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Synthesis and DNA photocleavage by a pyridine-linked bis-acridine chromophore in the presence of copper(II): Ionic strength effects

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ABSTRACT

We report the synthesis of photonuclease **3** consisting of two acridine rings joined by a 2,6-bis(aminomethyl)pyridine copper-binding linker. In reactions containing micromolar concentrations of **3**, irradiation at 419 nm produces efficient, copper(II)-dependent cleavage of plasmid DNA in the presence of the high concentrations of salt that exist in the cell nucleus (150 mM NaCl and 260 mM KCl). The DNA interactions of **3** are compared to an analogous bis-acridine (**4**) containing a more flexible 2,6-bis{[(methoxycarbonylamino)-ethyl]methylaminomethyl}pyridine unit.

In recent years, there has been increasing interest in the development of copper(II) complexes that cleave DNA.[1] These reagents are important as potential chemotherapeutic drugs for a number of reasons.[1a,c] Amounts of tissue, and/or serum copper show a significant elevation in cancer patients compared to healthy control subjects.[1a] Other studies demonstrate that tissue and/or serum samples taken from cancer patients have significantly lower levels of zinc, iron, and selenium and 2- to 3-fold higher levels of copper compared to normal control samples.[1a] In living systems, copper is closely associated with nucleic acids and chromosomes.[1a,2] While the majority of copper is tightly bound by these and other macromolecules,[3] the kinetically labile nature of this metal enables it to be mobilized by external metal chelating agents.[1a,3a] Thus, a chemotherapeutic agent that binds to copper has the potential to be of great utility in living systems.

As early as 1979, Sigman et al. showed that mixtures of 1, 10-phenanthroline and copper(II) sulfate could oxidatively cleave DNA in the presence of the external reducing agent 3-mercaptopropionic acid.[4] Hecht and Oppenheimer generated DNA strand scission by adding dithiothreitol to solutions containing the anticancer antibiotic bleomycin A_2 and copper(II) acetate.[5] Notwithstanding, the addition of an external reducing agent to copper is not necessarily required for efficient oxidative cleavage of DNA. A good alternative is to use light to trigger nuclease activity.[6] An early example is the copper(II) complex of the anticancer alkaloid camptothecin: oxidative DNA cleavage was achieved upon irradiation of the drug/DNA complex at 365 nm.[6h]

In a previous paper, we reported the synthesis of bis-acridine **4**, containing a flexible copper-binding 2,6-bis{[(methoxycarbonylamino)-ethyl]methylaminomethyl}pyridine linking unit.[6d] Oxidative photocleavage of plasmid DNA was markedly enhanced when 50 μM of **4** was irradiated in the presence of 1 mol equiv of copper(II) chloride (419 nm, 22 °C, pH 7.0).[6d] In the present study, we describe the synthesis and DNA interactions of pyridine-linked bis-acridine photonuclease **3**, equipped with a more compact 2,6-bis(aminomethyl)pyridine unit. By comparison to **4**, herein we show that bis-acridine **3** produces superior levels of photocleavage (50–2 μM dye, 419 nm, 22 °C, pH 7.0). The addition of 1 mol equiv of copper(II) chloride to 20–2 μM of **3** inhibits DNA photocleavage under the standard, low salt conditions used in many in vitro DNA reactions (0 mM NaCl).[5,6b,d–h] Alternatively, the addition of 1 mol equiv of Cu(II) to 30–10 μM of **3** enhances photocleavage under the conditions of high ionic strength that exist in the cell nucleus (~150 mM Na(I) and 260 mM K(I)).[7]

Synthesis of bis-acridine **3** was carried out by reaction of (6-amino-3-acridinyl)carbamic acid *tert*-butyl ester (**1**),[8] as a sodium salt, with 2,6-bis(bromomethyl)pyridine in DMF to afford the bis-acridine **2** with good yield (Scheme 1).[9] The *tert*-butoxycarbonyl

