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Synthesis and biological evaluation of cepharadiones A and B and related dioxoaporphines

Mark A. Elban,[a] Jean C. Chapuis,[b] Mei Li[a] and Sidney M. Hecht[a,b,*]

[a] *Departments of Chemistry and Biology, University of Virginia, Charlottesville, VA 22904, USA*
[b] *Center for BioEnergetics, The Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA*

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Abstract—Described herein is the first total synthesis and structural confirmation of cepharadione A, a naturally occurring DNA damaging agent. Also reported is the synthesis of cepharadione B, a closely related natural product, as well as the biological evaluation of both natural products. Finally, the preparation and biological evaluation of novel dioxoaporphine analogues is described.
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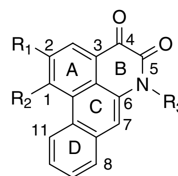
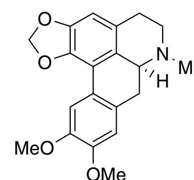
1. Introduction

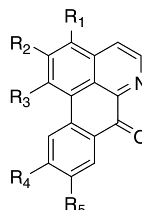
Cepharadione A (**2**) (Fig. 1), a known 4,5-dioxoaporphine alkaloid, has been isolated from a number of plant sources.[1] Although originally isolated from the callus tissue of *Stephania cepharantha* more than 30 years ago,[2] neither the synthesis nor any report of the biological activity of this compound had been described. Recently, we isolated cepharadione A from *Piper caninum*[3] using bioassay-guided fractionation based on DNA damaging activity in a sensitive yeast assay. Specifically, cepharadione A potently inhibited the growth of a yeast strain lacking RAD52 (IC_{50} 50.2 nM), which is associated with the repair of double-strand DNA breaks.[3] In contrast with the dearth of biological activity reported for cepharadione A, other members of the (oxo) aporphine family of alkaloids have demonstrated interesting biological activities. Cepharadione B (**1**) has been shown to be cytotoxic to a variety of cell lines including colon and breast cancer cell lines.[4] Artabotrine (**3**), a dioxoaporphine identical with cepharadione A except for an N–OMe instead of N–Me substituent, was also cytotoxic to repair deficient yeast (IC_{12} 1.2 μg/mL), as well as cultured wild-type and camptothecin-resistant cultured P-388 cells (IC_{50} 1.59 and 1.12 μg/mL, respectively).[5] Other bioactive oxoaporphine alkaloid family members include dicentrine (**4**)[6] and dicentrinone (**5**)[7] which have been found to be DNA topoisomerase II and topoisomerase I inhibitors, respectively. Further, the oxoaporphine alkaloids oxophoebine (**6**) and liriodenine (**7**) were found to be cytotoxic to yeast deficient in DNA recombinational repair, while lauterine (**8**) and 10-hydroxyliriodenine (**9**) inhibited the DNA unknotting activity of topoisomerase II.[8] The





(**1**) cepharadione B R_1=R_2= OMe, R_3=Me
(**2**) cepharadione A R_1R_2 = OCH_2O, R_3=Me
(**3**) artabotrine R_1R_2 = OCH_2O, R_3=OMe

(**4**) dicentrine



(**5**) dicentrinone R_1=H, R_2R_3=OCH_2O, R_4=R_5=OMe
(**6**) oxophoebine R_1=R_2=R_3=OMe, R_4R_5=OCH_2O
(**7**) liriodenine R_1=R_4=R_5=H, R_2R_3=OCH_2O
(**8**) lauterine R_1=R_5=H, R_2R_3=OCH_2O, R_4=OMe
(**9**) 10-hydroxyliriodenine R_1=R_5=H, R_2R_3=OCH_2O, R_4=OH

Figure 1. Cepharadiones A and B, and structurally related molecules.

Keywords: Cepharadiones A and B; Oxoaporphines; Suzuki coupling; Cytotoxicity.

* Corresponding author. Tel.: +1 434 924 3906; e-mail: smh9u@virginia.edu

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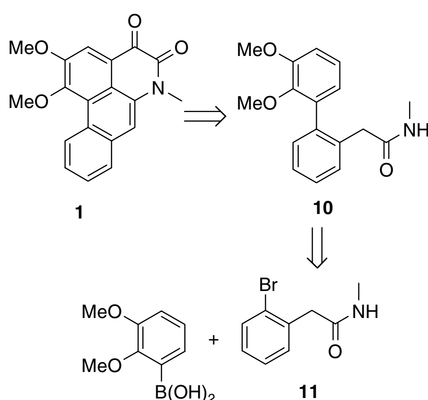
methylenedioxy group could be important for the putative DNA damaging properties of **6** and **7**, as structurally related compounds lacking this feature were much less cytotoxic (for tables comparing biological activities, see Ref. 8b).

