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Syntheses and copper(II)-dependent DNA photocleavage by acridine and anthracene 1,10-phenanthroline conjugate systems

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We report the syntheses and characterization of a series of compounds based on 1,10-phenanthroline covalently tethered, at the 2 and 9 positions, to either two benzene, naphthalene, acridine or anthracene chromophores. The acridine and anthracene derivatives are shown to efficiently cleave pUC19 plasmid DNA upon irradiation with ultraviolet light (pH = 7.0, 22 °C, 350 nm). Furthermore, photocleavage levels are markedly increased by the addition of Cu^{2+} to the DNA photolysis reactions. Interestingly, when the concentrations of the anthracene compounds are lowered from 35 μM to 0.25 μM, the reverse trend is observed. DNA photocleavage is markedly reduced in the presence of copper(II).

Introduction

There is continuing interest in the design and syntheses of small molecules that efficiently bind to and cleave DNA. In particular, the study of organic molecules and their coordination complexes has become a subject of intensive investigation. In this regard, the use of chelating agents such as 2,2′-bipyridine (bipy) and 1,10-phenanthroline (phen) has played an important role in bioinorganic chemistry.[1] Reagents of this kind have been widely employed, especially in combination with different transition metals, finding applications in therapeutics and as DNA cleaving agents and DNA footprinting tools.

The 1,10-phenanthroline moiety constitutes a ligand of considerable interest. The discovery of the nuclease activity of the cuprous complex $(phen)_2Cu(I)$ by Sigman and coworkers[2] has prompted an intense investigation aimed at establishing the underlying mechanism(s) of the DNA cleavage process.[3] It was found that the binding of $(phen)_2Cu(I)$ to DNA in the presence of H_2O_2 as coreactant induced single-strand cleavage, mainly by oxidative attack at C1′ of deoxyribose in the DNA minor groove.[4]

However, whereas great effort has been devoted to the development of new oxidative and hydrolytic[5] nucleases based on 1,10-phenanthroline, less attention has been paid to the use of 1,10-phenanthroline systems as DNA photocleavers. Despite their interesting photochemical properties,[6] only recently have examples of DNA photocleavage by these systems been reported in the literature.[7–9] Notwithstanding, DNA photocleaving agents have proven to be extremely useful tools in nucleic acid chemistry and medicine, with applications in biotechnology, photodynamic therapy (PDT) and in photofootprinting to study DNA–drug and DNA–protein interactions. Moreover, the analyses of acridine, anthracene and other chromophores endowed with the ability to photocleave DNA under physiological conditions can be used to provide a rational basis for the design and syntheses of new, more complex structures particularly well suited for PDT and other *in vivo* applications.

As part of our research program focused on the study of DNA binding coordination complexes, we have recently described the syntheses and DNA photocleaving properties of naphthalene, acridine and anthracene-bound 2,2′-bipyridine ligands and their corresponding platinum(II) complexes.[10,11] We now report the preparation of a new series of ligands based on a central 1,10-phenanthroline moiety (Fig. 1) covalently tethered to either two acridine or anthracene chromophores through an amide containing linker. We have also synthesized benzene, naphthalene

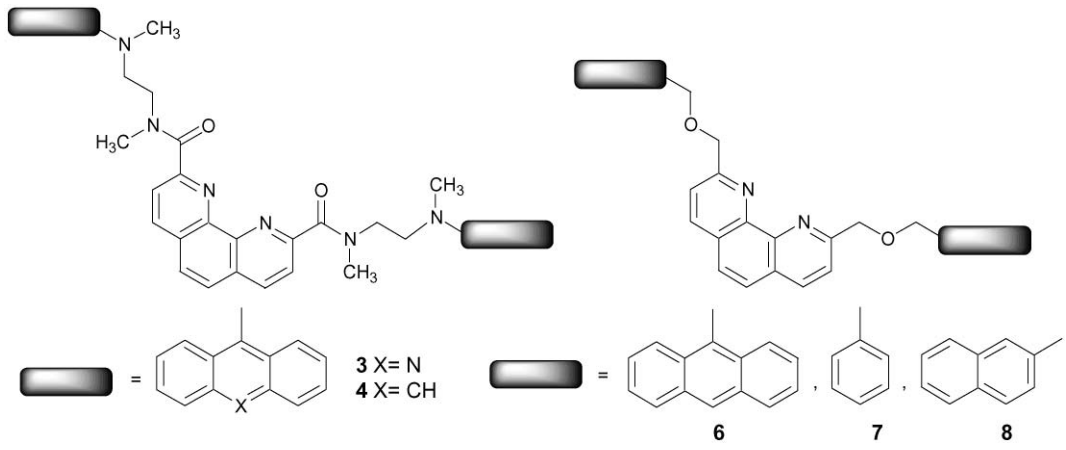

Fig. 1

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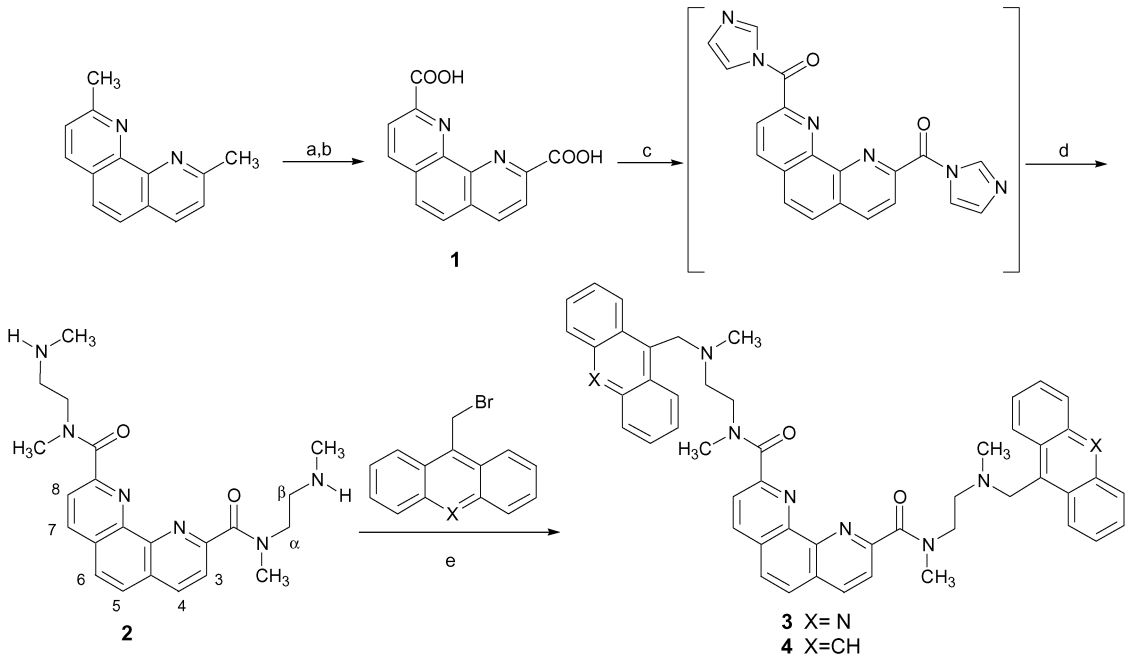