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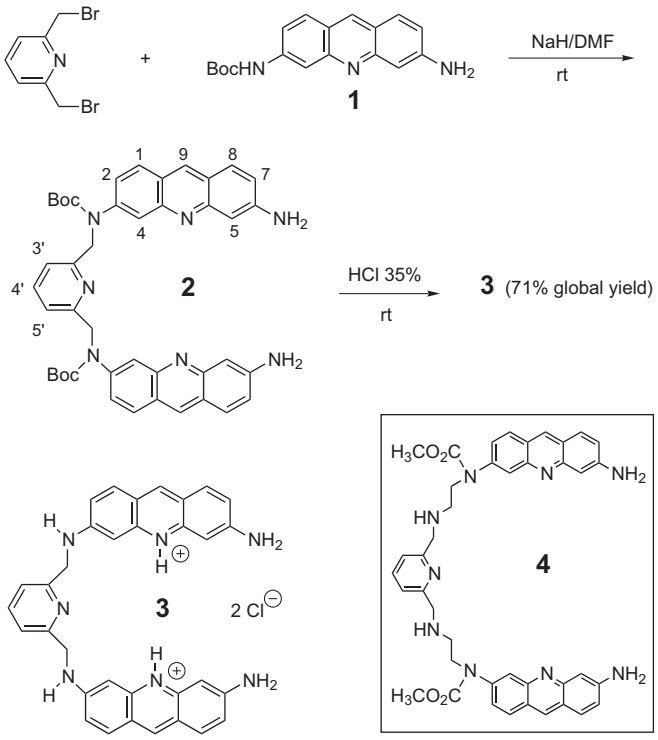

Scheme 1. Synthetic route of the pyridine-linked bis-acridine dye **3**. Pyridine-linked bis-acridine dye **4** is inset.

protecting groups were then easily removed by treating **2** with hydrochloric acid, affording compound **3** (95%) as a dihydrochloride derivative. The structures of **2** and **3** were confirmed by ^{1}H and ^{13}C NMR, 2D NMR, mass spectra, and microanalytical data.[9] Bis-acridine **4** was prepared using a previously reported procedure.[6d]

In photocleavage experiments, 50–2 μM concentrations of a bis-acridine (**3** or **4**) were equilibrated with 38 μM bp of pUC19 plasmid DNA and 10 mM sodium phosphate buffer pH 7.0, in the absence and presence of 1 mol equiv of $CuCl_2$, 150 mM of NaCl, and/or 260 mM of KCl (Fig. 1). The samples were irradiated for 50 min at 419 nm and 22 °C and then electrophoresed on a 1.0% or a 1.5% non-denaturing agarose gel. The conversion of uncleaved supercoiled plasmid DNA (S) to its nicked (N) and linear (L) forms was visualized by ethidium bromide staining. Minimal levels of DNA cleavage were observed in parallel control reactions in which 38 μM bp of pUC19 plasmid, 50 μM of $CuCl_2$, and/or 50 μM of bis-acridine **3** or **4** were kept in the dark for 60 min (no hν: Fig. 1, Lane C3; Fig. S1, Supplementary data; for compound **4**, Fig. 4, Lanes 11 and 12, in Fernández et al., 2007).[6d]

The bis-acridine reactions in 10 mM sodium phosphate buffer pH 7.0 (no NaCl and no KCl) were studied first. From an examination of the gels in Figures 1A and 1E, it is evident that, when copper(II) is absent, compound **3** produces significantly more DNA photocleavage than compound **4** (Fig. 1A, Lanes 1–6 vs Fig. 1E, Lanes 1–6). Notably, irradiation of concentrations of **3** as low as 10 μM results in the near-complete conversion of the plasmid DNA to its nicked and linear forms (Fig. 1A, Lane 4). Upon the addition of 1 mol equiv of copper(II) chloride, different trends were observed: DNA photocleavage by **3** was decreased (20–2 μM concentrations of dye; Fig. 1A, Lanes 9–12 vs Lanes 3–6), while photocleavage by **4** was enhanced (50–20 μM concentrations of dye; Fig. 1E, Lanes 7–9 vs Lanes 1–3). Notwithstanding, in the presence of Cu(II), **3** was still the more reactive compound (Fig. 1A, Lanes 7–12 vs Fig. 1E, Lanes 7–12): a total of 20 μM of **3** and