Given these interesting biological results and the lack of a synthetic route to cepharadione A (**2**) we focused on this synthetic target as well as the structurally related cepharadione B (**1**). Previous synthetic efforts have resulted in preparation of a number of related natural products by a variety of methods. The first reported syntheses of oxoaporphines involved the oxidation of the B ring from the appropriate precursors.[9] Later synthetic efforts by Castedo et al. involved forming the C ring as the last step. The initial synthesis of pontevedrine was accomplished under photochemical conditions;[10] later, using a radical ring closing methodology, pontevedrine and other 5-oxoaporphines were prepared.[11] A quite different approach employing benzynes also provided access to several 4,5-dioxoaporphines.[12] Subsequently, Suau and co-workers used photochemistry to form the B ring after forming rings A, C, and D.[4]

Perhaps the most versatile and efficient synthesis of this class of natural products to date has been realized via the sequential formation of the C and B rings using $(COCl)_2$ and $SnCl_4$.[13] This synthetic approach seemed the best option for the synthesis of cepharadione A, but the previously reported approach to the advanced intermediate **10** seemed cumbersome. During our initial synthetic studies on this class of molecules, Suau and co-workers reported the use of a Suzuki coupling to prepare a biaryl ester, which was elaborated to give the desired dioxoaporphines.[14] We describe herein the application of this approach to give the advanced biaryl amide **10** directly (Scheme 1) en route to cepharadione B. In addition, cepharadione A has been prepared for the first time by elaboration of the same advanced intermediate. Further, we describe the screening of several other boronic acids under the Suzuki coupling conditions followed by elaboration to the corresponding dioxoaporphines. The cytotoxic activities of these compounds have been studied and are discussed.



Scheme 1. Retrosynthetic analysis of cepharadione B (**1**).

