹) v: 3275, 2983, 2953, 2932, 2889, 2857, 1633, 1614, 1599, 1479, 1384, 1361, 1331, 1221, 1158, 1092, 841. ¹H NMR (CDCl₃, 300 MHz) δ : 7.43 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 6.65 (dd, J = 9.8, 3.4 Hz, 1H), 6.22 (s, 1H), 5.92 (d, J = 1.5 Hz, 1H), 5.82 (d, J = 1.5 Hz, 1H), 5.74 (dd, J =

9.8, 1.5 Hz, 1H), 4.59 (d, J = 8.8 Hz, 1H), 4.28 (m, 1H), 4.11 (s, 1H), 4.02–3.99 (m, 1H), 3.97 (s, 3H), 3.80–3.70 (m, 1H), 2.79–2.78 (m, 1H), 2.61–2.56 (m, 1H), 2.41 (s, 3H), 1.73 (s, 1H), 1.45 (s, 3H), 1.34 (s, 3H), 0.88 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) &: 147.5, 142.1, 139.5, 138.6, 135.5, 129.4, 129.8, 126.8, 125.3, 120.5, 119.7, 109.2, 104.8, 100.7, 79.1, 78.3, 70.3, 59.7, 53.7, 42.4, 39.1, 27.8, 26.3, 25.7, 21.5, 18.0, -5.03, -5.056. MS (+FAB) *m/z* (%) [M]⁺: 629 (3), 129 (13), 111 (12), 99 (13), 57 (100). HR-MS (+FAB) calcd for C₃₂H₄₃NO₈SSi [M]⁺: 629.2479; found: 629.2472.

N-[(3aS,4*R*,5*R*,6S,7*S*,7aS)-7-{[*Tert*-butyl(dimethyl)silyl] oxy}-6,6'-bis(hydroxymethyl)-7'-methoxy-2,2-dimethyl-3,4,5,6,7,7a-hexahydro-5,5'-bi-1,3-benzodioxol-4-yl]-4methylbenzenesulfonamide (19)

To a solution of 18 (254 mg, 0.404 mmol) in MeOH (50 mL) was added a few crystals of Sudan Red 7B. The solution was cooled down to -80 °C and a oxygen-ozone mixture was bubbled through until the disappearance of the pink color. The consumption of the starting material was also checked by TLC. A stream of nitrogen was bubbled through the reaction mixture for 5 min. NaBH₄ (250 mg, 6.67 mmol) was slowly added and reaction mixture was gradually warmed from -80 °C to room temperature. The solvent was removed in vacuo and the residue was redissolved in CH₂Cl₂ (50 mL), neutralized by 10% citric acid, and washed with water (50 mL). The organic phase was separated, dried over Na₂SO₄, filtered, and evaporated. The crude product was subjected to column chromatography (hexanes/EtOAc, 1:1) to give 19 as a white crystalline solid (164.3 mg, 61%) and 20 as a mixture of anomers (80 mg, 30%); mp 121–123 °C (CHCl₃). $[\alpha]_{D}^{20}$ -30.8 (c 1.09, CHCl₃). $R_f = 0.45$ (hexanes/ EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3472, 3386, 3172, 2927, 2855, 1622, 1482, 1385, 1332, 1255, 1220, 1158, 1095, 1057, 837. ¹H NMR (CDCl₃, 600 MHz) δ : 7.51 (d, J = 7.8 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H), 6.57 (s, 1H), 5.96 (s, 1H), 5.90 (s, 1H), 5.44 (d, J = 7.0 Hz, 1H), 4.77 (d, J =11.8 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 4.27–4.24 (m, 1H), 4.17-4.10 (m, 2H), 4.00 (s, 3H), 3.95-3.86 (m, 1H), 3.70 (dd, J = 11.9, 6.1 Hz, 1H), 3.59 (dd, J = 11.8, 6.3 Hz, 1H), 3.37 (dd, J = 11.8, 3.9 Hz, 1H), 2.92 (br s, 2H), 2.39 (s, 3H), 2.00–1.96 (m, 1H), 1.56 (s, 3H), 1.32 (s, 3H), 0.92 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) & 149.1, 142.1, 141.6, 139.2, 135.1, 132.4, 128.8, 126.8, 124.6, 109.7, 103.5, 101.0, 79.9, 79.0, 71.5, 61.3, 60.0, 57.0, 55.2, 47.3, 38.2, 27.4, 25.9, 25.8, 21.5, 18.0, 21.6, 21.0, 18.0, -4.8, -5.0. MS (+FAB) m/z (%): 664 ([M -H]+, 6), 648 (7), 372 (11), 302 (11), 254 (21), 248 (12), 73 (100). HR-MS (+EI) calcd for $C_{32}H_{47}NO_{10}SSi$ [M]+: 665.2690; found: 665.2803. Anal. calcd for C₃₂H₄₇NO₁₀SSi: C 57.72, H 7.11; found: C 57.76, H 6.99.