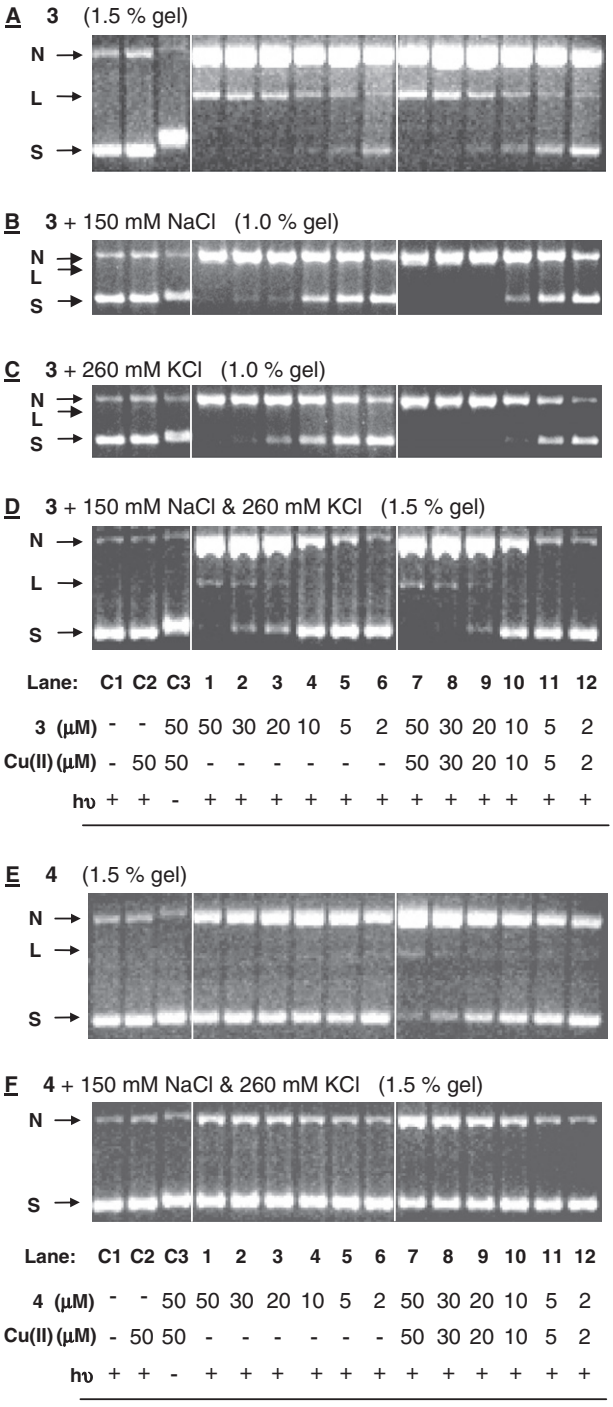

Figure 1. Photographs of 1.0% and 1.5% non-denaturing agarose gels showing photocleavage of pUC19 plasmid DNA by bis-acridine **3** with (A) no salt, (B) 150 mM NaCl, (C) 260 mM KCl, (D) 150 mM NaCl and 260 mM KCl; and by bis-acridine **4** with (E) no salt, (F) 150 mM NaCl and 260 mM KCl. Samples contained 10 mM sodium phosphate buffer pH 7.0, 38 μM bp DNA, and 50–2 μM concentrations of **3** or **4** without (Lanes **1**–**6**) and with (Lanes **7**–**12**) 50–2 μM of $CuCl_2$ (total volume 40 μL). Prior to photocleavage, the reactions were pre-equilibrated for 1 h in the dark at 22 °C. The samples in Lanes **C1**, **C2**, and **1**–**12** were then irradiated for 50 min at 22 °C in 1.7 mL microcentrifuge tubes in an aerobically ventilated Rayonet Photochemical Reactor fitted with nine RPR-4190 Å lamps.

50 μM of **4** were needed for near-complete photo-degradation of DNA, respectively (Fig. 1A, Lane 9 vs Fig. 1E, Lane 7). In order to account for the opposing trends produced by copper(II) chloride, we conducted cleavage inhibition experiments in which 38 μM bp of