Scheme 1 Syntheses of compounds **3** and **4**. *Reagents and conditions*: a) SeO_2, 1,4-dioxane, reflux, 3 h, 76%; b) 60% HNO_3, reflux, 4 h, 75%; c) 1,1′-carbonyldiimidazole, THF, 60 °C, 18 h; d) *N*,*N*′-dimethyethylenediamine, rt, 24 h, 75% (for steps c and d); e) K_2CO_3, CH_3CN, 24 h, rt (52–66%).

and anthracene analogues attached to 1,10-phenanthroline with ether linking chains. Substitution at the α-2,9 positions of 1,10-phenanthroline was chosen to preclude oxidative nuclease activity in the absence of light, since this substitution pattern clearly disfavours the H_2O_2-based Cu^{2+}/Cu^{1+} redox cycle needed to cleave DNA under the conditions reported by Sigman *et al*.[3a,3e]

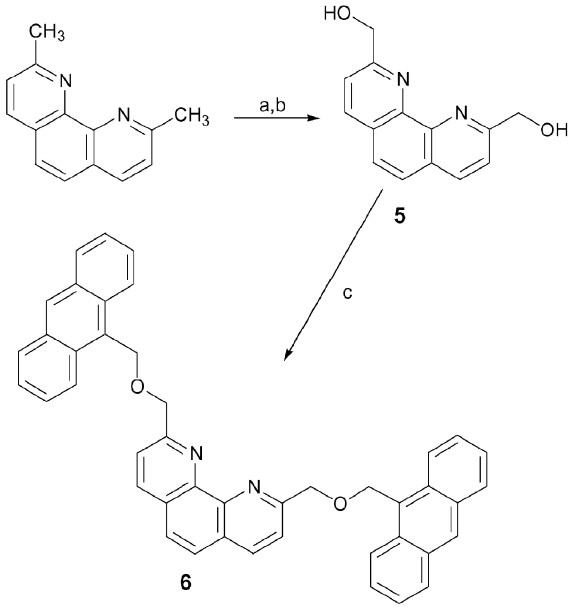
Because acridine and anthracene chromophores are known to be more efficient photocleaving agents than their naphthalene counterparts,[10] we have concentrated our efforts on the study of the acridine and anthracene containing ligands and we have evaluated their DNA photocleaving capabilities in the absence and in the presence of Cu^{2+}. We have found that the addition of copper metallic salt can be conveniently used to modulate levels of DNA photocleavage at pH 7.0 and 22 °C.

Results and discussion

Chemistry

The synthetic route used for the preparation of compounds **3** and **4** is shown in Scheme 1. Because previous attempts to obtain the requisite 1,10-phenanthroline-2,9-dicarboxylic acid (**1**) through direct oxidations from 2,9-dimethyl-1,10-phenanthroline (dmp, neocuproine) with concentrated HNO_3 and Jones' reagent proved unsuccessful, compound **1** was prepared in two steps (57% overall yield) from 2,9-dimethyl-1,10-phenanthroline through oxidation of the corresponding dialdehyde. The resulting diacid was first reacted with an excess of 1,1′-carbonyldiimidazole at 60 °C and then with *N*,*N*′-dimethylethylenediamine at rt. This procedure readily afforded precursor amide **2**, *via* the imidazolide, in a facile, one-pot type process. Subsequent reactions of the amide **2** with alkylating reagents 9-bromomethylacridine[12] and 9-bromomethylanthracene[13] provided the desired ligands **3** and **4** as a mixture of *Z*- and *E*-isomers in solution, with moderate yields. The compounds were fully characterized by NMR, IR, MS and combustion analyses. The establishment of the isomer proportion and stereochemistry in solution was made based on NMR studies and by correlation with results obtained with analogous amides.[11]

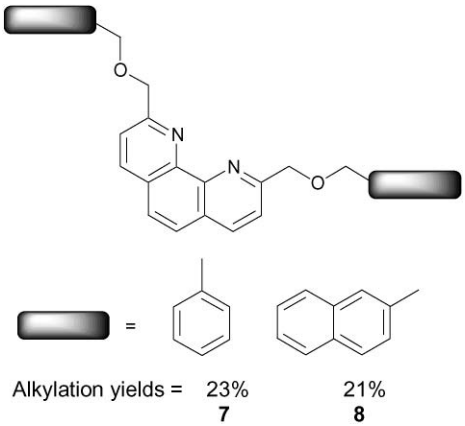
Compounds **6**, **7** and **8** were synthesized next. As shown in Scheme 2, the methodology described by Chandler and coworkers was used to obtain 2,9-bis(hydroxymethyl)-1,10-phenanthroline **5** from 2,9-dimethyl-1,10-phenanthroline.[14] To prepare anthracene containing derivative **6**, efforts were then made to perform the Williamson reaction according to conditions previously described,[10] but with no results. The reaction did not proceed with 9-chloromethylanthracene as an alkylating reagent, even in the presence of strong base. Thus, the use of 9-bromomethylanthracene was required. Compound **6** could only be obtained in low yield upon sequential addition of this alkylating agent in NaH–DMF. An attempt to improve the reaction by employing the crown ether 15-crown-5 was unsuccessful.



Scheme 2 Synthesis of **6**. *Reagents and conditions:* a) SeO_2, 1,4-dioxane, reflux, 3 h, 76%; b) $NaBH_4$, EtOH, reflux, 3.5 h, 76%; c) 9-bromomethyl anthracene, NaH, DMF, rt, 5 days, 20%.

Possible explanations that could account for the lower yield observed in this case compared to analogous 2,2′-bipyridine compounds[10] might be either the lower diol acidity of **5** arising from α-nitrogen deactivation or limited diol solubility. No

evidence of dependence on the alkylating agent was found, because the reactions used to obtain the benzene and naphthalene derivatives (compounds **7** and **8**) employed essentially identical chemistry and gave rise to similar, low yields (Scheme 3).



Scheme 3 *Reagents and conditions:* diol **5**, benzylbromide or 2-bromomethylnaphthalene, NaH, rt, 24 h.

DNA photocleavage

DNA photocleavage by the acridine and anthracene compounds **3**, **4** and **6** in the absence and presence of Cu^{2+} (1 : 1 stoichiometric ratio) was assessed using agarose gel electrophoresis to monitor the conversion of supercoiled pUC19 plasmid DNA (Form I) to its nicked (II) and linear (III) forms. In initial experiments, the benzene (**7**) and naphthalene (**8**) derivatives did not show appreciable photocleaving activities. Therefore, no additional analyses of these compounds were undertaken.

