Synthesis and DNA-binding Properties of 1,10-Phenanthroline Analogues as Intercalating-Crosslinkers

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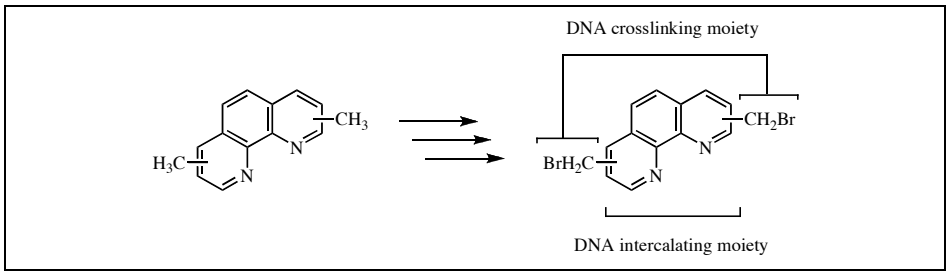
Received April 22, 2008



We synthesized a series of bis(bromomethyl)-1,10-phenanthrolines as novel anticancer lead compounds and examined their DNA-binding properties. 5,6-Bis(bromomethyl)-1,10-phenanthroline showed DNA intercalating activity and DNA crosslinking activity, furthermore it is stable in aqueous solution.

J. Heterocyclic Chem., **45**, 1889 (2008).

INTRODUCTION

Cisplatin is a crosslinking agent with high anticancer activity. However, it causes very serious side effects such as renal toxicity and nausea. We suspect that some of these side effects are caused by platinum, a component of cisplatin, and that a crosslinking agent lacking heavy metals would therefore be useful as a drug in cancer chemotherapy.

We have reported the synthesis of DNA crosslinking agents containing the acridine skeleton [1]. These compounds possess DNA intercalating and interstrand crosslinking activities, and inhibit cell proliferation in a human T cell leukemia cell line. However, these compounds were hydrolyzed rapidly in aqueous solution. Therefore, the purpose of the present study was to synthesize crosslinking compounds which are stable in aqueous solution.

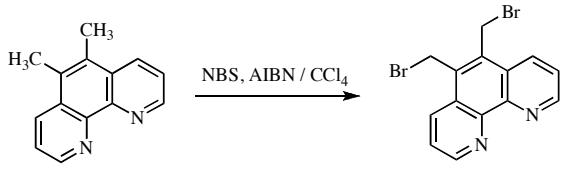
The metal-complexing characteristics of 1,10-phenanthroline have been well-characterized. Some 1,10-phenanthroline-cuprous complexes oxidatively cleave DNA [2-6], and possess anticancer activity towards a human cervical epidermoid carcinoma cell line [7]. Ruthenium [8], lanthanum [9,10], osmium [11] and vanadium [12] complexes demonstrate similar effects. 5,6-Dimethyl-1,10-phenanthroline metal complexes are even more effective, especially when complexed with cuprous or ruthenium ions [13-16]. Furthermore, phenanthroline derivatives, which are crescent-shaped planar aromatic compounds, have been shown to interact specifically with a quadruplex and to inhibit telomerase activity [17-19]. Clearly, phenanthroline derivatives possess very interesting biological activities.

In the current study, we synthesized a novel crosslinking agent based on the 1,10-phenanthroline skeleton, and examined both its DNA intercalating and interstrand crosslinking activity and stability in aqueous solution.

RESULTS AND DISCUSSION

5,6-Bis(bromomethyl)-1,10-phenanthroline **2** was synthesized *via* a radical reaction of 5,6-dimethyl-1,10-phenanthroline **1** with NBS [20-22] (Scheme 1).