pUC19 plasmid DNA was equilibrated with the singlet oxygen ($^1O_2$) scavenger sodium azide or with the hydroxyl radical (·OH) scavenger sodium benzoate. The scavengers were added prior to photocleavage by 10 μM of **3** or by 50 μM of **4** in the presence and absence of 1 mol equiv of $CuCl_2$ (no NaCl and no KCl). When compared to parallel photocleavage reactions run in the absence of the scavengers, sodium azide and sodium benzoate reduced DNA photocleavage by **3** and **4** to a significant degree. The percent inhibition data in Table S1 (Supplementary data) show that, depending on the reaction conditions, the scavengers either inhibited the formation of linear DNA, so that more nicked DNA was produced in the photocleavage reaction, or reduced the amounts of the linear and/or nicked plasmid forms. These results indicate that hydroxyl radicals and singlet oxygen contribute to DNA photocleavage by **3** and by **4**. In the case of compound **4**, sodium benzoate produces slightly more inhibition than sodium azide, with the percent inhibition values of both scavengers being higher when 1 mol equiv of Cu(II) is present in the photocleavage reaction. This result indicates that the addition of Cu(II) increases the production of hydroxyl radicals and singlet oxygen and is consistent with our observation that bis-acridine **4** generates more DNA photocleavage in the presence of 1 mol equiv of Cu(II). In the case of compound **3**, sodium azide causes more inhibition than sodium benzoate, with the percent inhibition value of sodium azide being significantly higher when Cu(II) is omitted from the photocleavage reaction. This result indicates that the addition of Cu(II) decreases the production of singlet oxygen and is consistent with our finding that bis-acridine **3** generates less DNA photocleavage in the presence of 1 mol equiv of Cu(II).

The bis-acridine photocleavage reactions containing 150 mM NaCl and 260 mM KCl were studied next (Figs. 1D and 1F). These NaCl and KCl concentrations were selected in order to simulate the conditions of high ionic strength that exist in the cell nucleus where genomic DNA is contained.[7] Sodium(I), potassium(I), and other cations can induce the dissociation of ligands from DNA in two ways. By decreasing phosphate–phosphate repulsion between DNA strands, counter cations decrease minor groove width, decrease effective helical diameter, and in the case of supercoiled DNA, increase negative writhe.[10] Cations can also directly compete with positively charged ligands for DNA binding sites.[11] As expected, the gel images show that the combination of 150 mM NaCl and 260 mM KCl lowered overall photocleavage yields, both in the presence and absence of Cu(II) (Fig. 1D vs 1A for **3**; Fig. 1F vs 1E for **4**). It was also evident that compound **3** continued to produce more DNA photocleavage than **4** (Fig. 1D vs 1F). Considered next were the effects of adding 1 mol equiv of copper(II) chloride to the bis-acridine reactions. In the presence of 150 mM NaCl and 260 mM KCl, levels of DNA photocleavage by **3** and **4** were both increased (30–10 μM of **3**, Fig. 1D, Lanes 8–10 vs Lanes 2–4; 50–10 μM of **4**, Fig. 1F, Lanes 7–10 vs Lanes 1–4). However, as discussed in the previous section, when NaCl and KCl were omitted from photocleavage reactions, copper(II) produced an enhancement in cleavage only for bis-acridine **4**. In the case of bis-acridine **3**, cleavage was reduced.

In order to better document the differential effects of Cu(II) as a function of ionic strength, the individual salts, either 150 mM of NaCl (Fig. 1B) or 260 mM of KCl (Fig. 1C) were tested against **3**. It was found that the addition of 1 mol equiv of Cu(II) increased DNA photocleavage by **3** under both sets of reaction conditions (Fig. 1B, Lanes 8–10 vs Lanes 2–4 for 150 mM NaCl; Fig. 1C, Lanes 9 and 10 vs Lanes 3 and 4 for 260 mM KCl). An overall comparison could then be made of the bis-acridine **3** photocleavage reactions run in the presence of copper (Figs. 1A–D, Lanes 7–12) to those run in the absence of copper (Figs. 1A–D, Lanes 1–6). Four sets of reaction conditions were considered: no salt (Fig. 1A); 150 mM NaCl (Fig. 1B); 260 mM KCl (Fig. 1C); 150 mM NaCl and 260 mM KCl (Fig. 1D). The addition of 1 mol equiv of Cu(II) was found to alter DNA photocleavage yields differentially, according to salt composition: 260 mM KCl (Fig. 1C; increased cleavage in Lanes 9 and 10 vs Lanes 3 and 4) >150 mM NaCl and 260 mM KCl (Fig. 1D; increased cleavage in Lanes 7–10 vs Lanes 1–4) >150 mM NaCl (Fig. 1B; increased cleavage in Lanes 8–10 vs Lanes 2–4) >>>> no salt (Fig. 1A; decreased cleavage in Lanes 9–12 vs Lanes 3–6). In the presence of 260 mM of KCl, concentrations of **3** and copper(II) as low as 10 μM produced near-complete conversion of the plasmid DNA to its nicked and linear forms (Fig. 1C, Lane 10).