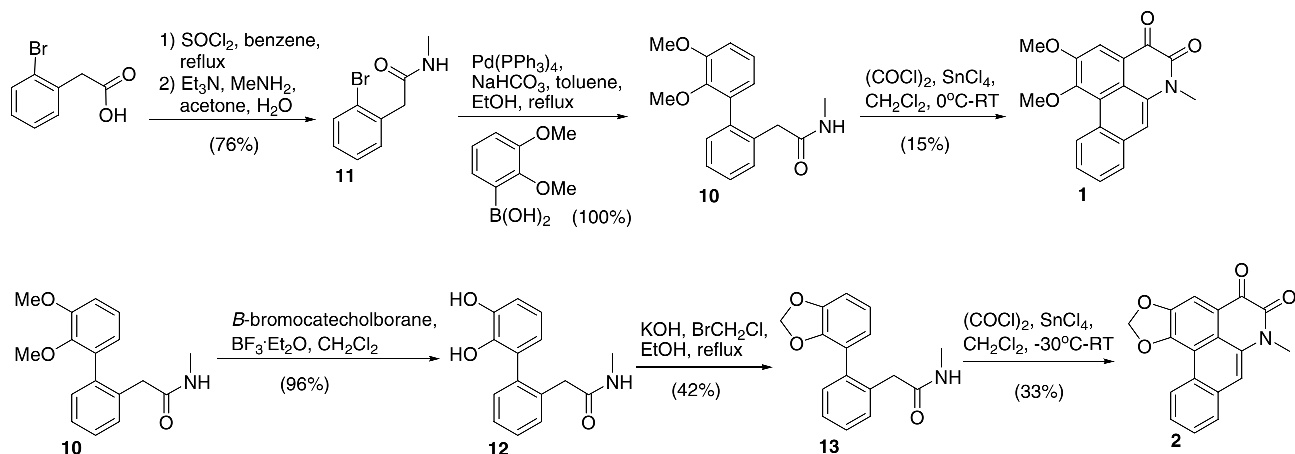
2. Results and discussion

The synthesis of cepharadione B (Schemes 1 and 2) began with commercially available (2-bromophenyl)acetic acid, which was treated with thionyl chloride to form the acid chloride and then with methylamine under basic conditions to afford *N*-methylamide **11** in 76% yield. Key intermediate **10** was then obtained in quantitative yield by Suzuki coupling[15] of **11** with commercially available 2,3-dimethoxyphenylboronic acid.[16] It may be noted that pure **10** was obtained in quantitative yield, in contrast with an earlier finding[14] in which triphenylphosphine oxide hindered the purification of a series of related compounds, resulting in poor yields. The C and B rings were then formed sequentially with $(COCl)_2$ and $SnCl_4$ according to the method of Suau and co-workers[14] to give cepharadione B in 15% yield. In order to synthesize cepharadione A (Scheme 2) intermediate **10** was deprotected using B-bromocatechol borane and $BF_3{\cdot}Et_2O$[17] to afford the unstable diphenol **12** in 96% yield. Formation of the corresponding benzodioxole **13** was accomplished in 42% yield by treatment of diphenol **12** with $BrCH_2Cl$ and KOH in EtOH at reflux.[18] Completion of the synthesis of cepharadione A (**2**) was accomplished using the same ring closure procedure described above[14] in 33% yield. The material obtained was identical in all respects to that isolated previously.[1,3]

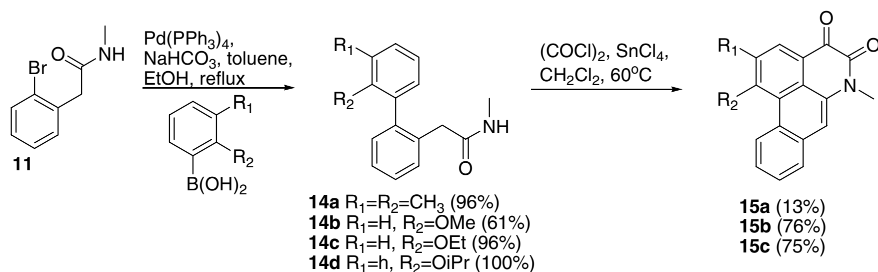
In order to probe the scope of the Suzuki coupling to give advanced biaryl amide intermediates, several boronic acids were studied (Scheme 3). Gratifyingly, the 2,3-dimethyl, 2-ethoxy, and 2-isopropoxy boronic acids all underwent coupling to give the biaryl species **14a**, **c**, **d** in excellent yields. The low yield associated with the preparation of **14b** was due to the contamination of the initially isolated product with triphenylphosphine oxide. A separate purification step was required to remove most of this contaminant, which resulted in the loss of significant desired material.

The subsequent formation of the corresponding dioxoaporphines was attempted by treating **14a–d** with $(COCl)_2$ and $SnCl_4$ according to the method of Suau.[14] The ring closure to give **15a** proceeded in low yield, possibly due to the change in electronic character of the A ring. The methoxy and ethoxy derivatives proceeded in good yield to give the desired dioxoaporphines **15b** and **c**, respectively. The final attempt using the isopropoxy biaryl intermediate **14d** failed to give any product, possibly due to the increased steric bulk preventing the ring system from aligning properly for ring closure.

We have previously evaluated cepharadione A[3] in a yeast strain (RS321 NpRAD52) which harbors a plasmid containing a RAD52 recombinant gene under the control of a galactose promoter. When this strain was grown on glucose, cepharadione A (**2**) inhibited the growth of this yeast strain with an IC_{50} value of 50.2 nM. When the strain was grown on galactose (i.e., under DNA repair proficient conditions), the IC_{50} value for **2** was 293 nM. The synthetic sample prepared as described here produced comparable results in this as-



Scheme 2. Syntheses of cepharadione A (**2**) and B (**1**).



Scheme 3. Suzuki couplings and subsequent cyclization.

say. In contrast, cepharadione B (**1**) was essentially without activity in the yeast assay.

Cepharadione B as well as 2-demethoxycepharadione B have been tested previously using several cell lines; the resulting IC_{50} values ranged from 1.9 to 6.6 μg/mL.[4] In light of this promising data, we tested cepharadiones A and B as well as **15a**–**c** against a different series of cancer cell lines (Table 1). Comparing **1** and **2** against the cell lines tested revealed a similar trend to that of the DNA damaging yeast assay; **2** was generally considerably more active than **1**. The dimethylated species **15a** also proved to be cytotoxic against several of the cell lines tested with toxicities similar to that of cepharadione A. Perhaps the most interesting biological result involved the cytotoxicity of 2-demethoxycepharadione B (**15b**), which was potently cytotoxic toward all six cell lines tested with GI_{50} values ranging from 0.42 to 1.6 μg/mL. In particular, **15b** was the only compound tested which possessed significant activity against BXPC-3, the pancreatic adenocarcinoma cell line. When the methyl ether was replaced with an ethyl ether (**15c**), the cytotoxicity was greatly reduced or eliminated, a result somewhat incongruous with previous findings.[4]