In Fig. 2, DNA cleaved by irradiation of 50 μM of acridine amide derivative **3** in the absence and presence of 50 μM $CuCl_2$ is resolved on a 1.0% agarose gel stained with ethidium bromide (0.5 μg mL^{-1}). The reactions were conducted as follows. After a 1 h equilibration (22 °C, pH 7.0), samples were irradiated at 350 nm at time intervals from 10 to 50 min, under aerobic conditions in a Rayonet Photochemical Reactor. Derivative **3** was found to produce time-dependent photocleavage (lanes 3–7). In addition, there were notable cleavage enhancements in the presence of Cu^{2+} at all time points (lanes 8–12) and the observation of linear DNA after only 20 min of irradiation (lane 9). Background cleavage at the maximum irradiation time (50 min) produced either by 50 μM $CuCl_2$ (lane 2) or 50 μM 2,9-dimethyl-1,10-phenanthroline (a reference compound which lacks the chromophore units, data not shown) was negligible. Thus, the interaction between compound **3** and the metal appears to be synergistic, as the photocleavage induced by the resulting complex (lanes 8–12) exceeds the theoretical yields that would be produced by the addition of copper(II)-induced cleavage (lane 2) to compound **3**-induced cleavage (lanes 3–7) (essentially no reactivity was observed in the two dark controls, in which DNA reactions containing 50 μM of **3** and 50 μM of **3** with 50 μM of $CuCl_2$ were kept in the dark for 50 min; lanes 13 and 14 respectively).

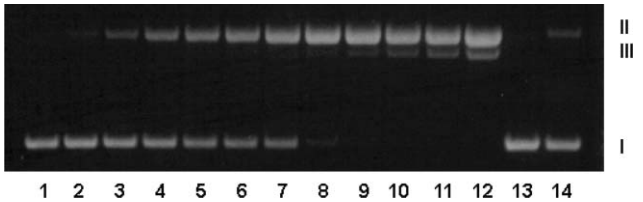


Fig. 2 A photograph of a 1% agarose gel showing photocleavage of 38 μM base pair (bp) pUC19 plasmid DNA by 50 μM **3** in the absence and presence of 50 μM $CuCl_2$ (20 mM sodium phosphate buffer, pH 7.0). Prior to electrophoresis, all reactions were equilibrated in the dark (1 h, 22 °C) and were then either irradiated with 13 RPR-3500 Å 24 W Rayonet lamps at specific time intervals up to 50 min or kept in the dark for 50 min. Lane 1: DNA control irradiated for 50 min; lane 2: 50 μM $CuCl_2$ irradiated for 50 min; lanes 3–7: 50 μM compound **3** irradiated for 10, 20, 30, 40 and 50 min, respectively; lanes 8–12: 50 μM **3** + 50 μM $CuCl_2$ irradiated for 10, 20, 30, 40, 50 min, respectively; lane 13: 50 μM compound **3** in the dark for 50 min; lane 14: 50 μM **3** + 50 μM $CuCl_2$ in the dark for 50 min.

Parallel experiments carried out with 50 μM of anthracene derivatives **4** and **6** in the presence of 50 μM of $CuCl_2$ led to the production of overdamaged DNA. Consequently, we reduced the reagent concentrations and irradiation times to identify conditions that were optimal for observation of synergistic, Cu^{2+}-enhanced DNA cleavage. Shown in Fig. 3 are the results of time course experiments in which 35 μM concentrations of compounds **3**, **4** and **6** were irradiated at time intervals from 5 to 25 min, in the absence and presence of 35 μM $CuCl_2$ (350 nm, 22 °C, pH 7.0). From examination of the data it is evident that anthracene amide derivative **4** is the most efficient DNA photocleaver under the reaction conditions employed, followed by anthracene ether **6** and then the acridine amide **3**. DNA photocleavage by all three compounds was increased in the presence of $CuCl_2$, enabling **4** and **6** to produce linear DNA after only 5 min of irradiation (lane 9).

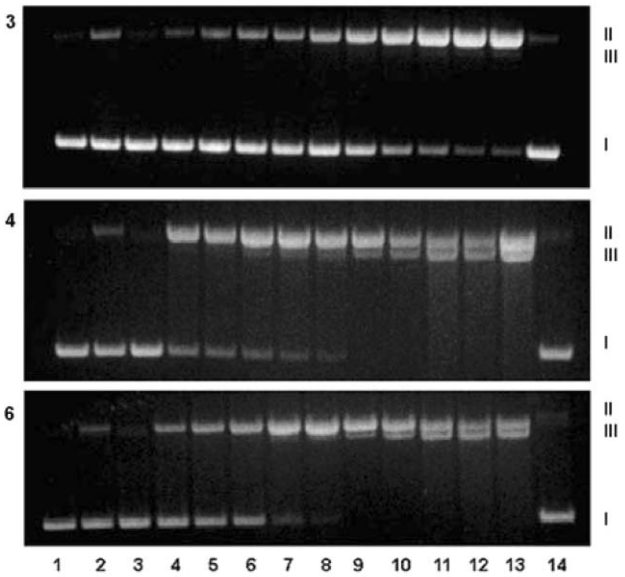


Fig. 3 Photographs of 1% agarose gels showing photocleavage of 38 μM bp pUC19 plasmid DNA by 35 μM of compounds **3**, **4** and **6** (from top to bottom), in the absence and presence of 35 μM $CuCl_2$ (20 mM sodium phosphate buffer, pH 7.0). Prior to electrophoresis, all reactions were equilibrated in the dark (1 h, 22 °C) and were then either irradiated with 13 RPR-3500 Å 24 W Rayonet lamps at specific time intervals up to 25 min or kept in the dark for 25 min. Lane 1: DNA control irradiated for 25 min; lane 2: 35 μM $CuCl_2$ irradiated for 25 min; lane 3: 35 μM of compound in the dark for 25 min; lanes 4–8: 35 μM of compound irradiated for 5, 10, 15, 20, 25 min, respectively; lanes 9–13: 35 μM of compound + 35 μM $CuCl_2$ irradiated for 5, 10, 15, 20 and 25 min, respectively; lane 14: 35 μM of compound + 35 μM $CuCl_2$ in the dark for 25 min.

Because the photoreduction of 1,10-phenanthroline-Cu(II) complexes has been documented,[15] the goal of our next experiment was to determine if the observed enhancements in DNA photocleavage by compounds **3**, **4** and **6** could be associated with photoreduction of Cu^{2+} to Cu^{1+}. Subsequent redox cycling of Cu^{1+} would be expected produce reactive oxygen species capable of cleaving DNA.[16] In order to detect Cu^{1+} formation, we employed a spectrophotometric assay in which bathocuproinedisulfonic acid disodium salt hydrate (BCS) forms a stable, brightly coloured 2 : 1 complex with Cu^{1+} (λ_{max} = 480 nm; ε = 13 500 M^{-1} cm^{-1}).[17] DNA reactions containing

35 μM of compound and 70 μM of BCS were irradiated in the absence and presence of 35 μM $CuCl_2$ for 25 min (20 mM sodium phosphate buffer pH 7.0, 22 °C, 350 nm). Parallel reactions run in the dark were used as negative controls. UV-visible spectra were then recorded (Fig. 4). It is evident that the only reactions to exhibit the 480 nm absorption maximum indicative of the formation of a BCS complex with Cu^{1+} were those in which any of the three compounds and copper were irradiated in combination. These results point towards the involvement of Cu^{1+} in DNA photocleavage by acridine derivative **3** and the anthracenes **4** and **6**.