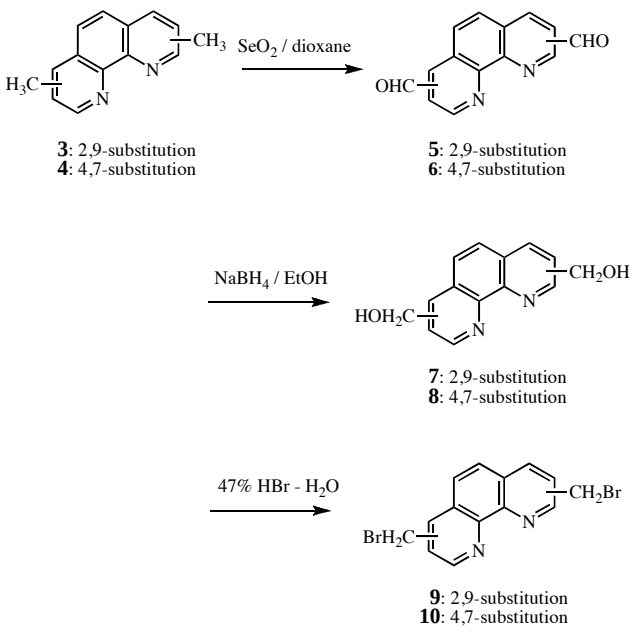
Scheme 1



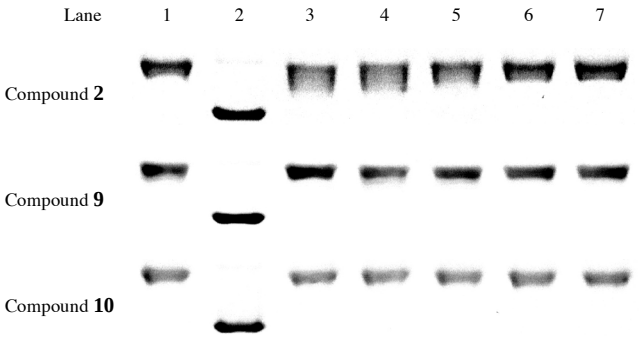
1 **2**

1,10-Phenanthroline-2,9-dicarboxaldehyde **5** was synthesized *via* the oxidation of 2,9-dimethyl-1,10-phenanthroline **3** with selenium dioxide [23]. Compound **5** on hydrolysis afforded 2,9-bis(hydroxymethyl)-1,10-phenanthroline **7**. 2,9-Bis(bromomethyl)-1,10-phenanthroline **9** was synthesized by a substitution reaction of compound **7**. 4,7-Substitution products **6**, **8**, **10** were synthesized by the same routes as compounds **5**, **7**, **9** (Scheme 2).

Scheme 2

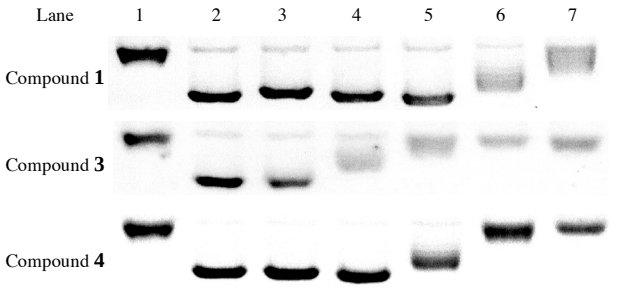


We examined the DNA intercalating activity of these compounds using an unwinding assay [24]. Compound **2** caused a partial transition from open circular to supercoiled plasmid DNA, and demonstrated higher DNA intercalating activity than did compounds **9** or **10** (Fig. 1). Comparison of methyl compounds **1**, **3** and **4** showed that compound **1** has the highest activity (Fig. 2). Maheswari *et al*. previously demonstrated that 5,6-dimethyl-1,10-phenanthroline has high affinity for DNA surfaces due to hydrophobic interactions [13-15]. Our results are in accordance with their report.



Lane 1, open circular plasmid DNA; lane 2, supercoiled plasmid DNA; lanes 3-7, plasmid DNA treated with 1 mM, 500 μM, 250 μM, 100 μM or 50 μM of the indicated compound. Reactions were performed for 30 minutes.

