Synthesis of Lakshminine and Antiproliferative Testing of Related Oxoisoaporphines

Vicente Castro-Castillo,*† Marco Rebolledo-Fuentes,‡ Cristina Theoduloz,§ and Bruce K. Cassels†§

Faculty of Basic Sciences, Metropolitan Educational Sciences University, Avenida J.P. Alessandri 774, Núñoa, Santiago, Chile, Department of Chemistry, Faculty of Sciences, University of Chile, Santiago, Chile, Faculty of Health Sciences, University of Talca, Talca, Chile, and Millennium Institute for Cell Dynamics and Biotechnology, Santiago, Chile

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Lakshminine (6-amino-1-aza-5-methoxy-7H-dibenzo[de,h]quinolin-7-one, 1) is a recent addition to the small family of oxoisoaporphine alkaloids and a member of an even smaller set bearing an amino group at C-6. This rare natural product has now been synthesized in order to have sufficient amounts for biological testing. Lakshminine, its 4-amino isomer (2), their 6- and 4-nitro precursors (8 and 10, respectively), the intermediate 5-methoxy-7H-dibenzo[de,h]quinolin-7-one (6), and the unsubstituted skeleton (11) were tested against normal human fibroblasts and three human solid tumor cell lines. Only compound 10 showed marginal antiproliferative activity.

The oxoisoaporphines constitute a small family of alkaloids that are only known to occur in two species of Menispermaceae,1–8 plants characterized by the accumulation of “regular” isoquinoline alkaloids. Their planar 7H-dibenzo[de,h]quinolin-7-one skeleton (commonly known as 1-azabenzanthrone, particularly in the dye and pigment industries) could be assumed to intercalate between DNA base pairs. Their redox-active iminoquinone structure could also be expected to interfere with mitochondrial electron transport. Therefore, these compounds might inhibit cell replication and also be expected to interfere with DNA replication. For a straightforward preparation of 1-aza-9-(substituted amino)benzan-thrones, we decided to use this substance as starting material. Though our initial approach involved reaction of benzanthrones with sulfuric acid, nitrobenzanthrones were obtained in unsatisfactory yields.

Scheme 1. Synthesis of Lakshminine (1) a

20 g of dried, powdered ketoconazole-supplemented M. dauricum root culture.6 Starting from 20 kg of M. dauricum roots, Yu et al. were only able to obtain 20, 9, and 10 mg of dauriosoisoaporphines A, B, and C, respectively.7 Finally, Killmer et al. reported the isolation of 2 mg of 1 from 4.2 kg of S. toxifera vines.8 As we already had experience in the synthesis of 2,3-dihydro-5-methoxy-7H-dibenzo[de,h]quinolin-7-one (1-aza-2,3-dihydro-5-methoxybenzanthrone), we decided to use this substance as starting material for a straightforward preparation of 1 in sufficient amount to determine its antiproliferative activity against a panel of normal and cancer cell lines.

1-Aza-2,3-dihydro-5-methoxybenzanthrone (3) and its 6-methoxy (4) and 6-hydroxy (5) derivatives were obtained by polyphosphoric acid (PPA)-catalyzed cyclization of homoveratrylaminophthalide, (4) and (5) respectively. Nitration of 1-aza-2,3-dihydro-5-methoxybenzanthrone in trifluoroacetic acid (TFA) produced a mixture of isomers (7 and 9). Subsequent oxidation of 3, 7, and 9 with air, over Pd/C in toluene, affor ded 6, 8, and 10 in approximately 90% yields, and selective reduction of the nitro groups of the latter products with sodium sulfide in alkaline solution gave lakshminine (1).

Aromatic electrophilic substitution reactions of the 1-azabenzanthrone system and its 2,3-dihydro analogue have been described

The scanty biological data on these alkaloids may, at least in part, be a consequence of the small quantities in which they have been isolated. Thus, Sugimoto et al. isolated 20 mg of crude 5 from

a Reagents and conditions: (a) H2SO4/HNO3, TFA, rt, 2 h; (b) air, Pd/C, toluene, reflux, 24 h; (c) Na2S, NaOH, H2O, reflux, 5 h.

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extensively.11 With regard to nitration of the 5-methoxy-substituted compounds, it is worth pointing out that, although 1-aza-2,3-dihydro-5-methoxybenzantrane undergoes reaction with similar efficiency at both positions neighboring the OCH₃ group, its aromatized analogue gives the 4-nitro compound (10) as the major product. In our hands, the latter reaction occurred in 68% yield. Considering that 10 was reduced to 2, the regiosomer of 1, in 70% yield, this alternative route is clearly preferable for the synthesis of 2 (Scheme 2).