$\label{eq:sigma} \begin{array}{l} (3aS,3bR,10bR,11S,12S,12aS)-12-\{[\textit{Tert-butyl}(dimethyl) \\ silyl]oxy\}-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-5-ol (20) \end{array}$

To a solution of alcohol **19** (100 mg, 0.15 mmol) in CH_2Cl_2 (100 mL) was added MnO_2 (268 mg, 3 mmol). The reaction mixture was vigorously stirred until total consump-

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tion of the starting material was observed (TLC). The reaction mixture was filtered through a plug of Celite® and washed with CH_2Cl_2 (3 × 100 mL). The solvent was removed in vacuo, affording 20 as a white solid compound (87 mg, 87%, mixture of anomers); mp 106-116 °C (CHCl₃). $R_f = 0.5$ and 0.65 (hexanes/EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3452, 2985, 2954, 2931, 2894, 2857, 1624, 1483, 1384, 1341, 1251, 1163, 1077, 839. NMR of the major anomer: ¹H NMR (CDCl₃, 300 MHz) δ : 7.55 (d, J = 8.1 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 6.65 (s, 1H), 6.08 (s, 1H), 5.92–5.91 (m, 2H), 5.26 (dd, J = 9.9, 5.4 Hz, 1H), 4.38 (t, J = 3.0 Hz, 1H) 4.25-4.21 (m, 2H), 4.11 (s, 3H), 3.74(dd, J = 11.0, 7.9 Hz, 1H), 3.37 (dd, J = 11.0, 3.6 Hz, 1H),2.87 (dd, J = 12.9, 5.1 Hz, 1H), 2.33 (s, 3H), 2.16 (br s, 1H), 2.15 (br s, 1H), 2.06 (s, 1H), 1.44 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) & 150.0, 142.9, 140.5, 138.3, 134.0, 130.0, 128.9, 127.2, 121.3, 109.4, 101.0, 100.2, 78.9, 76.4, 73.5, 68.1, 59.9, 59.3, 53.1, 48.1, 34.4, 27.9, 26.2, 25.7, 21.4, 17.9, -4.8, -5.1. MS (+EI) m/z (%) [M - Ts - H₂O]+: 491 (15), 432 (13), 302 (22.2), 302 (10.5), 247 (31.1), 246 (19.9), 43 (100). HR-MS (+EI) calcd for $C_{32}H_{45}NO_{10}SSi$ [M]+: 663.2533; found: 663.2549. Anal. calcd for C₃₂H₄₅NO₁₀SSi: C 57.90, H 6.83; found: C 58.02, H 7.03.

$\label{eq:sigma} \begin{array}{l} \{(3aS,3bR,10bR,11S,12S,12aS)-12-\{[\textit{Tert-butyl}(dimethyl) \\ silyl]oxy\}-5-hydroxy-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl\} \\ methyl acetate (21) \end{array}$

Pyridine (50 mg, 0.63 mmol) was added to a solution of hemiaminal 20 (48 mg, 0.072 mmol) in CH_2Cl_2 (3 mL), followed by the addition of acetic anhydride (29.5 mg, 0.29 mmol). The reaction mixture was stirred until consumption of the starting material was observed (TLC). The reaction mixture was quenched with water (5 mL) and extracted by CH_2Cl_2 (3 × 4 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The crude product was subjected to column chromatography (eluent hexanes/ EtOAc, 4:1) affording 21 as a colourless oil (43 mg, 87%) mixture of anomers). $R_f = 0.9$ and 0.8 (hexanes/EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3462, 2984, 2953, 2931, 2896, 2857, 1743, 1624, 1481, 1371, 1342, 1330, 1250, 1222, 1164, 1077, 840. NMR of the major anomer: ¹H NMR $(CDCl_3, 600 \text{ MHz}) \delta$: 7.56 (d, J = 8.2 Hz, 2H), 7.08 (d, J =8.0 Hz, 2H), 6.66 (s, 1H), 6.012 (s, 1H), 5.94 (m, 2H), 5.31 (dd, J = 10.0, 5.1 Hz, 1H), 4.37 (m, 1H) 4.22–4.21 (m, 1H), 4.08 (s, 3H), 3.96 (s, 1H), 3.91 (dd, J = 11.4, 4.4 Hz, 1H), 3.05 (s, 1H), 2.91 (dd, J = 13.2, 4.5 Hz, 1H), 2.35 (s, 3H), 2.15 (s, 1H), 1.93 (s, 3H), 1.47 (s, 3H), 1.38 (s, 3H), 0.93 (s, 9H), 0.15 (s, 3H), 0.11 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) & 170.8, 150.0, 143.0, 140.6, 138.5, 134.1, 129.8, 128.9, 127.2, 121.3, 109.3, 101.0, 100.1, 78.9, 76.3, 73.3, 67.2, 61.2, 59.9, 53.2, 45.6, 33.8, 29.7, 26.3, 25.7, 25.5, 21.4, 20.8, 17.9, -5.0, -5.2. MS (+FAB) m/z (%) [M - H₂O]+: 688 (47), 230 (12), 117 (17), 302 (11), 247 (31), 117 (17), 73 (100). HR-MS (+FAB) calcd for $C_{34}H_{46}NO_{10}SSi [M - H_2O]^+$: 688.2612;

found: 688.2642. Anal. calcd for $C_{34}H_{47}NO_{11}SSi$: C 57.85, H 6.71; found: C 57.80, H 6.67.