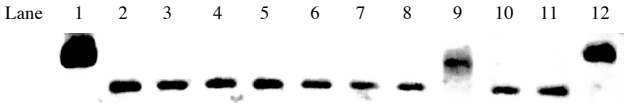
Figure 1. DNA intercalating activity of crosslinking compounds.



Lane 1, open circular plasmid DNA; lane 2, supercoiled plasmid DNA; lanes 3-7, plasmid DNA treated with 1 mM, 500 μM, 250μM, 100 μM or 50 μM of the indicated compound. Reactions were performed for 30 minutes.

Figure 2 DNA intercalating activity of methyl compounds.

Next, we examined DNA interstrand crosslinking activity [25]. Compound **2** at a concentration of 10 μ M generated a band of double stranded DNA, while compounds **9** and **10** had no effect at the tested concentrations (Fig. 3). These results suggest that compound **2** has high crosslinking activity due to its high DNA affinity, driven by intercalation. Furthermore, the position of the substitution affects activity.

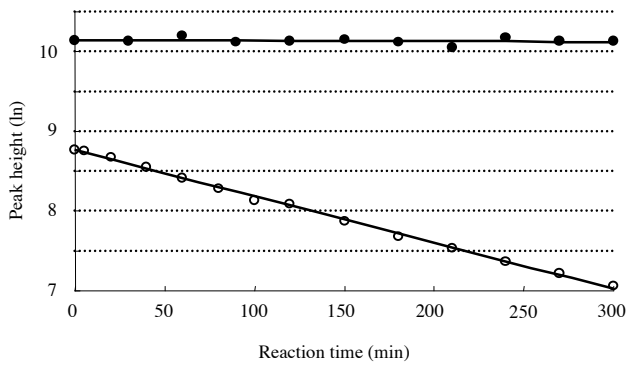


Lane 1, nondenatured (double-stranded) DNA; lane 2, denatured (single-stranded) DNA; lanes 3-5, plasmid DNA treated with 10, 1 or 0.1 μM compound **9**; lanes 6-8, plasmid DNA treated with 10, 1 or 0.1 μM compound **10**; lanes 9-11, plasmid DNA treated with 10, 1 or 0.1 μM compound **2**; lanes 12, plasmid DNA treated with 0.1 μM cisplatin.

Figure 3. DNA interstrand crosslinking activity.

We previously synthesized 4,5-bis(bromomethyl)-acridine, which has high DNA crosslinking activity and inhibits proliferation of a human T cell leukemia cell line [1]. However, this acridine compound hydrolyzed rapidly in aqueous solution. We examined the stability of compound **2** in aqueous solution (Fig. 4) and showed that it is not hydrolyzed. Hydrolysis is caused by a S_N1 reaction. The intermediate carbocation is more stable in the acridine compound than in 1,10-phenanthroline compounds because two heteroatoms in the 1,10-phenanthroline skeleton strongly withdraw electrons. Therefore, compound **2** is more stable in aqueous solution than the acridine compound.

In conclusion, we synthesized a novel intercalating-crosslinker with a 1,10-phenanthroline skeleton. Compound **2** has DNA intercalating and crosslinking activity, and is stable in aqueous solution. These characteristics make compound **2** a suitable leading compound as an anticancer agent. We are investigating the anticancer activity of compound **2** in a human T cell leukemia cell line.



Solid circle, compound **2**; open circle, 4,5-bis(bromomethyl)acridine.

Figure 4. Stability in aqueous solution.

EXPERIMENTAL

^{1}H NMR spectra were obtained on a Jeol JNM-A500 spectrometer using tetramethylsilane as an internal reference. Mass spectra were determined using a Shimadzu GCMS-QP5050A spectrometer. Compounds **1**, **3** and **4** were purchased from Tokyo Kasei, Inc. (Tokyo, Japan). Silica gel 60 and aluminum oxide 90 were purchased from Merck & Co. (Rahway, NJ, USA). pBR322 plasmid DNA and topoisomerase I were purchased from Takara-Bio, Inc. (Tokyo, Japan). L-column 2 was purchased from Chemicals Evaluation and Research Institute, Japan (Tokyo, Japan).