The effects of these compounds on proliferation were determined in four different human cell lines (MRC-5: normal lung fibroblasts (CCL-171); AGS: gastric adenocarcinoma cells (CRL-1739); SK-MES-1: lung cancer cells (HTB-58); and J82: bladder carcinoma (HTB-1)), using the MTT reduction assay. The concentrations of the compounds inhibiting cell growth by 50% (IC₅₀ values) were obtained adjusting the dose–response curves to a sigmoidal model. Only compound 10 was moderately active and slightly more toxic (IC₅₀ = 4.5 µM) toward gastric adenocarcinoma cells than toward normal fibroblasts.

For comparison, the IC₅₀ values obtained for daunorubicinophosphorines A and B, using similar methodology in different tumor cell lines, were reported to be in the range 3.0 µM to greater than 50 µM.17 Another similar study, in which 12 synthetic 1-azabenzenes were tested against three different tumor cell lines, gave IC₅₀ values between 2.09 and >100 µM.18 Our results, added to the previously published data, suggest that further modification of the 1-azabenzanthrene scaffold might lead to the development of cytotoxic anticancer drugs. Nevertheless, a rational series of compounds with a broader range of substitution patterns would have to be synthesized and tested before any reasonable structure–activity relationships could be discerned.

Experimental Section

General Experimental Procedures. All reagents and solvents were commercially available from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) and were used without further purification. Melting points are uncorrected and were determined with a commercially available from Sigma-Aldrich (St. Louis, MO, USA) or Thermo Finnigan MAT 95XP instrument, with electron impact ionization at 70 eV and with perfluorokerosene as reference. Purities of the compounds subjected to biological testing were >95% in every case (HPLC).

Synthesis of 2,3-dihydro-7H-dibenzo[d,h]quinolin-7-one Derivatives (3, 4, and 5). A solution of phthalaldehydic acid (10 g, 66.6 mmol) in toluene (50 mL) was treated with 3,4-dimethoxyphenethylamine (11.0 mL, 66.6 mmol). The solution was refluxed with stirring under a Dean–Stark trap for 2 h. The solvent was removed in vacuo, and the crude product was heated with phosphoric acid (40 g) at 100 °C for 20 min with stirring. The mixture was poured into ice water, made alkaline (pH 8–9) with 10% NH₄OH and extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with H₂O and dried over Na₂SO₄, and the solvent was evaporated to dryness to leave 0.086 g (0.5%); mp 156 °C; 1H NMR (CDCl₃) δ 2.63–2.73 (1H, m, CH₂), 2.84–3.01 (1H, m, CH₂), 3.33–3.48 (1H, m, CH₂), 3.84 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 4.13–4.23 (1H, m, CH₂), 6.57 (1H, s, ArH), 7.49 (1H, J = 7.6 Hz, ArH), 7.66 (1H, J = 7.3 Hz, ArH), 8.27 (1H, J = 7.6 Hz, ArH), 8.38 (1H, J = 7.8 Hz, ArH), 12.93 (1H, s, OH).

5-Methoxy-6-nitro-7H-dibenzo[d,h]quinolin-7-one (1-aza-5-methoxy-6-nitrobenzantrane, 2). A solution of phthalaldehydic acid (10 g, 66.6 mmol) in toluene (50 mL) was treated with Pd/C (400 mg) and refluxed under air for 48 h. The hot mixture was filtered through Celite and washed several times with hot benzene. The solvent was removed to afford 0.205 g (35%); mp 209–210 °C; 1H NMR (CDCl₃) δ 2.99 (2H, J = 7.9 Hz, CH₂), 4.00 (3H, s, OCH₃), 4.20 (2H, J = 7.8 Hz, CH₂), 7.19 (1H, s, ArH), 7.64 (1H, J = 7.6 Hz, ArH), 7.74 (1H, J = 7.4 Hz, ArH), 8.23 (1H, J = 7.8 Hz, CH) 8.38 (1H, d, J = 7.8 Hz, CH), 10.84 (1H, d, J = 7.8 Hz, CH), 13.13 (1H, s, CH) 131.3, 131.7, 134.9, 135.4, 131.3, 151.2, 151.5, 183.7 (CO); HREIMS m/z 308.1463 (calcd for C₁₇H₁₀N₂O₄, 308.0797) and 2,3-dihydro-5-methoxy-6-nitro-7H-dibenzo[d,h]quinolin-7-one (1-aza-2,3-dihydro-5,6-dimethoxybenzantrane, 3): red needles, 0.935 g (6%); mp 158 °C (lit.11 154–155 °C); 1H NMR (CDCl₃) δ 2.63–2.73 (1H, m, CH₂), 2.84–3.01 (1H, m, CH₂), 3.33–3.48 (1H, m, CH₂), 3.84 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 4.13–4.23 (1H, m, CH₂), 6.57 (1H, s, ArH), 7.49 (1H, J = 7.6 Hz, ArH), 7.66 (2H, m, ArH), 8.09 (1H, J = 7.6 Hz, ArH), 8.27 (1H, J = 7.8 Hz, ArH), 8.38 (1H, J = 8.1 Hz, CH), 7.6 (1H, J = 7.3 Hz, ArH), 7.71 (1H, J = 7.3 Hz, ArH), 8.27 (1H, J = 7.8 Hz, ArH), 8.38 (1H, J = 8.1 Hz, ArH), 12.93 (1H, s, OH).