{(3aS,3bR,10bR,11S,12S,12aS)-12-{[*Tert*-butyl(dimethyl) silyl]oxy}-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl) sulfonyl]-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3] dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl}methyl acetate (22)

A predried (by MgSO₄) solution of N-methylmorpholine-N-oxide (100 mg, 0.85 mmol) in CH₂Cl₂ (20 mL) was added to hemiaminal 21 (30 mg, 0.042 mmol) in CH₂Cl₂ (10 mL), followed by activated crushed molecular sieves (0.5 g, 4 Å). After stirring for 15 min, a few crystals of tetrapropylammonium perruthenate were added and the reaction was stirred until consumption of the starting material was observed (TLC). The reaction mixture was filtered through a plug of Celite and washed with CH_2Cl_2 (3 × 50 mL). The combined organic phases were evaporated and subjected to column chromatography (hexanes/EtOAc, 2:1) affording 22 as a colourless oil (25 mg, 84%). $[\alpha]_D^{24}$ +41.4 (c 0.9, CHCl₃). $R_f =$ 0.65 (eluent hexanes/EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3450, 2984, 2953, 2930, 2857, 1742, 1710, 1614, 1484, 1360, 1253, 1169, 1090, 1021, 839. ¹H NMR (CDCl₃, 300 MHz) δ: 8.20 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.3 Hz, 2H), 6.73 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 4.83 (dd, J = 7.9, 5.5 Hz, 1H), 4.46 (s, 1H), 4.26 (t, J = 11.1 Hz, 1H), 4.17–4.13 (m, 1H), 4.12-4.08 (m, 1H), 4.04 (s, 3H), 4.00-3.95 (m, 1H), 3.47 (dd, J = 13.1, 3.3 Hz, 1H), 2.66–2.63 (m, 1H), 2.43 (s, 3H), 2.08 (s, 1H), 1.48 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) δ: 170.8, 163.7, 153.4, 144.8, 143.4, 138.8, 136.7, 136.1, 128.9, 128.7, 126.8, 117.8, 109.1, 102.0, 100.1, 79.0, 76.3, 66.9, 60.9, 60.7, 60.4, 59.7, 43.4, 36.5, 27.9, 26.1, 25.7, 21.6, 20.9, 18.0, -4.9, -5.1. MS (+EI) m/z (%): 639 (3.1) 549 (6.6), 492 (3.4), 434 (2.9), 374 (3.9), 43 (100). HR-MS (+EI) calcd for C₃₃H₄₂NO₁₁SSi⁺ [M - CH₃]⁺: 688.2248; found: 688.2436.

$((3aS,3bR,10bR,11S,12S,12aS)-12-\{[Tert-butyl(dimethyl) silyl]oxy\}-6-methoxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl)methyl acetate (23)$

To a solution of 22 (64 mg, 0.09 mmol) in dry DME (7 mL) at -50 °C was added a solution of Na/naphthalene in DME (0.5 mol/L) dropwise until a light green colour persisted and total consumption of the starting material was observed (TLC). The solution was stirred for 10 min before the reaction was quenched with a saturated solution of NH_4Cl (aq, 1 mL). The reaction was warmed to room temperature and extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The products 23 and 24 were isolated by column chromatography (gradient hexanes/EtOAc, 2:1 to 1:1) as a clear and colourless oil for 23 (30 mg, 62%) and a white crystalline solid for 24 (2 mg; 5%). Repetition of this procedure on a 1.01 g gram scale provided 23 in a 48% and 24 in a 10% yield. $[\alpha]_D^{20}$ +32.4 (c 1.0, CHCl₃). $R_f = 0.28$ (hexanes/ EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3417, 3228, 3109, 2987, 2953, 2932, 2897, 2858, 1743, 1676, 1617, 1481, 1385, 1366, 1339, 1250, 1222, 1169, 1088, 1071, 1057, 1033, 840. ¹H NMR (CDCl₃, 600 MHz) δ: 6.69 (s, 1H), 6.06 (s,

1 H), 6.01 (s, 1H), 5.92 (s, 1H), 4.57 (s, 1H), 4.23 (d, J = 7.5 Hz, 1H), 4.19–4.18 (m, 1H), 4.16–4.14 (m, 1H), 4.07 (s, 3H), 3.42 (dd, J = 13.8, 8.3 Hz, 1H), 3.31 (dd, J = 13.8, 3.6 Hz, 1H), 2.66–2.65 (m, 1H), 2.11 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 0.91 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) δ : 170.9, 163.5, 152.4, 145.4, 137.5, 135.5, 116.2, 109.9, 101.8, 99.9, 78.3, 77.8, 67.0, 61.1, 60.9, 52.5, 41.9, 35.1, 28.2, 26.1, 25.69, 25.65, 20.9, 18.0, -5.0, -5.1. MS (+FAB) *mlz* (%): 552 (12), 551 (36), 550 ([M + 1]⁺, 100), 246 (10), 220 (11), 117 (101). HR-MS (+FAB) calcd for C₂₇H₄₀NO₉Si [M + 1]⁺: 550.2472; found: 550.2459.