These results indicate that the replacement of the 1,2-dimethyl ethers with a methylenedioxy group (**1** vs **2**) increases the cytotoxic potency of this dioxoaporphine. Since the assay depends explicitly on differences in DNA repair capability between two yeast strains that are otherwise isogenic, it is logical to think that the presence of the methylenedioxy functionality enhances DNA damage in some fashion. This observation, while surprising, is entirely consistent with observations made by Gunatilaka et al.[8b] for other aporphine alkaloids that damage DNA in the same yeast assay system. Further, these results demonstrate that substitution at C-2 of the dioxoaporphines is not absolutely required for potent biological activity. Finally, the ability of the dioxoaporphines to tolerate increased steric bulk at C-1 while maintaining potent growth inhibition seems to be rather limited.

Table 1. Human cancer cell line inhibition values (GI_{50}) expressed in μg/mL for **1**, **2**, **15a**–**c**

Cancer cell line[a]	1	2	15a	15b	15c
BXPC-3	>10	>10	>10	1.6	>10
MCF-7	>10	6.3	8.4	0.66	>10
SF-268	22.8	2.9	0.70	0.42	>10
NCI-H460	>10	2.5	2.3	0.46	>10
KM20L2	>10	17.3	4.2	0.82	>10
DU-145	36.1	4.3	>10	1.3	>10

[a] Cancer type: BXPC-3 (pancreatic adenocarcinoma); MCF-7 (breast carcinoma); SF-268 (CNS glioblastoma); NCI-H460 (lung large cell); KM20L2 (colon adenocarcinoma); DU-145 (prostate carcinoma).

3. Conclusions

In summary, we have described the first total synthesis of cepharadione A, permitting confirmation of the assigned structure as well as verification of its biological

activity. The synthesis was accomplished via the key biaryl amide intermediate **10**, which was prepared utilizing a Suzuki coupling. The preparation of additional biaryl intermediates was also accomplished using the same conditions, further demonstrating the utility of this approach for preparing congeners of the dioxoaporphine family of natural products. Finally, the biological activity of several new dioxoaporphine congeners is reported, further establishing the biological profile of this interesting class of natural products.

4. Experimental

4.1. General experimental procedures

Reagents and solvents were of reagent grade and used without further purification. Anhydrous grade solvents were purchased from VWR. All reactions involving air or moisture sensitive reagents or intermediates were performed under a nitrogen or argon atmosphere. Flash chromatography was performed using Silicycle 40–60 mesh silica gel. Analytical TLC was performed using 0.25 mm EM silica gel 60 F_{250} plates that were visualized by irradiation (254 nm) or by staining with Hanessian's stain (cerium molybdate). ^{1}H and ^{13}C NMR spectra were obtained using 300 and 500 MHz Varian instruments. Chemical shifts are reported in parts per million (ppm δ) referenced to the residual ^{1}H resonance of the solvent ($CDCl_3$, 7.26 ppm). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, 77.0 ppm). Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Michigan State University–NIH Mass Spectrometry Facility.

4.2. Specific procedures

4.2.1. 2-(2-Bromophenyl)-*N*-methylacetamide (11). To a solution containing 1.00 g (4.65 mmol) of (2-bromophenyl)acetic acid in 15 mL of benzene was added 0.83 g (6.90 mmol) of thionyl chloride. The reaction mixture was stirred at reflux for 3 h at which time excess solvent was removed under diminished pressure. The resulting residue was dissolved in 5 mL of acetone and added slowly to a solution containing 3.60 mL (4.65 mmol) of methylamine (40% in water), 1.85 mL (14.0 mmol) of triethylamine, and 3 mL of H_2O chilled to 0 °C. The reaction mixture was stirred at room temperature for 1 h at which time it was poured into 150 mL of benzene. The organic phase was washed with 25 mL of brine, 25 mL of 1 N HCl, and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a crude oil. The residue was purified by flash chromatography on a silica gel column (30 × 2 cm). Elution with 20% MeOH in CH_2Cl_2 gave **11** as a colorless solid: yield 0.80 g (76%); mp 102–104 °C; silica gel TLC R_f 0.38 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.74 (d, 3H, $J = 4.8$ Hz), 3.68 (s, 2H), 5.61 (br, 1H), 7.14 (m, 1H), 7.31 (m, 2H) and 7.55 (d, 1H, $J = 7.8$ Hz); ^{13}C NMR ($CDCl_3$) δ 26.48, 43.78, 124.92, 127.91, 129.02, 131.63, 133.02, 134.82 and 170.11; mass spectrum (FAB) *m/z* 228.0025 $(M+H)^+$ ($C_9H_{11}NOBr$ requires 228.0024).

