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Design, Synthesis, and Evaluation of *N*-Aroyloxy-2-thiopyridones as DNA Photocleaving Reagents

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Abstract—*N*-Benzoyloxy-2-thiopyridone (**12**) was shown to induce single-strand nicks in duplex DNA upon irradiation with visible light (λ&350 nm). This finding led to the design of a series of compounds, in which an acridinyl nucleus was covalently linked to the *N*-benzoyloxy-2-thiopyridone unit. These conjugates (**15, 16, 17** and **18**) were synthesized and evaluated as novel DNA photocleaving reagents. Optimal photocleaving activity was observed for conjugate **16**, in which a flexible polymethylene spacer of 4 carbons was used to connect the aminoacridine entity to the thiopyridone. This compound was shown to cleave DNA at low μM concentrations and was approximately two-orders of magnitude more efficient than the parent *N*-benzoyloxy-2-thiopyridone (**12**). Furthermore, the DNA cleavage ladders induced by **16** and **12** were found to be identical and of no significant sequence selectivity. These data suggest that the *N*-aroyloxy-2-thiopyridones can be used for the design of new DNA photocleaving reagents with potential use as 'photofootprinting agents' or as 'site-directed photonucleases'. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

The design of functional molecules has been a major goal of research in chemistry, biology, and medicine, encompassing topics such as catalysis, drug design, host–guest interactions, catalytic antibodies, and synthetic enzymes. This last topic includes, among others, research directed toward the development of nucleolytic reagents that react with DNA and/or RNA, ultimately leading to cleavage of the phosphodiester backbone.[1] These reagents, commonly referred to as 'artificial nucleases', hold great promise as tools in molecular biology[2] and as potential chemotherapeutic agents for cancer or antiviral treatment.[3] In addition, attachment of these reagents to selective carriers could produce 'artificial restriction enzymes' and specific gene-targeted drugs.[4]

The challenge in the design of artificial nucleases lies in choosing a suitable chemical moiety, which upon activation will generate reactive species, capable of damaging and cleaving nucleic acid biopolymers. Within this context, oxygen-centered radicals appear to be the most promising candidates, since they are characterized by an inherent reactivity profile and are of great biological importance.[5] Indeed, literature reports dated more than 15 years ago demonstrate the potential of these radicals as chemical nucleases. In the initial reports, generation of such radicals was accomplished by redox chemistry of organometallic reagents, a concept pioneered by Sigman,[6] Dervan,[7] and Tullius[8] using Cu(II) and Fe(II) complexes. More recently, the repertoire of organometallic reagents has been expanded by the work of Hecht[9] and Burrows[10] to include Co(II) and Ni(II) complexes. Although all these compounds have been extensively used over the years, they suffer from practical limitations, since they are sensitive to the presence of various biological species, including glycerol, thiols or metal ions, and require high concentrations of chemical initiators.

The search for alternative, perhaps more efficient, chemical nucleases led several groups to examine light-induced activation techniques.[11] These methods are advantageous since the cleavage proceeds using light, eliminating the need of external chemical initiators. These photoactivated reagents, commonly referred to as 'photonucleases', include complexes of Ru and Rh,[12] uranyl salts[13] and even simply UV light[14] and have been pioneered by Barton, Nielsen and Wang, respectively. However all the above methods have several drawbacks, particularly as related to the reagent concentration, the wavelength and intensity of the light source and the low quantum yield of the photoinitiation step. Owing to these limitations, despite the inherent advantages of light-activation, these reagents have not been widely applied.

The ability of organic (non-metal containing) molecules to cleave nucleic acids upon visible light activation has

Key words: DNA cleavage; photochemistry; thiopyridone; aminoacridine.