UV–visible spectra were acquired next, in order to examine the interactions of **3** and **4** with DNA (Fig. 2). A total of 38 μM bp of calf thymus DNA (CT DNA) was added to a set of solutions containing a bis-acridine (**3** or **4**) and 1 mol equiv of copper(II) chloride as well as to a second set of solutions containing **3** or **4**, 1 mol equiv of copper(II) chloride, and 260 mM KCl. The concentrations of **3** and **4** were set at 10 and 50 μM, respectively, due to the large cleavage enhancements produced by copper(II) (Fig. 1C, Lane 10 vs Lane 4 for **3**; Fig. 1E, Lane 7 vs Lane 1 for **4**). Chromicity was then quantitated by measuring each curve area from 330 to 520 nm, where there is minimal overlap with DNA absorption (Fig. 2). This analysis showed that, within each bis-acridine series, the ordering of DNA photocleavage yields corresponded with the relative bis-acridine chromicity produced by the addition of CT DNA: **3**/Cu(II)/KCl strong cleavage (Fig. 1C, Lane 10) and 40% hyperchromicity > **3**/Cu(II) moderately strong cleavage (Fig. 1A, Lane 10) and 1% hypochromicity; **4**/Cu(II) moderately strong cleavage (Fig. 1E, Lane 7) and 35% hypochromicity > **4**/Cu(II)/KCl moderate cleavage (Fig. 1F, Lane 7) and 4% hypochromicity. Taken together, these data suggest that the addition of KCl to solutions of bis-acridine and 1 mol equiv of copper(II) increases DNA interactions in the case of **3**, but decreases interactions in the case of **4**. It is conceivable that KCl induces a structural change in the nucleic acid duplex that differentially alters the DNA binding of the two bis-acridine ligands. Experiments aimed at testing this hypothesis will be the subject of future research in our laboratories.

Our data have shown that bis-acridine **3** consistently produces significantly more photocleavage than bis-acridine **4** (Fig. 1A vs E; Fig. 1D vs 1F). In order to account for this result, DNA melting isotherms were recorded from 25 to 95 °C (no NaCl and no KCl). Melting assays provide a straightforward way of comparing the relative binding affinities of compounds that interact with double-helical DNA. The π–π, van der Waals, electrostatic, and hydrogen bonding interactions arising from intercalation and/or groove binding stabilize helical DNA. These interactions increase the DNA binding affinity of the ligand, and increase the melting temperature of the DNA–ligand complex compared to the unbound duplex.[12] Melting isotherms of 15 μM bp calf thymus DNA in 20 mM sodium phosphate buffer pH 7.0 were recorded in the presence and absence of 10 μM of bis-acridine **3** and/or copper(II) (Fig. S2 in Supplementary data). In our previous paper, isotherms acquired under identical conditions showed that 10 μM of **4** increased the melting temperature (T_m) of calf thymus DNA by 4 and 10 °C in the absence and presence of 10 μM of Cu(II), respectively (Fig. 3, in Fernández et al., 2007).[6d] Compared to compound **4**, the DNA melting isotherms of **3** were shifted to higher temperatures (Fig. S3 in Supplementary data). Furthermore, the absence of any inflection points in the isotherms of **3** indicated that the DNA was not completed melted up to the maximum recordable temperature in the experiment (95 °C). Inflection points and T_m values could only be obtained by lowing the concentrations of compound **3** and Cu(II) from 10 to 2.5 μM (Fig. S4). These data indicate that, in the presence and absence of 1 mol equiv of Cu(II), compound **3** binds to DNA with significantly higher affinity than compound **4**. Thus, the data are consistent with our observation that compound **3** produces higher levels of photocleavage.