5-Methoxy-6-nitro-7H-dibenzo[d,h]quinolin-7-one (1-aza-5-methoxy-6-nitrobenzantrane, 4). A solution of phthalaldehydic acid (10 g, 66.6 mmol) in toluene (50 mL) was treated with 3,4-dimethoxyphenethylamine (11.0 mL, 66.6 mmol). The solution was refluxed with stirring under a Dean–Stark trap for 2 h. The solvent was removed in vacuo, and the crude product was heated with phosphoric acid (40 g) at 100 °C for 20 min with stirring. The mixture was poured into ice water, made alkaline (pH 8–9) with 10% NH₄OH and extracted with CH₂Cl₂. The
Hz, ArH), 8.70 (1H, d, J = 5.6 Hz, ArH), 8.90 (1H, d, J = 7.9 Hz, ArH); 13C NMR (CDCl3) δ 55.9 (CH3), 112.0, 118.6, 120.2, 120.8, 125.3, 127.1, 129.9, 130.7, 132.2, 134.1, 136.8, 137.3, 143.9, 148.1, 161.0, 183.1 (CO); HREIMS m/z 261.0813 (calcd for C17H14NO2, 261.0817).

5-Methoxy-4-nitro-7H-dibenzo[de,h]quinoline-7-one (1-aza-5-methoxy-4-nitrobenzanthrone, 10). To a solution of 6 (1.0 g, 3.8 mmol) in TFA (50 mL) was added carefully H2SO4/HNO3 1:1 (10 mL), and the resulting mixture was stirred at room temperature for 6 h. The reaction mixture was poured into water (150 mL), made alkaline with 10% NH4OH (pH 8–9), and extracted with CH2Cl2. The CH2Cl2 layer was washed with H2O and dried over Na2SO4, and the solvent was removed to leave a yellow residue, which was chromatographed on silica gel (AcOEt), giving compound 1: 0.802 g (68%); 1H NMR (DMSO-d6) δ 3.97 (3H, s, OCH3), 6.34 (1H, s, NH), 6.97 (1H, s, ArH), 7.44 (1H, d, J = 2.1 Hz, ArH), 7.66 (1H, t, J = 7.1 Hz, ArH), 7.80 (1H, t, J = 7.1 Hz, ArH), 8.53 (1H, d, J = 7.7 Hz, ArH), 8.60 (1H, d, J = 2.2 Hz, ArH), 9.00 (1H, d, J = 7.9 Hz, ArH), 10.59 (1H, s, NH); 13C NMR (CDCl3) δ 56.0 (CH3), 104.2, 108.3, 119.4, 120.2, 124.8, 126.4, 128.9, 129.5, 132.3, 132.8, 136.4, 141.9, 142.3, 148.0, 150.5, 183.8 (CO); HREIMS m/z 306.0871 (calcd for C15H13N2O2, 306.0864).

6-Amino-5-methoxy-7H-dibenzo[d,e]quinoline-7-one (lakshminine, 1). To a stirred suspension of 8 (1.5 g, 4.8 mmol) in EtOH (50 mL) was added a solution of Na2S·9H2O (2.7 g, 11.2 mmol) and NaOH (1.0 g, 25 mmol) in water (50 mL). The mixture was refluxed for 5 h and left to stand overnight. The EtOH was removed in vacuo, and the precipitate formed was collected by filtration, washed with water, and left to stand overnight. The EtOH was removed in vacuo, and the medium was changed every two days. The cells were stored in liquid nitrogen in media with 10% glycerol added, and their viability after thawing was higher than 90%, as assessed by the trypan blue exclusion test. For the assay, cells were plated in 96-well plates (100 µL/well) at a density of 5 × 104 cells/mL. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 to 100 µM, first dissolved in DMSO (final concentration of 1%), diluted with complete medium, and incubated for 72 h in a humidified incubator with 5% CO2 in air at 37 °C, after which the MTT reduction assay was performed as described previously.12 Etoposide (98% purity, Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. Each experiment was carried out three times in quadruplicate. The IC50 value was obtained adjusting the dose–response curve to a sigmoidal model (a + (b-a)/1 + e^(-c*x)), where c = log IC50.

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Supporting Information Available: T1: Antiproliferative activity of compounds 1, 2, 6, 8, 10, and 11. Figures S1–S10: 1H and 13C NMR spectra of compounds 1, 2, 7, 8, and 9. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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