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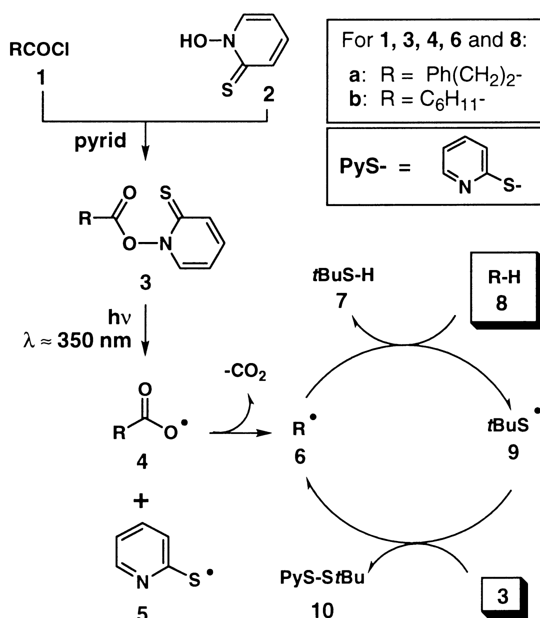
not yet been well explored.[15] Undoubtedly, such molecules could display a different, perhaps unique reaction profile and, thereby, expand and complement the repertoire of the already known DNA- and RNA-cleaving agents. Our own studies in this domain are directed toward the design and evaluation of *N*-hydroxy-2-thiopyridone and its acyloxyl derivatives as photoactivated DNA-cleaving species.[16–18]

Results and Discussion

Photochemistry of *N*-hydroxy-2-thiopyridone (2) and derivatives

Following the pioneering work of Barton and co-workers, the *O*-acyl derivatives of *N*-hydroxy-2-thiopyridone (such as **3**), commonly referred to as Barton's esters, have emerged as highly valuable sources of carbon-, nitrogen-, and oxygen-centered radicals.[19] The generally accepted mechanism of this process is shown in Scheme 1. Thus, photoexcitation of the thiocarbonyl group of **3** (λ&350 nm) results in homolytic cleavage of the N–O bond and generation of the acyloxyl radical **4** and the thiyl radical **5**. Rapid decarboxylation of **4** subsequently provides the carbon-centered radical **6**, which in the specific example of Scheme 1 is shown to abstract hydrogen from *tert*-butylthiol (**7**) thereby generating the hydrocarbon **8** and the propagating radical **9**. The synthetic efficiency of this process has been clearly demonstrated by the trapping of the carbon-centered radical **6** in a variety of ways, allowing a facile and high yielding entry into a variety of functionalized molecules.[19] Furthermore, this strategy permits the generation of radicals under mild conditions, using only visible light irradiation and without temperature restrictions.

Due to its efficiency and simplicity, the above method could be used for the generation and study of free radicals in biological systems. Indeed, early studies were focused on the employment of the *N*-hydroxy-2-thiopyridone (**2**) as a progenitor of hydroxyl radicals (**11**) in a biological environment (Scheme 2, eq (1)).[20] Although the reported results, by us[16] and others,[21] indicated that **2** could produce hydroxyl radicals, the photoexcitation process proved to be slow and rather inefficient. This drawback was attributed to the tendency of *N*-hydroxy-2-thiopyridone (**2**) to exist in equilibrium of different forms (Scheme 2, eq (2)).[22] This equilibrium decreases the concentration of the thiocarbonyl containing species and thereby hampers the homolytic cleavage of the N–O bond.

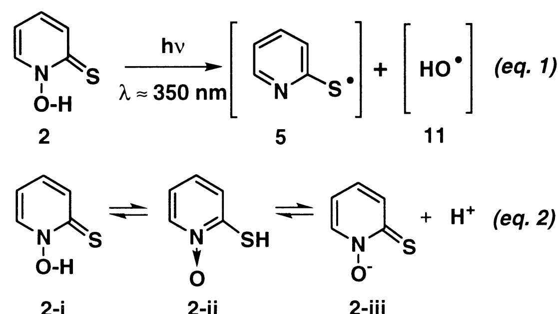


Scheme 1.