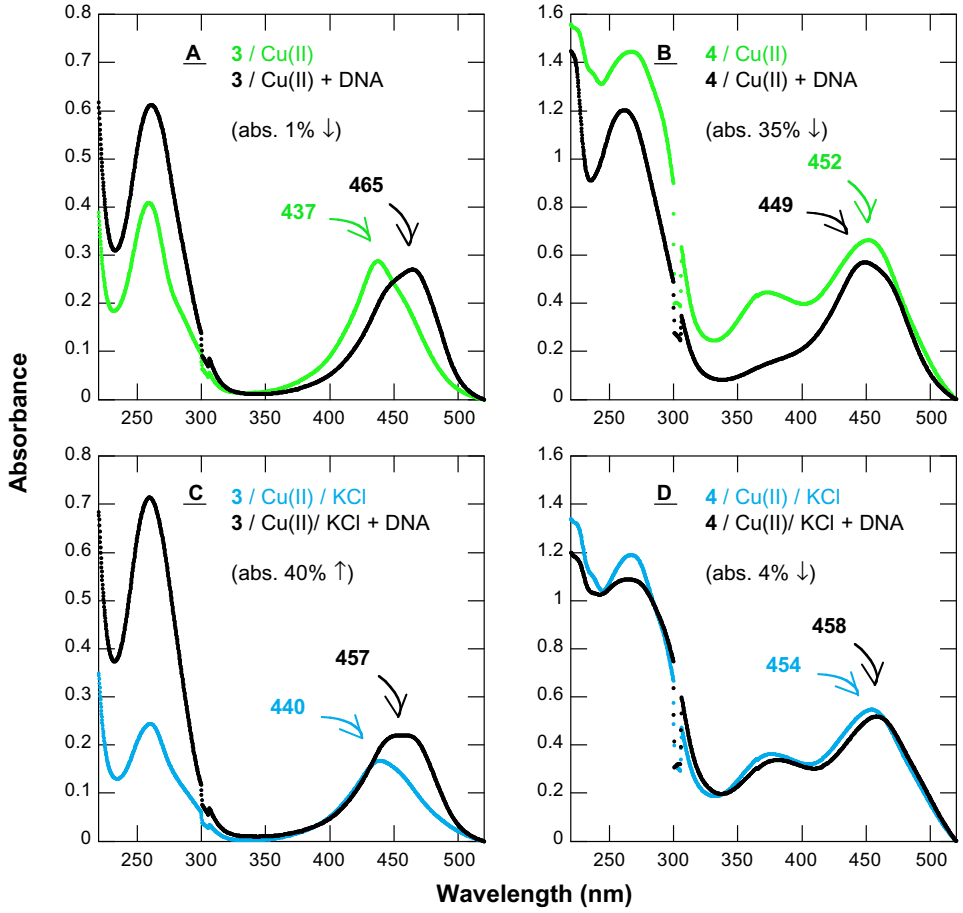

Figure 2. UV–visible absorbance spectra recorded at 22 °C in 10 mM sodium phosphate buffer pH 7.0 of pyridine-linked bis-acridine dyes **3** (10 μM, left panels) and **4** (50 μM, right panels) in the presence of: (A) and (B) 1 mol equiv Cu(II) (green), 1 mol equiv Cu(II) + 38 μM bp CT DNA (black); (C) and (D) 1 mol equiv Cu(II) + 260 mM KCl (blue), 1 mol equiv Cu(II) + 260 mM KCl + 38 μM bp CT DNA (black). Individual samples consisted of a total volume of 700 μL. Before absorbance was measured, the samples were pre-equilibrated for 1 h in the dark at 22 °C. Arrows indicate λ_{max}. The chromicity (% change in absorbance from 330 to 520 nm) produced by the addition of DNA appears in parenthesis. Chromicity = [(Total $Abs_{with\ DNA}$ − Total $Abs_{without\ DNA}$)/(Total $Abs_{without\ DNA}$)] × 100.

An ideal DNA photosensitizer for therapeutic applications should function optimally under the conditions of ionic strength that exist in the cell nucleus (~150 mM of Na(I) and 260 mM of K(I)).[7] Here, we have shown that DNA photocleavage by chromophore **3**, consisting of two acridine rings joined by a compact 2,6-bis(aminomethyl)pyridine copper-binding linker, is superior to bis-acridine **4**, equipped with a more flexible 2,6-bis{[(methoxycarbonylamino)-ethyl]methylaminomethyl}pyridine unit. In the presence of 150 mM of NaCl and 260 mM of KCl, DNA photocleavage by micromolar concentrations of **3** is enhanced by Cu(II), an ion that is closely associated with genomic DNA and chromosomes and is elevated in serum and tissue samples taken from cancer patients.[1a,2] Thus, the insights gained from the study of copper-binding acridine ligands may give rise to the development of new agents for use in photodynamic cancer therapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.009.