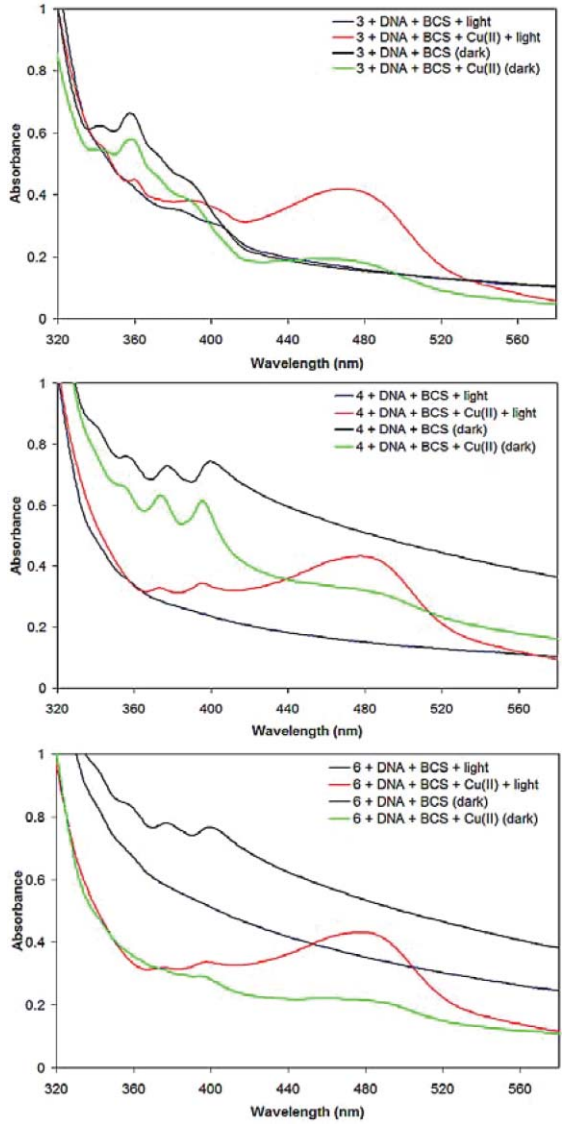


Fig. 4 UV-visible spectra to detect Cu^{1+}–BCS complex formation at 35 μM concentrations of compounds **3**, **4** and **6** (from top to bottom), in the absence and presence of 35 μM $CuCl_2$. All samples contain 38 μM bp pUC19 plasmid DNA, 70 μM BCS and 20 mM sodium phosphate buffer pH 7.0. The samples were irradiated at 350 nm for 25 min or kept in the dark. Legend: blue: compound, light; red: compound, Cu^{2+}, light; black: compound, dark; green: compound, Cu^{2+}, dark.

Preliminary experiments clearly showed that compound **3** was unable to cleave DNA significantly at concentrations below 1 μM of compound, both in the absence and in the presence of Cu^{2+} (data not shown). Between 1 μM and 50 μM of **3**, the addition of copper(II) always enhanced cleaving activity. Interestingly, when similar experiments were conducted at very low concentrations (0.25 μM) of the anthracene derivatives **4** and **6**, it was found that these compounds continued to produce very high levels of DNA photocleavage. Furthermore, the addition of $CuCl_2$ to both the photolysis reactions dramatically diminished cleavage levels. Shown in Fig. 5 are representative time course experiments in which 0.25 μM of compound **4** was reacted in the absence and presence of 0.25 μM of $CuCl_2$. After only 15 min of irradiation, compound **4** completely cleaved the plasmid to produce linear DNA, while the majority of the DNA remained uncut when $CuCl_2$ was included in the reaction (lane 5) (the behavior displayed by compound **6** was nearly identical, except that levels of photocleavage were slightly higher in the absence of copper). Thus, the trends observed at 0.25 μM concentrations of **4** and **6** were opposite those at 35 μM, in which photocleavage levels were markedly increased by Cu^{2+} (Fig. 3).

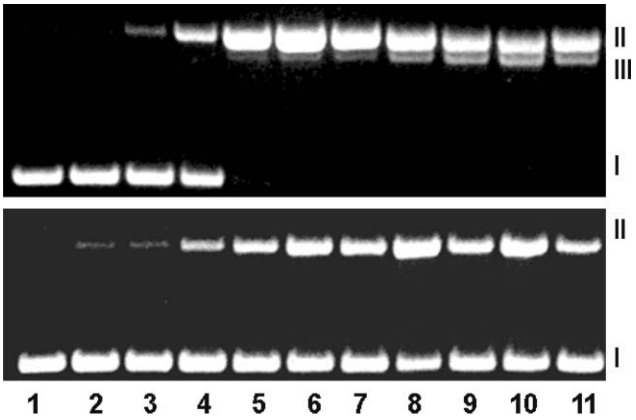


Fig. 5 Photographs of 1% agarose gels showing photocleavage of 38 μM bp pUC19 plasmid DNA by 0.25 μM of compound **4** in the absence (top) and in the presence (bottom) of 0.25 μM $CuCl_2$ (20 mM sodium phosphate buffer, pH 7.0). Prior to electrophoresis, all reactions were equilibrated in the dark (1 h to 1.5 h, 22 °C) and were then irradiated with 13 RPR-3500 Å 24 W Rayonet lamps at specific time intervals. Lane 1: DNA control irradiated for 50 min; lane 2: compound **4** in the dark for 50 min (top gel) or DNA irradiated for 50 min in the presence of $CuCl_2$ (bottom gel); lanes 3–11: 0.25 μM compound **4** (top) or 0.25 μM compound **4** + 0.25 μM $CuCl_2$ (bottom) irradiated for 5, 10, 15, 20, 25, 30, 35, 40 and 45 min, respectively.

The above results are of interest because they demonstrate that copper(II) can be used as a "photonuclease switch" to modulate the DNA cleaving activities of acridine amide **3** and anthracene ether derivatives **4** and **6**. Regarding the dual behaviour observed for **4** and **6**, one possible explanation might involve the existence of different DNA binding modes at the 0.25 μM and 35 μM concentration ranges studied.[18] In general, outside stacking of anthracene[19] and other aromatic compounds[20] in the DNA grooves is more favourable at high ligand concentrations, while an intercalative mode of binding is preferred as the concentration of ligand is lowered. Concentration-dependent interactions have also been reported by McMillin and coworkers[21] in their description of a copper 1,10-phenanthroline complex, where the ratio of complex to DNA played a crucial role in determining the major binding mode. Thus, we hypothesize that efficient intercalation at low concentrations of compounds **4** and **6** might account for the effective DNA cleaving activity in the anthracene series. It is also possible that the addition of Cu^{2+} might compromise this interaction, thereby inhibiting cleavage. Alternatively, as ligand concentrations are raised, it is conceivable that the presence of copper might favour binding interactions along the DNA grooves.