5,6-Bis(bromomethyl)-1,10-phenanthroline (**2**). A mixture of **1** (104 mg, 0.5 mmol), *N*-bromosuccinimide (270 mg, 1.5 mmol) and 2,2'-azobis(isobutyronitrile) (32 mg, 0.2 mmol) in tetrachloromethane (30 mL) was refluxed under nitrogen for 15 minutes, then the mixture was allowed to cool to room temperature. The solvent was evaporated, and the residue was purified by aluminum oxide column chromatography in dichloromethane to obtain **2** as yellow needles, m.p: 170 ºC dec., yield: 67.5 mg (36.9%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 5.08 (s, 4H, -CH_2-), 7.77 (dd, 2H, phenyl protons), 8.57 (dd, 2H, phenyl protons), 9.25 (dd, 2H, phenyl protons). EI MS: m/z: 368, 366, 364, 287, 285, 206.

1,10-Phenanthroline-2,9-dicarboxaldehyde (**5**). A mixture of **3** (545 mg, 2.5 mmol) and selenium dioxide (1.11 g, 10 mmol) in dioxane containing 4% H_2O (50 mL) was heated under reflux for an hour and then filtered through celite while hot. A solid separated in the cold filtrate and was recrystallized from dioxane containing 4% H_2O to give compound **5** as white needles, m.p: 237ºC dec., yield: 395.9 mg (67.1%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 8.05 (s, 2H, phenyl protons), 8.39 (d, 2H, phenyl protons), 8.52 (d, 2H, phenyl protons), 10.57 (s, 2H, -CHO). EI MS: m/z: 236, 208, 180.

1,10-phenanthroline-4,7-dicarboxaldehyde (**6**). A mixture of **4** (104 mg, 0.5 mmol) and selenium dioxide (224 mg, 2.0 mmol) in dioxane containing 4% H_2O (10 mL) was heated under reflux for 30 minutes and then filtered through celite while hot. A solid separated in the cold filtrate and recrystallized from dioxane containing 4% H_2O to give compound **6** as yellow needles, m.p: 160 ºC dec., yield: 212.5 mg (90.0%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 8.10 (d, 2H, phenyl protons), 9.23 (s, 2H, phenyl protons), 9.54 (d, 2H, phenyl protons), 10.65 (s, 2H, -CHO). EI MS: m/z: 236, 208, 179.

2,9-Bis(hydroxymethyl)-1,10-phenanthroline (**7**). A solution of **5** (118 mg, 0.5 mmol) and sodium borohydride (38 mg, 1.0 mmol) in ethanol (10 mL) was heated under reflux for half an hour. The mixture was extracted with ethyl acetate, and the organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give compound **7** as yellow needles, m.p: 184 ºC dec., yield: 78.2 mg (65.2%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 5.10 (s, 4H, -CH_2-), 7.60 (d, 2H, phenyl protons), 7.79 (s, 2H, phenyl protons), 8.24 (d, 2H, phenyl protons). EI MS: m/z: 240, 239, 210, 209, 193.

4,7-Bis(hydroxymethyl)-1,10-phenanthroline (**8**). A solution of **6** (118 mg, 0.5 mmol) and sodium borohydride (38 mg, 1.0 mmol) in ethanol (10 mL) was heated under reflux for half an hour. The mixture was extracted with ethyl acetate, and the organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give compound **8** as yellow needles, m.p: 220 ºC dec., yield: 31.2 mg (26.0%). ^{1}HNMR (CD_3OD, 500MHz): δ 5.23 (s, 4H, -CH_2-). EI MS: m/z: 240, 239, 210, 209, 193. HRMS: 240.0917 (calcd. for $C_{14}H_{12}O_2N_2$: 240.0900) *Anal.* Calcd. for $C_{14}H_{12}O_2N_2$ (240.26). 0.2 H_2O: C, 68.95; H, 5.12; Found: C, 68.86; H, 5.12.