References and notes

1. For review: (a) Hadi, S. M.; Ullah, M. F.; Azmi, A. S.; Ahmad, A.; Shamim, U.; Zubair, H.; Khan, H. Y. *Pharm. Res.* **2010**, *27*, 979; (b) François, J.-C.; Faria, M.; Perrin, D.; Giovannangeli, C. *Nucleic Acids Mol. Biol.* **2004**, *13*, 223; (c) Manderville, R. A. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 195.
2. (a) Bryan, S. E.; Vizard, D. L.; Beary, D. A.; LaBiche, R. A.; Hardy, K. J. *Nucleic Acids Res.* **1981**, *9*, 5811; (b) Cantor, K. P.; Hearst, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 642; (c) Wacker, W. E. C.; Vallee, B. L. *J. Biol. Chem.* **1959**, *234*, 3257.
3. (a) Peña, M. M.; Lee, J.; Thiele, D. J. *J. Nutr.* **1999**, *129*, 1251; (b) Linder, M. C. *Biochemistry of Copper*; Plenum Press: New York, 1991; (c) Halliwell, B.; Gutteridge, J. M. C. *Biochem. J.* **1984**, *219*, 1.
4. Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269.
5. Ehrenfeld, G. M.; Rodriguez, L. O.; Hecht, S. M.; Chang, C.; Basus, V. J.; Oppenheimer, N. J. *Biochemistry* **1985**, *24*, 81.
6. (a) Patra, A. K.; Bhowmick, T.; Roy, S.; Ramakumar, S.; Chakravarty, A. R. *Inorg. Chem.* **2009**, *48*, 2932; (b) González-Álvarez, M.; Arias, M.-S.; Fernández, M.-J.; Gude, L.; Lorente, A.; Alzuet, G.; Borras, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3286; (c) Maity, B.; Roy, M.; Chakravarty, A. R. *J. Organomet. Chem.* **2008**, *693*, 1395; (d) Fernández, M.-J.; Wilson, B.; Palacios, M.; Rodrigo, M.-M.; Grant, K. B.; Lorente, A. *Bioconjugate Chem.* **2007**, *18*, 121; (e) Gude, L.; Fernández, M.-J.; Grant, K. B.; Lorente, A. *Org. Biomol. Chem.* **2005**, *3*, 1856; (f) Benites, P. J.; Holmberg, R. C.; Rawat, D. S.; Kraft, B. J.; Klein, L. J.; Peters, D. G.; Thorp, H. H.; Zaleski, J. M. *J. Am. Chem. Soc.* **2003**, *125*, 6434; (g) Eppley, H. J.; Lato, S. M.; Ellington, A. D.; Zaleski, J. M. *Chem. Commun.* **1999**, 2405; (h) Kuwahara, J.; Suzuki, T.; Funakoshi, K.; Sugiura, Y. *Biochemistry* **1986**, *25*, 1216.
7. (a) Hooper, G.; Dick, D. A. T. *J. Gen. Physiol.* **1976**, *67*, 469; (b) Moore, R. D.; Morrill, G. A. *Biophys. J.* **1976**, *16*, 527; (c) Billett, M. A.; Barry, J. M. *Eur. J. Biochem.* **1974**, *49*, 477; (d) Naora, H.; Naora, H.; Izawa, M.; Allfrey, V. G.; Mirsky, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 853.
8. Lorente, A.; Vázquez, Y. G.; Fernández, M.-J.; Ferrández, A. *Bioorg. Med. Chem.* **2004**, *12*, 4307.