4.2.2. 2-(2′,3′-Dimethoxybiphenyl-2-yl)-*N*-methylacetamide (10). To a solution containing 1.00 g (4.38 mmol) of **11** in 20 mL of toluene were added 0.30 g (0.26 mmol) of $Pd(PPh_3)_4$ and 8.76 mL (17.5 mmol) of 2.0 M aq Na_2CO_3. The reaction mixture was heated to reflux while degassing with N_2. At this point a degassed solution containing 1.59 g (8.77 mmol) of 2,3-dimethoxyphenylboronic acid in 10 mL of EtOH was added. The reaction mixture was maintained at reflux for 16 h at which time it was cooled and washed with two 50-mL portions of 2.0 M aq Na_2CO_3. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a crude dark oil. Chromatography on a flash silica gel column (30 × 4 cm) using 1:2 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **10** as a colorless solid: yield 1.25 g (100%); mp 116–118 °C, lit.[13b] 93–96 °C; silica gel TLC R_f 0.47 (10% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 2.65 (d, 3H, $J = 4.8$ Hz), 3.40 (s, 2H), 3.56 (s, 3H), 3.91 (s, 3H), 5.84 (br, 1H), 6.71 (dd, 1H, $J = 7.8$ Hz, 1.8 Hz), 6.93 (dd, 1H, $J = 8.1$ Hz, 1.4 Hz), 7.10 (t, 1H, $J = 7.8$ Hz) and 7.35 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 26.39, 41.19, 55.77, 60.80, 111.86, 122.75, 124.32, 126.72, 128.07, 129.60, 130.22, 134.07, 135.32, 138.13, 145.75, 152.75 and 171.89; mass spectrum (FAB) *m/z* 285.1442 $(M+H)^+$ ($C_{17}H_{20}NO_3$ requires 285.1443).

4.2.3. 1,2-Dimethoxy-6-methyl-6*H*-dibenzo[*de*,*g*]quinoline-4,5-dione (cepharadione B) (1).[13] To a solution containing 0.08 g (0.28 mmol) of **10** in 1 mL of CH_2Cl_2 at 0 °C was added 0.84 mL (1.68 mmol) of $(COCl)_2$. The reaction mixture was stirred for 15 min at which time 1.72 mL (1.68 mmol) of $SnCl_4$ was added. The reaction mixture was stirred at room temperature for 72 h and then quenched with 50 mL of 1 N HCl. The mixture was extracted with three 100-mL portions of ethyl acetate. The combined organic phase was washed with 100 mL of H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a dark red oil. Chromatography on flash silica gel (30 × 4 cm) using 20% MeOH in 1:1 ethyl acetate–hexanes as eluant gave **1** as an orange solid: yield 0.016 g (18%); mp 265–266 °C, lit.[2] 267–268 °C; silica gel TLC R_f 0.51 (10% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H), 4.10 (s, 3H), 4.11 (s, 3H), 7.48 (s, 1H), 7.65 (m, 2H), 7.85 (m, 1H), 8.23 (s, 1H) and 9.48 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 30.48, 56.47, 60.43, 112.54, 114.30, 119.45, 123.60, 124.51, 126.84, 127.58, 127.64, 128.01, 129.03, 131.69, 132.23, 152.79, 154.90, 156.23 and 175.45; mass spectrum (ESI) *m/z* 321.4 $(M)^+$ (theoretical *m/z* 321.10); mass spectrum (FAB) *m/z* 322.1080 $(M+H)^+$ ($C_{19}H_{16}NO_4$ requires 322.1079).