Conclusions

In summary, we have reported the syntheses of a series of compounds based on 1,10-phenanthroline covalently tethered, at the 2 and 9 positions, to either two benzene, naphthalene, acridine or anthracene chromophores. Among the synthesized compounds, acridine and anthracene derivatives were shown to be good DNA photocleavers (pH = 7.0, 22 °C, 350 nm), whereas benzene and naphthalene were inactive. Acridine

compound **3** showed copper(II)-enhanced photocleaving activity at micromolar concentrations, while only 0.25 μM of anthracene derivatives **4** and **6** was required to cleave DNA completely. Moreover, the effect of $CuCl_2$ addition to **4** and **6** was shown to be concentration-dependent. Low concentrations of these compounds demonstrated cleaving activity that was quenched by addition of the metal salt, while at higher, micromolar concentrations activity was increased in the presence of the metal.

Photonucleases have proven to be excellent tools for use in photofootprinting experiments aimed at elucidating DNA–drug and DNA–protein interactions. Furthermore, the analyses of acridine, anthracene and other model chromophores that photocleave DNA under physiologically relevant conditions can be used to provide valuable insights leading to the design of second generation compounds ideal for use in PDT and other *in vivo* applications. Thus, using the results reported here, our future experiments will be aimed at synthesizing new, more efficient DNA photocleaving agents.

Experimental

General information

All reactions were carried out under an argon atmosphere unless stated otherwise. Solvents were freshly distilled prior to use when anhydrous conditions were required (THF from sodium–benzophenone and acetonitrile from CaH_2). Chemicals were purchased from commercial suppliers and were used without further purification. TLC was performed on precoated aluminium silica gel plates (Merck or Macherey-Nagel 60F254 0.25 mm). TLC chromatograms were visualised using UV radiation at 254 or 366 nm. Flash column chromatography was performed using 0.1% Ca enriched silica gel (230–400 mesh) from Merck or Fluka. Melting points were determined in an Electrothermal digital IA9100 apparatus. Infrared spectra were taken on an FT-IR Perkin-Elmer 1725X spectrophotometer. All ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury V-X-300 or Varian UNITY-500 Plus spectrometer. Chemical shifts are reported in ppm using the residual peaks of either chloroform (δ 7.26 and 77.0 ppm) or benzene (δ 7.15 and 128.0 ppm) as an internal reference. Coupling constants (J values) are given in Hz. Carbon and proton assignments were based on HSQC and HMBC experiments. CI mass spectra were generated on a Hewlett-Packard HP-5988a spectrometer at 70 eV, while FAB mass spectra were recorded on a V.G. Autoexpec spectrometer with 3-nitrobenzyl alcohol as matrix. APCI mass spectra were done on an Automass Multi GC/API/MS Finnigan spectrometer. Elemental analyses were performed with a Heraeus CHN analyzer. UV-visible spectra were recorded using a Shimadzu UV-1601 spectrophotometer. Sodium hydride was used as a dispersion in mineral oil. The compound 9-bromomethylacridine was synthesized by modifying a procedure reported in the literature,[12] while 9-bromomethylanthracene,[13] 1,10-phenanthroline-2,9-dicarboxylic acid and 9-bis(hydroxymethyl)-1,10-phenanthroline[14] were prepared according to reported procedures. Stock solutions of compounds were made in pure DMF and stored in the dark at −20 °C until used. In photocleavage reactions, DMF content was 10% (v/v).

Distilled, deionized water was utilized in the preparation of all buffers and aqueous reactions. The metal salt $CuCl_2$ was purchased from the Aldrich Chemical Company (purity >99%). Copper coordination complexes were prepared *in situ* by the addition of freshly made $CuCl_2$ solutions to a diluted ligand stock solution. Transformation of *E. coli* competent cells (Stratagene, XL-1 blue) with pUC19 plasmid DNA (Sigma) and growth of bacterial cultures in Lauria–Bertani broth were performed using standard laboratory protocols.[22] The plasmid DNA was purified with a Qiagen Plasmid Mega Kit. DNA photocleavage reactions were carried out in an aerobically ventilated Rayonet Photochemical Reactor fitted with 13 RPR-3500 Å lamps (The Southern New England Ultraviolet Company).

Synthesis of 9-bromomethylacridine

A mixture of commercially available 9-methylacridine (1.50 g, 7.8 mmol), *N*-bromosuccinimide (1.50 g, 8.3 mmol) and benzoyl peroxide (130 mg, 0.54 mmol) was heated in anhydrous chloroform at reflux (40 mL) for 5 h, after which the solvent was removed under a reduced pressure. The product was then purified by flash column chromatography in silica gel using hexane–ethyl acetate (4 : 1) as eluent to give 9-bromomethylacridine (2.09 g, 99%) as a yellow solid, mp 166–168 °C decomp. [lit.[12] mp 169–170 °C decomp.].