9. (a) [2,6-pyridinediylbis(methylene)]bis[(6-amino-3-acridinyl)]carbamic acid di-*tert*-butyl ester (**2**). To a suspension of 80% NaH (26 mg, 0.86 mmol) in dry DMF (20 mL) under argon, (6-amino-3-acridinyl)carbamic acid *tert*-butyl ester (**1**) (240 mg, 0.77 mmol) was added. The mixture was stirred at rt for 20 min and then 2,6-bis(bromomethyl)pyridine (104 mg, 0.39 mmol) was transferred to the reaction. After 2 h, the solvent was evaporated in vacuo and the crude product purified by flash column chromatography (diameter: 3 cm) using silica gel as adsorbent and ethyl acetate–acetone–triethylamine (5:5:1) as eluent. The resulting oily product was dissolved in CH_2Cl_2 (5 mL) and precipitated by addition of hexane (40 mL). The solid thus obtained was filtered and dried, affording 209 mg (75%) of **2**, mp 145–150 °C. ^{1}H NMR (DMSO-d_6): δ 1.32 (s, 18H, $CO_2{}^t$Bu), 5.02 (s, 4H, CH_2), 6.19 (br s, 4H, NH_2), 6.83 (d, 2H, J = 1.98 Hz, H-5 acridine), 7.04 (dd, 2H, J = 9.06, J' = 1.98 Hz, H-7 acridine), 7.32 (d, 2H, J = 7.58 Hz, H-3′,5′), 7.38 (dd, 2H, J = 9.06, J' = 1.98 Hz, H-2 acridine), 7.68 (d, 2H, J = 1.98 Hz, H-4 acridine), 7.74 (d, 2H, J = 9.06 Hz, H-8 or H-1 acridine), 7.75 (d, 2H, J = 9.06 Hz, H-1 or H-8 acridine), 7.79 (t, 1H, J = 7.58 Hz, H-4′), 8.52 (s, 2H, H-9 acridine). ^{13}C NMR (DMSO-d_6): δ 157.62, 153.77, 151.49, 144.42, 137.67, 135.25, 129.71, 128.32, 123.36, 121.63, 120.85, 119.42, 102.15, 80.51, 55.18, 27.97. FAB MS: m/z = 722 [(M+H)$^+$, 61%]. Anal. Calcd for $C_{43}H_{43}N_7O_4$: C, 71.55; H, 6.0; N, 13.58. Found: C, 71.23; H, 6.14; N, 13.87. (b) *N*,*N*′-bis(6-amino-3-acridinyl)-2,6-pyridinedimethanamine dihydrochloride (**3**). A solution of **2** (100 mg, 0.14 mmol) in 35% HCl (5 mL) was stirred at rt for 5 min. Then, the solvent was concentrated in vacuo and the resulting residue was triturated with CH_2Cl_2. The solid thus obtained was filtered and dried over NaOH. Yield: 95%; mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 4.72 (s, 4H, CH_2), 6.82 (br s, 2H, H-5 acridine), 6.89 (br s, 2H, H-4 acridine), 6.99 (dd, 2H, J = 9.06, J' = 1.81 Hz, H-7 or H-2 acridine), 7.21 (dd, 2H, J = 9.06, J' = 1.81 Hz, H-2 or H-7 acridine), 7.46 (d, 2H, J = 7.91, H-3′,5′), 7.81 (d, 2H, J = 9.23 Hz, H-1 or H-8 acridine), 7.82 (d, 2H, J = 9.23, H-8 or H-1 acridine), 7.94 (t, 1H, J = 7.91 Hz, H-4′), 8.75 (s, 2H, H-9 acridine), 14.34 (s, 2H, N$^+$–H). ^{13}C NMR (DMSO-d_6): δ 156.63, 156.39, 154.27, 142.77, 142.53, 140.63, 131.94, 131.27, 121.15, 118.39, 117.02, 116.66, 93.63, 46.98. ESI-TOF HRMS obsd 522.2583, calcd 522.2406 [(M+H)$^+$, M = $C_{33}H_{27}N_7$]; obsd 261.6321, calcd 261.6242 [(M+2H)$^{2+}$, M = $C_{33}H_{27}N_7$]. Anal. Calcd for $C_{33}H_{29}N_7Cl_2$: C, 66.67; H, 4.92; N, 16.49. Found: C, 66.25; H, 4.81; N, 16.21.

10. (a) Hamelberg, D.; Williams, L. D.; Wilson, W. D. *J. Am. Chem. Soc.* **2001**, *123*, 7745; (b) Bednar, J.; Furrer, P.; Stasiak, A.; Dubochet, J.; Egelman, E. H.; Bates, A. D. *J. Mol. Biol.* **1994**, *235*, 825.
11. Schelhorn, T.; Kretz, S.; Zimmermann, H. W. *Cell. Mol. Biol.* **1992**, *38*, 345.
12. Wilson, W. D.; Tanious, F. A.; Fernández-Saiz, M. In *Drug-DNA Interaction Protocols*; Fox, K. R., Ed.; Humana Press: New Jersey, 1997; pp 219–240.