((3aS,3bR,10bR,11S,12S,12aS)-12-{[*Tert*-butyl(dimethyl) silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5*c*:4',5'-*j*]phenanthridin-11-yl)methyl acetate (25)

To a solution of 23 (39 mg, 0.071 mmol) in DMF (5 mL) was added LiCl (50 mg, 1.2 mmol) followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 120 °C for 2.5 h. The reaction mixture was cooled to room temperature, diluted with water (100 mL), and extracted with diethyl ether (10×15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The product was isolated by column chromatography (hexanes/ EtOAc, 3:1) as a clear and colourless oil that solidifies after drying (25.7 mg, 68%). Repetition of this procedure on a 0.296 g gram scale provided 25 in a 67% yield; mp 68-69 °C (CHCl₃). $[\alpha]_D^{20}$ -35.2 (c 1.0, CHCl₃). $R_f = 0.8$ (hexanes/ EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3402, 3348, 3285, 3212, 3087, 2987, 2953, 2933, 2899, 2859, 2795, 1743, 1627, 1601, 1464, 1389, 1366, 1353, 1341, 1301, 1250, 1226, 1171, 1081, 1032, 940, 838, 778. ¹H NMR (CDCl₃, 600 MHz) & 12.68 (s, 1H), 6.53 (s, 1H), 6.26 (s, 1H), 6.07 (s, 2H), 4.57 (s, 1H), 4.30 (dd, J = 11.1, 3.3 Hz, 1H), 4.22 (t, J = 11.0 Hz, 1H), 4.21–4.18 (m, 2H), 3.50 (dd, J = 14.4, 7.8 Hz, 1H), 3.36 (dd, J = 14.4, 3.7 Hz, 1H), 2.70–2.69 (m, 1H), 2.11 (s, 3H), 1.49 (s, 3H), 1.41 (s, 3H), 0.91 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H). ¹³C NMR (CDCl₃, 150 MHz) & 170.8, 169.9, 153.1, 146.9, 133.9, 133.0, 110.0, 107.3, 102.3, 97.3, 78.4, 77.6, 67.0, 61.10, 53.2, 41.7, 33.9, 28.3, 26.0, 25.7, 20.9, 18.0, -5.0, -5.1. MS (+EI) m/z (%): 536 ($[M + 1]^+$, 9), 535 (25), 360 (17), 256 (11), 231 (10), 218 (19), 205 (21), 149 (25), 43 (100). HR-MS (+EI) calcd for C₂₆H₃₇NO₉Si [M]+: 535.2238; found: 535.2248.

[(3aS,3bR,10bR,11S,12S,12aR)-6,12-Dihydroxy-2,2dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3] dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl]methylacetate(26)

Compound **25** (52 mg, 0.097 mmol) was taken up in THF (2.5 mL) and cooled to 0 °C. A solution of tetrabutylammonium fluoride (TBAF) in THF (1 mol/L, 0.107 mL, 0.107 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of the starting material was observed (TLC). The reaction mixture was quenched with water (5 mL) and extracted with CH₂Cl₂ (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc, 1:1) to give **26** as a white crystalline solid (38 mg, 95%); mp > 200 °C (CH₂Cl₂-CH₃OH). $[\alpha]_{24}^{24}$ +6.2 (*c* 0.48, DMSO). $R_f =$ 0.3 (hexanes / ethyl acetate, 1:1). IR (KBr, cm^{-1}) v: 3449, 3270, 2988, 2911, 1743, 1672, 1625, 1600, 1466, 1443, 1357, 1307, 1245, 1231, 1166, 1087, 1071, 1032, 844. ¹H NMR (DMSO-d₆, 600 MHz) δ: 13.35 (s, 1H), 8.55 (s, 1H), 6.61 (s, 1H), 6.08 (s, 1H), 6.06 (s, 1H), 5.50 (d, J = 3.6 Hz, 1H), 4.34 (br s, 1H), 4.25 (d, J = 4.8 Hz, 1H), 4.20 (dd, J =11.4, 3.6 Hz, 1H), 4.16–4.15 (m, 1H), 4.13 (d, J = 11.4 Hz, 1H), 3.51 (dd, J = 15.0, 8.4 Hz, 1H), 3.19 (dd, J = 14.4, 3.6 Hz, 1H), 2.03 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H). ¹³C NMR (DMSO-d₆, 150 MHz) δ: 170.9, 169.8, 152.7, 146.3, 135.0, 132.6, 109.0, 107.7, 102.4, 97.6, 77.9, 76.9, 65.2, 61.2, 53.4, 34.3, 28.3, 26.4, 21.2. MS (+EI) m/z (%): 422 $([M + 1], 24), 421 ([M^+], 100), 248 (34), 247 (60), 232 (27),$ 231 (32), 218 (11), 206 (17), 145 (14). HR-MS calcd for C₂₀H₂₃NO₉ [M]⁺: 421.13728; found: 421.13792. Anal. calcd for C₂₀H₂₃NO₉: C 57.00, H 5.50; found: C 57.18, H 5.48.