***N*,*N*′-Dimethyl-*N*,*N*′-bis[2-(methylamino)-ethyl]-[1,10-phenanthroline]-2,9-dicarboxamide (2).** A solution of 1,1′-carbonyldiimidazole (1.47 g, 9 mmol) in dry tetrahydrofuran (40 mL) was added dropwise to a suspension of the 1,10-phenanthroline-2,9-dicarboxylic acid (604 mg, 2.1 mmol) in tetrahydrofuran (260 mL) under an argon atmosphere. The mixture was heated at 60 °C for 18 h, after which it was cooled and *N*,*N*′-dimethylethylenediamine (2.5 mL, 22.8 mmol) was added. The reaction was stirred at rt for 24 h and the solvent removed under a reduced pressure. The oily product was then dissolved in chloroform (30 mL), washed with brine (3 × 30 mL) and then with water (2 × 15 mL). The organic phase was dried with $MgSO_4$ and concentrated, to give a yellow oil (784 mg, 75%), which was used in the next step without further purification. IR ($CHBr_3$/NaCl), ν_{max}/cm^{-1} 3466, 2935, 1690 and 1630; δ_H (500 MHz; $CDCl_3$) 8.34 (m, 4-H, 7-H *EZ* + *EE* + *ZZ*), 8.11 (d, *J* 8.3, 3-H, 8-H *EZ*), 8.05 (d, *J* 8.3, 3-H, 8-H *EE*), 8.00 (d, *J* 8.3, 3-H, 8-H *ZZ*), 7.97 (d, *J* 8.3, 3-H, 8-H *EZ*), 7.85 (m, 5-H, 6-H *EZ* + *EE* + *ZZ*), 3.90 (t, *J* 6.5, α-CH_2 *EZ*), 3.78 (t, *J* 6.5, α-CH_2 *EE*), 3.76 (t, *J* 6.5, α-CH_2 *EZ*), 3.71 (t, *J* 6.5, α-CH_2 *ZZ*), 3.37 (s, CH_3NCO *EZ*), 3.27 (s, CH_3NCO *EZ*), 3.22 (s, CH_3NCO *EE*), 3.21 (s, CH_3NCO *ZZ*), 2.97 (m, β-CH_2 *EZ* + *EE*), 2.91 (m, β-CH_2 *EZ* + *ZZ*), 2.52 (s, CH_3N *EZ*), 2.51 (s, CH_3N *EZ*), 2.27 (s, CH_3N *EE*), 2.25 (s, CH_3N *ZZ*); [*EE*/*EZ*/*ZZ* (NMR) = 19/42/39]; δ_C (125 MHz, $CDCl_3$) 169.0, 168.8 and 168.5 (C=O), 154.3, 154.1 and 153.8 (C-2, C-9), 144.5, 144.3, 144.1 and 144.0 (C-10a, C-10b), 137.1 and 137.0 (C-4, C-7), 129.1, 129.0 and 129.0 (C-4a, C-6a), 127.3 and 127.2 (C-5, C-6), 123.9 (C-3, C-8 *EZ*), 123.6 (C-3, C-8 *EE*), 123.4 (C-3, C-8 *ZZ*), 123.1 (C-3, C-8 *EZ*), 50.9 (CH_2-α *EZ*), 50.9 (CH_2-α *ZZ*), 50.0 (CH_2-β *ZZ*), 49.4 (CH_2-β *EZ*), 49.1 (CH_2-β *EZ* + *EE*), 48.4 (CH_2-α *EE*), 48.2 (CH_2-α *EZ* + *EE*), 37.9 (CH_3NCO *EE*), 37.8 (CH_3NCO *EZ*), 36.4, 36.3 and 36.2 (CH_3N), 34.4 (CH_3NCO *EZ*), 34.0 (CH_3NCO *ZZ*); *m*/*z* (CI) 364 ($M^+ - 44$, 11), 320 (10), 294 (7), 249 (13), 180 (100).

***N*,*N*′-Bis[2-[(9-acridinylmethyl)methylamino]-ethyl]-*N*,*N*′-dimethyl-[1,10-phenanthroline]-2,9-dicarboxamide (3).** Solid K_2CO_3 (220 mg, 1.6 mmol) and a solution of the 9-bromomethylacridine (200 mg, 0.74 mmol) in dry acetonitrile (50 mL) were added under argon to a solution of the amide **2** (145 mg, 0.36 mmol). The reaction was stirred at rt for 16 h, after which the solvent was removed under a reduced pressure. The crude product thus obtained was purified by flash column chromatography using 0.1% Ca enriched silica gel and ethyl acetate as eluent to give the product (185 mg, 66%) as an oil that turns into a yellow foam when dried, mp (AcOEt) 64–65 °C. (Found C, 75.61; H, 5.69; N, 14.56. $C_{50}H_{46}N_8O_2$ requires C, 75.93; H, 5.86; N, 14.17%); IR (KBr), ν_{max}/cm^{-1} 2930, 2852, 1632 and 1601; δ_H (500 MHz; C_6D_6) 8.47–8.40 (m, 4-H, 5-H Acr), 8.37–8.29 (m, 1-H, 8-H Acr), 7.72 (d, *J* 8.3, 3-H, 8-H *EZ*), 7.61 (d, *J* 8.3, 3-H, 8-H *EZ*), 7.58 (d, *J* 8.3, 3-H, 8-H *EE*), 7.55 (d, *J* 8.3, 3-H, 8-H *ZZ*), 7.41–7.27 (m, 2-H, 7-H, 3-H, 6-H Acr and 4-H, 7-H), 7.23–7.20 (m, 2-H, 7-H Acr), 7.04 (s, 5-H, 6-H), 4.17 (s, CH_2Acr *EZ*), 4.11 (s, CH_2Acr *EE*), 4.10 (s, CH_2Acr *ZZ*), 4.06 (s, CH_2Acr *EZ*), 3.52 (t, *J* 6.3, α-CH_2 *EE*), 3.47 (t, *J*

6.3, α-CH_2 *EZ*), 3.37 (t, *J* 6.3, α-CH_2 *EZ*), 3.30 (t, *J* 6.3, α-CH_2 *ZZ*), 2.83 (s, CH_3NCO *EE*), 2.82 (s, CH_3NCO *EZ*), 2.80 (t, *J* 6.3, β-CH_2 *EZ*), 2.75 (t, *J* 6.3, β-CH_2 *ZZ*), 2.72 (s, CH_3NCO *ZZ*), 2.63 (t, *J* 6.3, β-CH_2 *EE*), 2.60 (s, CH_3NCO *EZ*), 2.55 (t, *J* 6.3, β-CH_2 *EZ*), 2.07 (s, CH_3N *EE*), 2.03 (s, CH_3N *EZ*), 1.58 (s, CH_3N *EZ*), 1.51 (s, CH_3N *ZZ*); [*EE*/*EZ*/*ZZ* (NMR) = 15/41/44]; δ_C (125 MHz, C_6D_6) 169.1 and 168.7 (C=O), 155.3 (C-2, C-9), 149.7 and 149.4 (C-4a, C-10a Acr), 144.3 (C-10a, C-10b), 141.2 and 141.1 (C-9 Acr), 136.50 (C-4, C-7), 131.1 (C-4, C-5 Acr), 129.6, 129.5 and 129.2 (C-4a, C-6a and C-3, C-6 Acr), 126.8 and 126.4 (C-5, C-6), 126.0, 125.9 and 125.7 (C-2, C-7 Acr), 125.3 (C-1, C-8 Acr), 123.8, 123.7, 123.4 and 123.1 (C-3, C-8 and C-8a, C-9a Acr), 57.4 (CH_2-β *EZ*), 57.3 (CH_2-β *ZZ*), 55.3 (CH_2-β *EE*), 54.9 (CH_2-β *EZ*), 54.3 (CH_2Acr *EZ*), 54.2 (CH_2Acr *ZZ*), 53.7 (CH_2Acr *EE* + *EZ*), 49.2 (CH_2-α *EZ* + *ZZ*), 46.6 (CH_2-α *EE*), 45.9 (CH_2-α *EZ*), 41.9 (CH_3N *EE* + *EZ*), 41.2 (CH_3N *EZ* + *ZZ*), 37.7 (CH_3NCO *EE*), 37.3 (CH_3NCO *EZ*), 34.9 (CH_3NCO *EZ*), 34.5 (CH_3NCO *ZZ*); *m*/*z* (FAB) 791 $[M + H]^+$ ($C_{50}H_{46}N_8O_2$ requires 790).