4.2.4. 2-(2′,3′-Dihydroxybiphenyl-2-yl)-*N*-methylacetamide (12). To a solution containing 0.50 g (1.75 mmol) of **10** in 10 mL of CH_2Cl_2 were added 2.89 g (10.5 mmol) of *B*-bromocatechol borane and 0.28 mL (1.75 mmol) of $BF_3{\cdot}Et_2O$. The reaction mixture was stirred at room temperature for 5 h at which time it was

poured into 50 mL of 2 N HCl and extracted with two 50-mL portions of ethyl acetate. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a crude brown oil. Chromatography on flash silica gel (30 × 4 cm) using 78:20:2 CH_2Cl_2–MeOH–AcOH as eluant gave **12** as a brown foam which was used immediately in the subsequent step to avoid decomposition: yield 0.43 g (96%); silica gel TLC R_f 0.23 (10% MeOH in 1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.56 (d, 3H, $J = 4.8$ Hz), 3.37 (s, 2H), 6.52 (dd, 1H, $J = 7.5$ Hz, 1.5 Hz), 6.77 (t, 1H, $J = 7.8$ Hz), 6.84 (dd, 1H, $J = 8.1$ Hz, 1.5 Hz) and 7.24 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 26.41, 40.53, 114.64, 120.56, 121.67, 127.32, 128.00, 128.89, 129.42, 130.75, 133.44, 138.47, 141.32, 145.48 and 173.74; mass spectrum (ESI) *m/z* 257.3 $(M)^+$ (theoretical *m/z* 257.11).

4.2.5. 2-(2-Benzo[1,3]dioxol-4-yl-phenyl)-*N*-methylacetamide (13). To a solution containing 0.10 g (0.39 mmol) of **12** in 3 mL of EtOH were added 0.03 mL (0.43 mmol) of CH_2BrCl and 0.04 g (0.78 mmol) of KOH. The reaction was maintained at reflux for 48 h at which time the reaction mixture was concentrated under diminished pressure. Chromatography on a flash silica gel column (30 × 4 cm) using 10% MeOH in 1:1 ethyl acetate–hexanes as eluant gave **13** as a colorless oil: yield 0.040 g (42%); silica gel TLC R_f 0.44 (20% MeOH in 1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.62 (d, 3H, $J = 4.8$ Hz), 3.52 (s, 2H), 5.61 (br, 1H), 5.87 (s, 2H), 6.69 (dd, 1H, $J = 7.2$ Hz, 1.5 Hz), 6.82 (m, 2H), and 7.34 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 26.36, 41.27, 100.64, 108.10, 121.94, 123.11, 127.51, 128.58, 130.50, 130.72, 132.00, 133.39, 136.38, 144.48, 147.16 and 171.57; mass spectrum (ESI) *m/z* 269.3 $(M)^+$ (theoretical *m/z* 269.1); mass spectrum (FAB) *m/z* 270.1129 $(M+H)^+$ ($C_{16}H_{16}NO_3$ requires 270.1130).

4.2.6. 1,2-Benzodioxol-6-methyl-*6H*-dibenzo[*de,g*]quinoline-4,5-dione (cepharadione A) (2). To a solution containing 0.14 g (0.54 mmol) of **13** in 5 mL of CH_2Cl_2 at −30 °C was added 0.52 mL (5.94 mmol) of $(COCl)_2$. The reaction mixture was stirred for 15 min at which time 0.58 mL (4.95 mmol) of $SnCl_4$ was added. The reaction mixture was stirred at room temperature for 72 h and then quenched with 50 mL of 1 N HCl. The mixture was extracted with three 100-mL portions of ethyl acetate. The combined organic phase was washed with 100 mL of H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a dark red oil. Chromatography on flash silica gel column (30 × 4 cm) using 5% MeOH in $CHCl_3$ as eluant gave **2** as an orange solid: yield 0.050 g (33%); mp 338–340 °C, lit.[1a] 340–342 °C; silica gel TLC R_f 0.70 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 6.46 (s, 2H), 7.52 (s, 1H), 7.66 (m, 2H), 7.89 (m, 1H), 8.14 (s, 1H) and 8.98 (m, 1H); mass spectrum (ESI) *m/z* 305.0 $(M)^+$ (theoretical *m/z* 305.3); mass spectrum (FAB) *m/z* 306.0765 $(M+H)^+$ ($C_{18}H_{12}NO_4$ requires 306.0766).

4.2.7. General procedure for preparation of 14a–d. To a solution containing 0.20 g (0.88 mmol) of **11** in 20 mL of toluene were added 0.06 g (0.05 mmol) of $Pd(PPh_3)_4$ and 1.75 mL (3.50 mmol) of 2.0 M aq Na_2CO_3. The reaction mixture was degassed with N_2 for 15 min at which time a degassed solution containing 1.75 mmol of the substituted boronic acid in 10 mL of EtOH was added. The reaction mixture was heated at reflux for 16 h. The cooled reaction mixture was extracted with two 100-mL portions of ethyl acetate and the organic extract was washed with two 50-mL portions of 2.0 M aq Na_2CO_3. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a crude oil. The product was purified by flash chromatography on silica gel to give the desired product.