[(1*S*,2*S*,3*R*,4*S*,4*aR*,11b*R*)-2,3,4,7-Tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-*j*] phenanthridin-1-yl]methyl acetate (8)

Compound 26 (38 mg, 0.09 mmol) was taken up in a CH_2Cl_2 - CH_3OH mixture (1:1, 4 mL) and cooled to 0 °C. Trifluoroacetic acid (2 mL) was added dropwise and the reaction mixture was stirred until consumption of the starting material was observed (TLC). The reaction mixture was dried in vacuo, triturated with CH_2Cl_2 (3 × 15 mL) and finally dried under high vacuum. The final product was isolated by column chromatography on silica gel (deactivated by 10% w/w of water, eluent CH₂Cl₂-CH₃OH, 10:1) to give 8 as a white crystalline compound (31 mg, 90%); mp > 200 °C $(CH_2Cl_2-CH_3OH)$. $[\alpha]_D^{24}$ +36.8 (*c* 0.2, THF). $R_f = 0.3$ (CH_2Cl_2-CH_3OH 10:1). IR (KBr, cm⁻¹) v: 3459, 3287, 3214, 2991, 2923, 1750, 1709, 1670, 1628, 1595, 1466, 1436, 1384, 1342, 1264, 1227, 1196, 1090, 1070, 1034. ¹H NMR (DMSO-*d*₆, 600 MHz) δ: 13.26 (s, 1H), 7.40 (s, 1H), 6.59 (s, 1H), 6.08 (s, 1H), 6.06 (s, 1H), 5.17 (m, 1H), 5.10–5.09 (m, 2H), 4.40 (m, 1H), 4.16 (dd, J = 10.93.5 Hz, 1H), 4.11 (s, 1H), 3.84 (m, 1H), 3.71 (m, 1H), 3.52 (dd, J = 13.6, 9.8 Hz, 1 H), 3.26 (m, 1H), 2.66 (m, 1H),2.03 (s, 3H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ: 171.0, 169.9, 152.8, 146.3, 135.7, 132.5, 107.5, 102.4, 97.8, 73.1, 71.2, 68.9, 61.8, 51.6, 40.5, 36.5, 21.3. MS (+EI) m/z (%): 381 ([M]⁺, 12), 321 (16), 279 (12), 277 (99), 276 (11), 256 (12), 247 (11), 205 (13), 201 (16), 199 (12), 185 (13), 183 (11), 179 (21), 167 (14), 149 (37), 129 (12), 123 (11), 69 (100). HR-MS (+EI) calcd for $C_{17}H_{19}NO_9$: 381.1060; found: 381.1055.

(1*S*,2*S*,3*R*,4*S*,4*aR*,11b*R*)-2,3,4,7-Tetrahydroxy-1-(hydroxymethyl)-1,3,4,4a,5,11b-hexahydro[1,3]dioxolo[4,5*j*]phenanthridin-6(2*H*)-one (7)

Compound **26** (48 mg, 0.11 mmol) was taken up in a $CH_2Cl_2-CH_3OH$ mixture (1:1, 4 mL) and two drops of concentrated HCl were added. The reaction was stirred until total consumption of the starting material was observed (TLC). The reaction mixture was neutralized by the dropwise addition of a saturated solution of NaHCO₃ and evaporated to dryness. The final product was isolated by column chromatography on silica gel (deactivated by 10% w/w of water, eluent $CH_2Cl_2-CH_3OH$, 5:1) to give **7** as a white crystalline compound (36 mg, 92%); mp > 200 °C ($CH_2Cl_2-CH_3OH$).

[α]²⁰₂ +48.0 (*c* 0.5, abs DMSO). $R_f = 0.4$ (CH₂Cl₂-CH₃OH 5:1). IR (KBr, cm⁻¹) ν : 3386, 2917, 1670, 1638, 1598, 1466, 1439, 1384, 1352, 1304, 1228, 1089, 1077, 1064, 1032. ¹H NMR (DMSO- d_6 , 300 MHz) δ: 13.25 (s, 1H), 7.31 (s, 1H), 6.55 (s, 1H), 6.07 (s, 1H), 6.06 (s, 1H), 5.02 (m, 3H), 4.47 (dd, J = 6.4, 4.0 Hz, 1H), 4.18 (m, 1H), 3.91 (m, 1H), 3.82 (m, 1H), 3.67 (m, 1H), 3.43 (m, 1H), 3.14 (m, 1H), 2.37 (br. s, 1H). ¹³C NMR (DMSO- d_6 , 150 MHz) δ: 169.9, 152.8, 146.1, 136.7, 132.2, 107.4, 102.4, 97.9, 73.2, 71.4, 69.5, 57.7, 51.8, 44.3, 36.9. MS (+EI) m/z (%): 339 [M]⁺: (0.4), 85 (39), 84 (80), 83 (57), 68 (13), 66 (100). HR-MS (+EI) calcd for C₁₅H₁₇NO₈: 339.0954; found: 339.0925.