***N*,*N*′-Bis[2-[(9-anthracenylmethyl)methylamino]-ethyl]-*N*,*N*′-dimethyl-[1,10-phenanthroline]-2,9-dicarboxamide (4).** To a solution of amide **2** (106 mg, 0.26 mmol) in acetonitrile (10 mL) under argon, K_2CO_3 (170 mg, 1.23 mmol) was added, followed by dropwise addition of 9-bromomethylanthracene (145 mg, 0.53 mmol) in dry acetonitrile (25 mL). The reaction was stirred at rt for 16 h, after which the solvent was removed under a reduced pressure. The crude product was purified by flash column chromatography using 0.1% Ca enriched silica gel and ethyl acetate as eluent to give the product (107 mg, 52%) as a yellow foam, mp (AcOEt) 104 °C. (Found C, 79.29; H, 6.09; N, 10.51. $C_{52}H_{48}N_6O_2$ requires C, 79.16; H, 6.13; N, 10.65%); IR (KBr), ν_{max}/cm^{-1} 3048, 2934, 2846 and 1632; δ_H (500 MHz; C_6D_6) 8.48 (m, 1-H, 8-H Anthr *EE* + *EZ* + *ZZ*), 8.15 and 8.12 (s, 10-H Anthr *EE* + *EZ* + *ZZ*), 7.78 (m, 4-H, 5-H Anthr and 3-H, 8-H), 7.66 (d, *J* 8.3, 3-H, 8-H), 7.54 (d, *J* 8.3, 4-H, 7-H), 7.43 (m, 2-H, 7-H Anthr), 7.38 (d, *J* 8.3, 4-H, 7-H), 7.32 (d, *J* 8.3, 4-H, 7-H), 7.26 (m, 3-H, 6-H Anthr), 7.09, 7.07 and 7.02 (s, 5-H, 6-H), 4.29 (s, CH_2 Anthr *EE*), 4.28 (s, CH_2 Anthr *EZ*), 4.26 (s, CH_2 Anthr *EZ*), 4.24 (s, CH_2 Anthr *ZZ*), 3.58 and 3.56 (2t overlapped, *J* 6.3, α-CH_2 *EE* + *EZ*), 3.52 (t, *J* 6.3, α-CH_2 *EZ*), 3.44 (t, *J* 6.1, α-CH_2 *ZZ*), 2.84 (s, CH_3NCO *EE*), 2.81 (s, CH_3NCO *EZ*), 2.80 (m, β-CH_2), 2.78 (s, CH_3NCO *ZZ*), 2.71 (m, β-CH_2), 2.71 (s, CH_3NCO *EZ*), 2.21 (s, CH_3N *EE*), 2.17 (s, CH_3N *EZ*), 1.68 (s, CH_3N *EZ*), 1.64 (s, CH_3N *ZZ*); [*EE*/*EZ*/*ZZ* (NMR) = 11/45/44]; δ_C (125 MHz, C_6D_6) 169.2, 168.8, 168.2 and 167.8 (C=O), 155.5, 155.3 and 155.0 (C-2, C-9), 144.8 and 144.4 (C-10a, C-10b), 136.4 and 136.3 (C-4, C-7), 131.9, 131.8 and 131.7 (C-4a, C-10a, C-8a, C-9a Anthr), 129.2 and 129.1 (C-4, C-5 Anthr), 128.8 and 128.7 (C-4a, C-6a), 128.1 (C-9 Anthr solvent overlap), 127.5 (C-10 Anthr), 127.1, 126.9 and 126.8 (C-5, C-6), 126.0 and 125.9 (C-2, C-7 Anthr), 125.8 and 125.7 (C-1, C-8 Anthr), 125.2 and 125.1 (C-3, C-6 Anthr), 123.9 (C-3, C-8 *EZ* + *EE*), 123.7 (C-3, C-8 *ZZ*), 123.3 (C-3, C-8 *EZ*), 56.9 and 56.8 (CH_2-β), 54.8, 54.7, 54.5 and 54.4 (CH_2-β + CH_2Anthr), 49.1 (CH_2-α *EZ* + *ZZ*), 46.5 (CH_2-α *EE*), 46.0 (CH_2-α *EZ*), 41.8 (CH_3N *EE*), 41.6 (CH_3N *EZ*), 41.0 (CH_3N *EZ*), 40.9 (CH_3N *ZZ*), 37.4 (CH_3NCO *EE*), 37.2 (CH_3NCO *EZ*), 34.5 (CH_3NCO *EZ*), 34.3 (CH_3NCO *ZZ*); *m*/*z* (FAB) 789 $[M + H]^+$ ($C_{52}H_{48}N_6O_2$ requires 788).

2,9-Bis[(9-anthracenylmethoxy)methyl]-1,10-phenanthroline (6). Sodium hydride (90 mg, 2.25 mmol; 60% dispersion in mineral oil) was added to a suspension of 2,9-bis(hydroxymethyl)-1,10-phenanthroline (240 mg, 1 mmol) in anhydrous dimethylformamide (30 mL) and stirred for 30 min at rt. Then, a solution of 9-bromomethylanthracene (600 mg, 2.2 mmol) in dimethylformamide (15 mL) was added dropwise. The reaction was stirred for 24 h, after which additional amounts of NaH (60% dispersion in mineral oil; 14 mg, 0.35 mmol) and 9-bromomethylanthracene (110 mg, 0.4 mmol) were added. The reaction mixture was stirred for another 48 h and then more 9-bromomethylanthracene (100 mg, 0.37 mmol) was added. After 24 h of stirring, a last addition of NaH (13 mg, 0.33 mmol) and 9-bromomethylanthracene (110 mg, 0.4 mmol) was performed and the reaction was stirred at rt for another 24 h. After quenching with water, the solvent was removed under a high vacuum. The crude product thus obtained was purified by silica gel flash column chromatography using hexane–ethyl acetate (1 : 1, v/v) as the initial eluent. The ethyl acetate content was gradually increasing up to 100% to give pure product (124 mg, 20%) as a yellow–brown solid, mp (AcOEt) 109 °C. (Found C, 85.29; H, 5.11; N, 4.42. $C_{44}H_{32}N_2O_2$ requires C, 85.14; H, 5.20; N, 4.51%); IR (KBr), ν_{max}/cm^{-1} 3050, 2922, 2853, 1724, 1622, 1591, 1524 and 1446; δ_H (300 MHz; $CDCl_3$) 8.67 (2H, s, 10-H Anthr), 8.43 (4H, d, *J* 8.6, 1-H, 8-H Anthr), 8.17 (2H, d, *J* 8.4, 4-H, 7-H), 8.00 (4H, d, $J_{3\text{-}4}$ 8.6, 4-H, 5-H Anthr), 7.82 (2H, d, *J* 8.4, 3-H, 8-H), 7.71 (2H, s, 5-H, 6-H), 7.56–7.41 (8H, m, H-2, H-7, H-3, H-6 Anthr), 5.96 (4H, s, CH_2Anthr), 5.25 (4H, s, CH_2Phen); δ_C (75 MHz, $CDCl_3$) 159.2 (C-2, C-9), 144.3 (C-10a, C-10b), 136.6 (C-4, C-7), 131.1 (C-4a, C-10a Anthr), 130.7 (C-8a, C-9a Anthr), 128.7 (C-4, C-5 Anthr), 128.3 (C-10 Anthr), 128.1 (C-9 Anthr), 127.7 (C-4a, C-6a), 126.0 (C-2, C-7 Anthr), 125.8 (C-5, C-6), 124.7 (C-3, C-6 Anthr), 124.0 (C-1, C-8 Anthr), 121.0 (C-3, C-8), 73.9 (CH_2Phen), 65.2 (CH_2Anthr); *m*/*z* (FAB) 621 $[M + H]^+$ ($C_{44}H_{32}N_2O_2$ requires 620).