4.2.7.1. 2-(2′,3′-Dimethylbiphenyl-2-yl)-*N*-methylacetamide (14a). Flash chromatography on a silica gel column (20 × 2 cm) using 2:1 hexanes–ethyl acetate → 100% ethyl acetate afforded **14a** as a colorless solid: yield 0.212 g (96%); mp 126–128 °C; silica gel TLC R_f 0.30 (2:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.93 (s, 3H), 2.31 (s, 3H), 2.59 (d, 3H, $J = 4.8$ Hz), 3.26 (dd, 2H, $J = 24.3$ Hz, 15.3 Hz), 5.35 (br, 1H), 6.14 (m, 1H), 7.14 (m, 3H) and 7.36 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 16.32, 20.37, 26.11, 40.84, 125.04, 126.94, 127.49, 128.99, 129.92, 130.00, 132.99, 134.16, 136.94, 140.11, 142.22 and 171.18; mass spectrum (FAB) *m/z* 254.1546 $(M+H)^+$ ($C_{17}H_{20}NO$ requires 254.1545).

4.2.7.2. 2-(2′-Methoxybiphenyl-2-yl)-*N*-methylacetamide (14b). In order to remove triphenylphosphine oxide (which comigrated with the product on TLC), the crude oil was dissolved in cold hexanes, which deposited a white precipitate. The suspension was filtered through a silica gel pad and washed with 200 mL of ether. The filtrate was concentrated under diminished pressure to afford a crude oil. Flash chromatography on a silica gel column (20 × 2 cm) using 2:1 hexanes–ethyl acetate → 100% ethyl acetate afforded **14b** as a colorless solid: yield 0.135 g (61%); mp 126–128 °C, lit.[13b] 125–126 °C; silica gel TLC R_f 0.13 (2:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.65 (d, 3H, $J = 4.8$ Hz), 3.42 (s, 2H), 3.72 (s, 3H), 5.56 (br, 1H), 7.00 (m, 2H), 7.11 (m, 1H), 7.23 (m, 1H) and 7.34 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 26.23, 41.15, 55.19, 110.60, 120.67, 127.07, 128.86, 129.08, 129.26, 129.93, 130.76, 130.90, 133.71, 138.85, 155.395 and 171.68; mass spectrum (FAB) *m/z* 256.1339 $(M+H)^+$ ($C_{16}H_{18}NO_2$ requires 256.1338).

4.2.7.3. 2-(2′-Ethoxybiphenyl-2-yl)-*N*-methylacetamide (14c). Flash chromatography on a silica gel column (20 × 2 cm) using 2:1 hexanes–ethyl acetate → 100% ethyl acetate afforded **14c** as a colorless solid: yield 0.225 g (96%); mp 112–116 °C; silica gel TLC R_f 0.40 (2:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, $J = 6.9$ Hz), 2.61 (d, 3H, $J = 5.1$ Hz), 3.44 (d, 2H, $J = 4.2$ Hz), 3.99 (m, 2H), 5.58 (br, 1H), 6.98 (m, 2H), 7.08 (m, 1H), 7.21 (m, 1H) and 7.32 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.55, 26.11, 41.16, 63.84, 112.27, 120.68, 126.87, 127.71, 128.96, 129.74, 130.77, 130.93, 133.68, 138.87, 155.32 and 171.75; mass spectrum (FAB) *m/z* 270.1493 $(M+H)^+$ ($C_{17}H_{20}NO_2$ requires 270.1494).

4.2.7.4. 2-(2′-Isopropoxybiphenyl-2-yl)-*N*-methylacetamide (14d). Flash chromatography on a silica gel column (20×2 cm) using 2:1 hexanes–ethyl acetate → 100% ethyl acetate afforded product as a colorless solid: yield 0.247 g (100%); mp 114–116 °C; silica gel TLC R_f 0.36 (2:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.05 (d, 3H, $J = 6.0$ Hz), 1.20 (d, 3H, $J = 6.0$ Hz), 2.62 (d, 3H, $J = 5.1$ Hz), 3.42 (s, 2H), 4.40 (dq, 1H, $J = 6.0$, 6.0 Hz), 5.71 (br, 1H), 7.00 (m, 2H), 7.09 (m, 1H), 7.19 (m, 1H) and 7.32 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 21.75, 22.12, 26.09, 41.15, 71.53, 115.40, 121.15, 126.67, 127.66, 128.84, 129.49, 130.67, 131.09, 131.46, 133.88, 138.95, 154.52, and 171.87; mass spectrum (FAB) *m*/*z* 284.1649 (M+H)$^+$ ($C_{18}H_{22}NO_2$ requires 284.1650).