$(3aS,3bR,10bR,11S,12S,12aS)-12-\{[Tert-butyl(dimethyl) silyl]oxy\}-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydrobis[1,3]dioxolo[4,5-c:4',5'-j] phenanthridin-5(3aH)-one (24)$

To a solution of 23 (170 mg, 0.309 mmol) in methanol (5 mL) was added NaOH (aq 40%, 0.5 mL) and stirred until total consumption of the starting material was observed (TLC). The reaction was quenched with a saturated solution of NH₄Cl (1 mL). The mixture was evaporated and the residue was extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes/EtOAc, 2:1 to 1:1) to give 24 as a white crystalline solid (125 mg; 80%); mp 148-149 °C (CHCl₃). $[\alpha]_D^{20}$ +32.6 (c 0.63, CHCl₃). $R_f = 0.2$ (hexanes/ EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3416, 2987, 2952, 2932, 2893, 2857, 1660, 1618, 1501, 1481, 1439, 1384, 1350, 1295, 1250, 1220, 1169, 1083, 1056, 841. ¹H NMR (CDCl₃, 600 MHz) δ: 6.61 (s, 1H), 6.05 (s, 1H), 6.00 (s, 1H), 5.86 (s, 1H), 4.68(s, 1H), 4.20 (d, J = 4.5 Hz, 1H), 4.14 (dd, J = 8.2, 5.0 Hz, 1H), 4.08 (s, 3H), 3.95 (dt, J = 10.1, 6.0 Hz, 1H), 3.67-3.66 (m, 1H), 3.49 (dd, J = 13.8, 8.4 Hz, 1H), 3.28(dd, J = 13.8, 3.7 Hz, 1H), 2.53-2.52 (m, 1H), 1.91 (s, 1H),1.46 (s, 3H), 1.40 (s, 3H), 0.91 (s, 9H), 0.18 (s, 6H). ¹³C NMR (CDCl₃, 150 MHz) & 163.6, 152.2, 145.4, 137.3, 136.3, 116.3, 101.7, 99.8, 78.3, 78.0, 67.4, 60.9, 58.8, 52.7, 45.2, 35.3, 28.1, 26.2, 25.7, 17.9, -4.92, -4.94. MS (+FAB) m/z (%): 508 ([M + 1], 14), 507 ([M+], 37), 450 (16), 449 (10), 434 (17), 433 (16), 392 (22), 374 (12), 345 (12), 261 (100). HR-MS (+FAB) calcd for $C_{25}H_{37}NO_8Si$ [M]+: 507.2289; found: 507.2287.

$((3aS,3bR,10bR,11S,12S,12aS)-12-\{[Tert-butyl(dimethyl) silyl]oxy\}-6-methoxy-2,2-dimethyl-5-oxo-$ 3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5c:4',5'-j]phenanthridin-11-yl)methyl benzoate (27)

To a solution of **24** (121 mg, 0.24 mmol) in CH_2Cl_2 (25 mL) was added triethylamine (0.04 mL, 0.48 mmol) at 0 °C followed by benzoyl chloride (0.03 mL, 0.26 mmol) and crystals of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at 0 °C until total consumption of the starting material was observed (TLC). The reaction mixture was quenched with distilled water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with a solution of citric acid (10%, 10 mL), dried over sodium sulfate, filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes/EtOAc 2:1 to 1:1) to give **27** as a white

crystalline powder (94 mg, 64%); mp 90-92 °C (CHCl₃). $[\alpha]_{D}^{20}$ -15.1 (c 1, CHCl₃). $R_f = 0.4$ (hexanes/EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3467, 3416, 3387, 3069, 2986, 2952, 2932, 2896, 2857, 1722, 1674, 1616, 1502, 1481, 1453, 1385, 1339, 1273, 1221, 1093, 1071, 1028, 838. ¹H NMR (CDCl₃, 300 MHz) δ : 8.06 (d, J = 7.2 Hz, 2H), 7.63–7.58 (m, 1H), 7.48 (t, J = 7.5 Hz, 2H), 6.74 (s, 1H), 6.01–6.02 (m, 3H), 4.68 (s, 1H), 4.53-4.45 (m, 2H), 4.20-4.16 (m, 2H), 4.07 (s, 3H), 3.53 (dd, J = 13.8, 7.5 Hz, 1H), 3.40–3.35 (m, 1H), 2.86-2.83 (m, 1H), 1.50 (s, 3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.18 (s, 6H). ¹³C NMR (CDCl₃, 75 MHz) & 166.4, 163.6, 152.4, 145.4, 135.6, 133.2, 129.8, 129.6, 128.4, 116.1, 110.0, 101.7, 99.9, 78.3, 77.9, 67.2, 61.6, 60.9, 52.5, 42.1, 35.2, 28.2, 26.1, 25.6, 17.9, -4.9, -5.0. MS (+FAB) m/z (%): 614 ([M + 2]⁺, 12), 613 (36), 612 (89), 179 (12), 105 (100). HR-MS calcd for C₃₂H₄₂NO₉Si⁺ [M]⁺: 612.2251; found: 612.2653.