2,9-Bis[(phenylmethoxy)methyl]-1,10-phenanthroline (7). To a suspension of crushed KOH (450 mg, 8.0 mmol) in anhydrous dimethylsulfoxide (2 mL), a solution of the 2,9-bis(hydroxymethyl)-1,10-phenanthroline (200 mg, 0.83 mmol) in dimethylsulfoxide (10 mL) was added dropwise. The mixture was stirred at rt for 30 min, after which benzyl bromide (568 mg, 0.4 mL, 3.32 mmol) was slowly added. The reaction was stirred at rt for 1.5 h and quenched with water (2 mL). The solvent was evaporated under a vacuum and the crude product was dissolved in dichloromethane (20 mL) and washed with brine (2 × 10 mL). The organic phase was dried with $MgSO_4$, concentrated and purified by flash column chromatography in silica gel using hexane–ethyl acetate (1 : 1) as eluent to give the desired product (82 mg, 23%) as a yellow solid, mp (AcOEt) 115 °C. δ_H (300 MHz; $CDCl_3$) 8.25 (2H, d, *J* 8.4, 4-H, 7-H), 7.93 (2H, d, *J* 8.4, 3-H, 8-H), 7.75 (2H, s, 5-H, 6-H), 7.47–7.31 (10H, m, Ph), 5.14 (4H, s, CH_2Ph or CH_2Phen), 4.74 (4H, s, CH_2Ph or CH_2Phen); δ_C (75 MHz, $CDCl_3$) 159.7 (C-2, C-9), 145.1 (C-10a, C-10b), 138.1 (C-4_{ipso} Ph), 136.7 (C-4, C-7), 128.3 (C_o Ph), 128.0 (C-4a, C-6a), 129.8 (C_m Ph), 127.7 (C_p Ph), 126.0 (C-5, C-6), 120.8 (C-3, C-8), 73.8 (CH_2), 73.1 (CH_2); *m*/*z* (APCI) 421 (M^+ + H) ($C_{28}H_{24}N_2O_2$ requires 420).

2,9-Bis[(2-naphthalenylmethoxy)methyl]-1,10-phenanthroline (8). To a suspension of NaH (80% dispersion in mineral oil; 66 mg, 2.2 mmol) in anhydrous dimethylformamide (5 mL), a solution of 2,9-bis(hydroxymethyl)-1,10-phenanthroline (240 mg, 1 mmol) in dimethylformamide (17 mL) was added dropwise. The mixture was stirred at rt for 30 min, after which a solution of 2-bromomethylnaphthalene (460 mg, 2 mmol) in dimethylformamide (3 mL) was slowly added. The reaction was stirred at rt for 24 h and quenched with water (2 mL). The solvent was evaporated *in vacuo* and the crude product thus obtained was purified by flash column chromatography in silica gel using hexane–ethyl acetate (1 : 1, v/v) as eluent to give the desired product (110 mg, 21%) as a white solid mp, (AcOEt) 109–111 °C. (Found C, 82.90; H, 5.36; N, 5.56. $C_{36}H_{28}N_2O_2$ requires C, 83.05; H, 5.42; N, 5.38%); IR (KBr), ν_{max}/cm^{-1} 3051, 2888, 1593, 1507 and 1450; δ_H (300 MHz; $CDCl_3$) 8.28 (2H, d, *J* 8.4, 4-H, 7-H), 7.97 (2H, d, *J* 8.4, 3-H, 6-H), 7.90 (2H, br s, 1-H Naph), 7.89–7.83 (6H, m, 4-H, 5-H, 8-H Naph), 7.78 (2H, s, 5-H, 6-H), 7.57 (2H, dd, *J* 8.4 and 1.8, 3-H Naph), 7.48 (4H, m, 6-H, 7-H Naph), 5.20 (4H, s, CH_2), 4.91 (4H, s, CH_2); δ_C (75 MHz, $CDCl_3$) 159.7 (C-2, C-9), 145.1 (C-10a, C-10b),

136.8 (C-4, C-7), 135.5 (C_{ipso} Naph), 133.3, 133.0 (C-4a, C-8a Naph), 128.2 (C-4 Naph), 128.1 (C-4a, C-6a), 127.9 (C-5, C-8 Naph), 126.6 (C-1 Naph), 126.1 (C-5, C-6), 126.1, 125.9 (C-6, C-7 Naph), 125.8 (C-3 Naph), 120.9 (C-3, C-8), 74.1 (CH_2), 73.1 (CH_2); *m/z* (CI) 521 (M^+ + 1, 93) ($C_{36}H_{28}N_2O_2$ requires 520), 381 (66), 377 (60), 225 (30), 171 (41), 143 (100).

Photocleavage of supercoiled plasmid DNA

A total of 38 μM bp pUC19 plasmid DNA in 20 mM sodium phosphate buffer pH 7.0 or in 20 mM sodium phosphate buffer pH 7.0, 0.25 μM to 50 μM of compound and/or 0.25 μM to 50 μM $CuCl_2$ was irradiated at 350 nm and 22 °C, or kept in the dark. Aliquots of 20 μL were removed at specific time intervals. After the addition of 3 μL of loading buffer (15% (w/v) Ficoll, 0.025% (w/v) bromophenol blue), cleavage products were electrophoresed at 4 V/cm on a 1.0% non-denaturing agarose gel stained with ethidium bromide (0.5 μg mL^{-1}). To determine the percent conversion of supercoiled plasmid DNA to nicked and linear forms, the gel was visualized on a transilluminator set at 305 nm, photographed and then quantitated using ImageQuant Mac v. 1.2 software (Amersham Biosciences).

Colorimetric detection of copper(I)

A series of 500 μL reactions containing 38 μM bp pUC19 plasmid DNA, 20 mM sodium phosphate buffer pH 7.0 and 35 μM of compound was prepared in the absence and presence of 35 μM $CuCl_2$. The samples were irradiated at 350 nm in the presence of 70 μM bathocuproinedisulfonic acid disodium salt hydrate, while a parallel set of reactions was kept in the dark. After 25 min, the solutions were visually examined for colour change, placed in 500 μL quartz cuvettes and monitored between 240 nm and 600 nm for evidence of Cu^{1+}–bathocuproine complex formation.

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