4.2.8. Procedures for preparation of 15a–c.

4.2.8.1. 1,2-Dimethyl-6-methyl-6*H*-dibenzo[*de,g*]quinoline-4,5-dione (15a). To a degassed solution containing 0.065 g (0.26 mmol) of **14a** in 3 mL of CH_2Cl_2 was added 0.056 mL (0.64 mmol) of $(COCl)_2$. The reaction mixture was stirred for 5 min and 0.075 mL (0.64 mmol) of $SnCl_4$ was added dropwise. The reaction mixture was sealed tightly with a septum and stirred at 60 °C for 18 h. The reaction mixture was quenched with 50 mL of 2 N HCl, extracted with two 100-mL portions of CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a dark oil. Step gradient elution chromatography on a flash silica gel column (15×2 cm) using 2% MeOH in 1:1 $CHCl_3$–hexanes → 2% MeOH in $CHCl_3$ as eluant gave **15a** as a yellow solid: yield 0.010 g (13%); mp 255–257 °C; silica gel TLC R_f 0.67 (10% MeOH in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.63 (s, 3H), 3.03 (s, 3H), 3.83 (s, 3H), 7.50 (s, 1H), 7.62 (m, 2H), 7.93 (m, 1H), 8.43 (s, 1H) and 8.54 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 21.57, 23.17, 30.60, 114.02, 122.21, 125.05, 125.72, 127.44, 127.83, 128.14, 128.96, 130.13, 131.09, 132.29, 132.95, 139.12, 144.22, 156.64 and 176.46; mass spectrum (FAB) *m*/*z* 290.1182 (M+H)$^+$ ($C_{19}H_{16}NO_2$ requires 290.1181).

4.2.8.2. 1-Methoxy-6-methyl-6*H*-dibenzo[*de,g*]quinoline-4,5-dione (15b).[14] Prepared as described above starting with 0.055 g (0.21 mmol) of **14b**. Flash chromatography on a silica gel column (15×2 cm) using 2% MeOH in $CHCl_3$ as eluant gave **15b** as a yellow solid: yield 0.048 g (76%); mp 267–269 °C, lit.[14] 256–258 °C; silica gel TLC R_f 0.44 (10% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3H), 4.26 (s, 3H), 7.62 (d, 1H, $J = 8.7$ Hz), 7.68 (m, 2H), 7.94 (s, 1H), 8.08 (m, 1H), 8.46 (d, 1H, $J = 8.7$ Hz) and 9.42 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 30.33, 56.81, 111.27, 115.81, 118.49, 120.76, 124.17, 126.16, 127.09, 127.22, 27.58, 129.10, 130.88, 131.75, 131.91, 155.96, 164.60 and 174.69; mass spectrum (FAB) *m*/*z* 292.0972 (M+H)$^+$ ($C_{18}H_{14}NO_3$ requires 292.0974).

4.2.8.3. 1-Ethoxy-6-methyl-6*H*-dibenzo[*de,g*]quinoline-4,5-dione (15c). Prepared as described above starting with 0.060 g (0.22 mmol) of **14c**. Flash chromatography on a silica gel column (15×2 cm) using 2% MeOH in $CHCl_3$ as eluant gave **15c** as a yellow solid: yield 0.051 g (75%); mp 279–281 °C; silica gel TLC R_f 0.49 (10% MeOH in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.75 (t, 3H, $J = 6.9$ Hz), 3.76 (s, 3H), 4.34 (q, 2H, $J = 7.2$ Hz), 7.30 (d, 1H, $J = 8.7$ Hz), 7.50 (s, 1H), 7.65 (m, 2H), 7.86 (m, 1H), 8.57 (d, 1H, $J = 8.7$ Hz) and 9.56 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.79, 30.61, 65.79, 111.02, 115.67, 119.83, 120.87, 124.75, 127.26, 127.32, 128.04, 128.96, 131.60, 131.81, 131.86, 156.76, 164.51, and 175.15; mass spectrum (FAB) *m*/*z* 306.1132 (M+H)$^+$ ($C_{19}H_{16}NO_3$ requires 306.1130).

4.3. Biological evaluation

4.3.1. SRB assay experimental procedure. Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.[19] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium solution were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software.

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