((3aS,3bR,10bR,11S,12S,12aS)-12-{[*Tert*-butyl(dimethyl) silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5*c*:4',5'-*j*]phenanthridin-11-yl)methyl benzoate (28)

To a solution of 27 (74.3 mg, 0.122 mmol) in dry DMF (5 mL) was added LiCl (50 mg, 1.2 mmol) followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 120 °C for 3.5 h. The reaction was then cooled to room temperature, diluted with distilled water (50 mL), and extracted with diethyl ether (6 \times 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The product was isolated by column chromatography (hexanes/EtOAc, 2:1) to give **28** as a white crystalline solid (60 mg, 83%); mp 141–145 °C (CHCl₃). $R_f = 0.8$ (hexanes/EtOAc, 1:1). $[\alpha]_{D}^{20}$ -63.0 (c 1.0, CHCl₃). IR (KBr, cm⁻¹) v: 3449, 2953, 2931, 2901, 2858, 1721, 1674, 1655, 1637, 1627, 1603, 1461, 1385, 1351, 1341, 1304, 1271, 1219, 1113, 1081, 837. ¹H NMR (CDCl₃, 600 MHz) & 12.70 (s, 1H), 8.07 (d, J = 8.0 Hz, 2H), 7.62-7.60 (m, 1H), 7.49-7.47 (m,)2H), 6.59 (s, 1H), 6.11 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 4.68 (s, 1H), 4.55–4.54 (m, 2H), 4.23–4.21 (m, 2H), 3.63– 3.59 (m, 1H), 3.43 (dd, J = 14.4, 3.5 Hz, 1H), 2.89 (s br,1H), 1.53 (s, 3H), 1.42 (s, 3H), 0.91 (s, 9H), 0.16-0.15 (m, 6H). ¹³C NMR (CDCl₃, 150 MHz) δ: 170.0, 166.4, 153.2, 146.9, 134.0, 133.2, 133.1, 129.8, 129.6, 129.4, 110.1, 107.3, 102.3, 97.3, 78.4, 77.7, 67.2, 61.6, 53.2, 41.8, 34.0, 30.3, 28.4, 26.1, 25.7, 17.9, -4.9, -5.0. MS (+FAB) m/z (%): 599 ($[M + 2]^+$, 11), 598 (29), 597 (8), 596 (4), 179 (11), 73 (100). HR-MS (+FAB) calcd for $C_{31}H_{40}N_1O_9Si^+$ $[M + 1]^+$: 598.2472; found: 598.2446.

[(3aS,3bR,10bR,11S,12S,12aR)-6,12-Dihydroxy-2,2dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3] dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl]methyl benzoate (29)

Compound **28** (42 mg, 0.07 mmol) was dissolved in THF (2.5 mL) and cooled to 0 °C. A 1 mol/L solution of TBAF in THF (0.077 mL, 0.077 mmol) was added dropwise and the reaction mixture was stirred until total consumption of the starting material was observed (TLC). The reaction mixture was quenched by distilled water (5 mL) and extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc,

1:1) to give **29** as a white crystalline solid (27.2 mg, 80%); mp > 200 °C (THF). $[\alpha]_{D}^{21}$ -40.1 (c 1.0, THF). $R_{f} = 0.3$ (hexanes/EtOAc, 1:1). IR (KBr, cm⁻¹) v: 3446, 3255, 2986, 2930, 2905, 2854, 1720, 1672, 1626, 1601, 1466, 1384, 1356, 1343, 1311, 1222, 1166, 1088, 1071, 1027, 713. ¹H NMR (DMSO-d₆, 600 MHz) & 13.36 (s, 1H), 8.57 (s, 1H), 7.97-7.96 (m, 2H), 7.69-7.67 (m, 1H), 7.56-7.53 (m, 2H), 6.74 (s, 1H), 6.07 (s, 1H), 5.99 (s, 1H), 5.56 (dd, J =4.3 Hz, 1H), 4.53 (dd, J = 11.3, 4.3 Hz, 1H), 4.46–4.45 (m, 1H), 4.43–4.39 (m, 1H), 4.29 (d, J = 5.0 Hz, 1H), 4.19 (dd, J = 8.3, 5.3 Hz, 1H), 3.63 (dd, J = 14.4, 8.4 Hz, 1H), 3.26 (dd, J = 14.4, 3.6 Hz, 1H), 2.97-2.95 (m, 1H), 1.45 (s, 3H),1.33 (s, 3H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ: 169.9, 166.1, 152.7, 146.3, 135.0, 133.9, 132.5, 130.0, 129.7, 129.2, 109.0, 107.7, 102.3, 97.9, 78.0, 77.1, 65.5, 62.0, 53.4, 34.3, 30.3, 28.3, 26.4. MS (+FAB) m/z (%): 484 ([M $([M]^+, 26), 248 (13), 247 (59), 232 (13), 231 (13), 231$ (28), 205 (11), 122 (16), 105 (100). HR-MS (+FAB) calcd for C₂₅H₂₅NO₉ [M]+: 483.1529; found: 483.1532.

[(1*S*,2*S*,3*R*,4*S*,4*aR*,11b*R*)-2,3,4,7-Tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-*j*] phenanthridin-1-yl]methyl benzoate (9)