2,9-Bis(bromomethyl)-1,10-phenanthroline (**9**). A solution of **7** (24 mg, 0.1 mmol) and hydrobromic acid (15 mL, 47%) was heated under reflux for an hour, then cooled on ice and treated with solid sodium carbonate at pH 10. The mixture was extracted with dichloromethane and the organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography in 5:1 dichloromethane / ethyl acetate to obtain **9** as yellow needles, m.p: 105 ºC dec., yield: 26.0 mg (71.0%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 4.97 (s, 4H, -CH_2-), 7.82 (s, 2H, phenyl protons), 7.92 (d, 2H, phenyl protons), 8.29 (d, 2H, phenyl protons). EI MS: m/z: 368, 366, 364, 287, 285, 206.

4,7-Bis(bromomethyl)-1,10-phenanthroline (**10**). A solution of **8** (12 mg, 0.05 mmol) and hydrobromic acid (10 mL, 47%) was heated under reflux for an hour and then cooled on ice and treated with solid sodium carbonate at pH 10. The mixture was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by aluminum oxide column chromatography in dichloromethane to obtain **10** as a yellow powder, m.p: 135 ºC dec., yield: 7.0 mg (38.3%). ^{1}HNMR ($CDCl_3$, 500MHz): δ 4.94 (s, 4H, -CH_2-), 7.66 (d, 2H, phenyl protons), 8.27 (s, 2H, phenyl protons), 9.18 (d, 2H, phenyl protons). EI MS: m/z: 368, 366, 364, 287, 285, 206. HRMS: 363.9197 (calcd. for $C_{14}H_{10}N_2Br_2$: 363.9211) *Anal.* Calcd. for $C_{14}H_{10}N_2Br_2$ (366.04): C, 45.93; H, 2.75; Found: C, 46.09; H, 3.01.

Unwinding assay. Negatively supercoiled pBR322 DNA (0.25 μg/reaction) was first relaxed at 37 ºC by incubation with 5 units of calf thymus topoisomeraseI at pH 8.0 for 30 minutes. Next, the DNA was treated with the test compound dissolved in DMSO at pH 8.0 for 30 minutes at 37 ºC. The reaction was terminated by the addition of sodium dodecyl sulfate (0.5% final concentration) followed by dilution and extraction to remove the drugs. The DNA samples were separated by 1% agarose gel electrophoresis, and the DNA bands were visualized by staining with ethidium bromide.

Crosslinking assay Linearized pBluescript® DNA (460 ng) was treated with each test compound dissolved in DMSO at 37 ºC for 6 hours. After the reaction, the DNA was precipitated with ethanol (95%) and cooled for 24 hours before being

centrifuged for 20 minutes. The supernatant was removed, the samples were washed with ethanol (70%) and spun for 20 minutes. The supernatant was removed and the DNA was lyophilized to dryness. The DNA was then dissolved in separation buffer (30% DMSO and 1 mM ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetic acid), heated at 95 ºC for 5 minutes, and immediately placed in an ice bath. The DNA samples were separated by 1% agarose gel electrophoresis, and the bands were visualized by staining with ethidium bromide.

Stability of intercalating-crosslinker The stability of compound **2** toward nucleophilic reagents was examined by measuring the decomposition rate in aqueous solution. Test compound (final concentration of 100 μM) was added to an aqueous solution (70:30 mixture of 0.01 M sodium phosphate buffer [pH 7.4] and acetonitrile), and 6 μL of the solution was injected onto an HPLC L-column 2 (5 μm) at regular intervals. Compounds were eluted with a 2:1 mixture of methanol and water.

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