Compound 29 (32 mg, 0.066 mmol) was dissolved in a mixture of CH₂Cl₂-CH₃OH (1:1, 4 mL) and cooled to 0 °C. Trifluoroacetic acid (1 mL) was added dropwise and the reaction was stirred until total consumption of the starting material was observed (TLC). The reaction mixture was dried in vacuo, triturated with CH_2Cl_2 (3 × 15 mL), and finally dried under high vacuum. The final product was isolated by column chromatography on silica gel (deactivated by 10% w/w of water, gradient CH₂Cl₂-CH₂Cl₂/CH₃OH (50:1) to CH₂Cl₂/CH₃OH (25:1)) to give 9 as a white crystalline compound (25 mg, 85%); mp > 200 °C (CH₂Cl₂). R_f = 0.6 (CH₂Cl₂/CH₃OH 10:1). $[\alpha]_D^{20}$ -24.9 (c 1, THF). IR (KBr, cm⁻¹) v: 3423, 3386, 2956, 2921, 2852, 1716, 1672, 1627, 1600, 1466, 1384, 1363, 1340, 1278, 1095, 1072, 1038, 711. ¹H NMR (DMSO-*d*₆, 600 MHz) δ: 13.27 (s, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.68 (t, J = 7.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.43 (s, 1H), 6.72 (s, 1H), 6.07 (s, 1H), 6.01 (s, 1H), 5.19 (m, 2H), 5.13 (m, 1H), 4.66 (t, J =10.7 Hz, 1H), 4.48 (dd, J = 10.9, 4.0 Hz, 1H), 4.24–4.23 (m, 1H), 3.88–3.87 (m, 1H), 3.75–3.73 (m, 1H), 3.62 (dd, J = 13.6, 9.9 Hz, 1H), 3.31 (dd, J = 13.8, 4.2 Hz, 1H), 2.85–2.84 (m, 1H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ: 170.0, 166.3, 152.8, 146.3, 135.8, 133.8, 132.5, 130.3, 129.7, 129.2, 107.5, 102.4, 98.0, 73.1, 71.2, 69.2, 62.5, 51.6, 36.6. MS (+FAB) m/z (%): 444 ([M + 1]⁺, 4), 219 (12), 136 (11), 121 (11), 109 (15), 107 (17), 105 (23), 97 (18), 95 (29), 55 (100). HR-MS (+FAB) calcd for $C_{22}H_{22}NO_9$ [M + 1]+: 444.1295; found: 444.1262.

Cell culture

The human non-small cell lung cancer line, NCI-H460 (American Type Culture Collection (ATCC) No. HTB-177), and human pancreatic adenocarcinoma cancer cell line, BxPC-3 (ATCC No. CRL-1687,) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (GIBCO BRL Carlsbad, California), 100 mg/L penicillin G, and 100 mg/L streptomycin (Cellgro, Manassas, Virginia). Human prostate carcinoma cells, DU-145 (ATCC No.

HTB-81), were cultured in Dulbecco's modified Eagle's medium (Cellgro) supplemented with 10% FBS, 100 mg/L penicillin G, and 100 mg/L streptomycin. Human mammary carcinoma cells, MCF-7 (ATCC No. HTB-22), were cultured using Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 mg/L penicillin G, 100 mg/L streptomycin, 1.0 mmol/L GlutaMAX, and 1.0 mmol/L sodium pyruvate (Gibco).

MTT assay

To evaluate the cytotoxic effects of the C-1 homologues of pancratistatin, mitochondrial dehydrogenase activities were measured. Briefly, BxPC-3, NCI-H460, DU-145, and MCF-7 lines were assessed by seeding 4×10^3 cells/well into microplates. The cells were grown for 24 h before treatment at concentrations ranging from 0.001 to 10 µmol/L and incubated for 48 h. MTT reagent (5 mg/mL, MP Biomedical, Solon, Ohio) was added to each well and incubated further for 2 h. The resulting formazan crystals were dissolved in DMSO and the optical density (OD) was determined at a wavelength of 490 nm. The experiments were repeated at least twice for each compound per cell line. Cells treated with 0.1% DMSO and narciclasine were used as negative and positive controls, respectively.

Cell culture

The human colorectal cancer cell line HCT 116 (ATCC, Cat. No. CCL-247, Manassas, Virginia) was grown and cultured with McCoy's mMedium 5a (Gibco BRL, VWR, Mississauga, Ontario) supplemented with 2 mmol/L L-glutamine, 10% FBS, and 10 mg/mL gentamicin (Gibco BRL, VWR). A human osterosarcoma cell line, Saos-2 (ATCC, Cat. No. HTB-85, Manassas, Virginia), was grown in McCoy's 5A mMedium Modified (Sigma-Aldrich Canada, Mississauga, Ontario) supplemented with 15% (ν/ν) FBS (Thermo Scientific, Waltham, Massachusetts) and 10 mg/mL gentamicin (Gibco BRL, VWR). All cells were grown at 37 °C and 5% CO₂.

WST-1 assay for cell viability

The WST-1-based colorimetric assay was conducted as per the manufacturer's protocol (Roche Applied Science, Indianapolis, Indiana) to determine cell viability as a function of active cell metabolism. Ninty-six well, clear bottom tissue culture plates were seeded with approximately 2.0×10^3 HCT 116 cells/well or 6.0×10^3 Saos-2 cells/well, and treated with various compounds at the indicated concentrations and durations. Following treatment, the WST-1 reagent was added to each well and incubated for 4 h at 37 °C. Absorbance readings were taken at 450 nm on a Wallac Victor³ 1420 multilabel counter (PerkinElmer, Woodbridge, Ontario) and were expressed as percentages of the solvent control group (Me₂SO).

Supplementary data

Supplementary data (copies of ¹H and ¹³C NMR spectra for compounds **7–9**, **12**, **13**, and **15–29**) are available with the article through the journal Web site at http://nrcresearchpress. com/doi/suppl/10.1139/v2012-073.

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