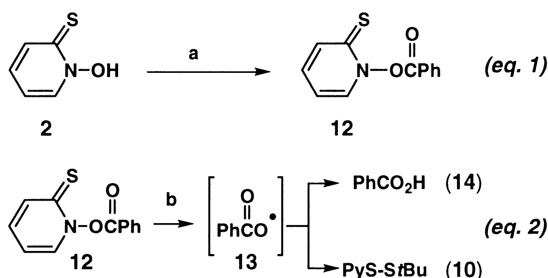
The undesirable effects of the above equilibrium can be minimized if one uses the *O*-acyl derivatives of *N*-hydroxy-2-thiopyridone (such as **3**) as sources of radicals. Among them, the *N*-aroyloxy-2-thiopyridones are particularly attractive, since they do not undergo rapid decarboxylation[23] and they could therefore be used as precursors of oxygen-centered radicals. With this in mind, we set out to examine the *N*-benzoyloxyl-2-thiopyridone (**12**) as a potential photoactivated DNA-cleaving agent. Photoexcitation of **12** was expected to produce benzoyloxyl radicals (**13**), which are more persistent than the acyloxyl species **4** and do not undergo efficient decarboxylation below 120 °C.[23] We anticipated that radicals **13** would exhibit similar reactivity with hydroxyl counterparts (**11**) and react with DNA, ultimately resulting in strand cleavage. Indeed, generation and trapping of benzoyloxyl radicals (**13**) was verified by photolysis of **12** in the presence of *tert*-BuSH. This resulted in the isolation of benzoic acid (**14**) and disulfide **10** in 92% and 89% yields, respectively, in accordance to a radical chain process (Scheme 3, eq (2)).[16]

DNA cleaving studies using *N*-benzoyloxy-2-thiopyridone (12)

Can the *N*-benzoyloxy-2-thiopyridone (**12**) induce DNA cleavage upon photoactivation? A comparison of concentration-dependent photocleavage of DNA by **2** and **12** is shown in Figure 1.[24] The results revealed an increased efficiency of strand scission using **12**, indicating that the generation of radicals happens before any saponification (that could afford **2** from **12**). The enhanced reactivity of **12** could be explained if we consider that: (a) compound **12** exists entirely in the thiocarbonyl containing form (shown in Scheme 3) and not



Scheme 2.



Scheme 3. Reagents and conditions: (a) 1.1 equiv of PhCOCl, 1.3 equiv of pyridine, CH_2Cl_2, 0 °C, 1 h, 92%; (b) 5.0 equiv of *t*BuSH, CH_2Cl_2, hν (GE, 300 W, > 350 nm), 0 °C, 1 h, 92% (for **14**) and 89% (for **10**).

in an equilibrium of forms, as it was proposed for **2**; and (b) the thiocarbonyl group of **12** is excited at higher wavelength, leading to faster N–O bond cleavage.[22] In fact, this difference is reflected in the color of **12**, which is bright yellow, while **2** is colorless and the UV-Vis absorption of the thiocarbonyl moieties of **12** ($\lambda_{max}=368$) and **2** ($\lambda_{max}=349$). Subsequent studies using **12** demonstrated that the observed DNA scission proceeds at a range of temperatures (0–25 °C) and is pH independent (pH range: 6–8.5).

Comparison of lanes 6 and 7 of Figure 1 indicate a strong inhibition of DNA-scission when the same experiment was conducted in the presence of glutathione (radical scavenger). This result further supports the notion that radical species, probably the benzoyloxyl radicals (**13**), are responsible for the cleavage. Subsequent time-controlled DNA cleavage studies demonstrated that these radicals are generated from **12** at a relatively linear rate.[18] This anticipated feature constitutes a fundamental difference between our method and those involving metal complexes, where radicals are usually produced in a rapid burst.[20]

We also compared the cleaving efficiency of **12** and **3b** (Fig. 2). Under the conditions tried, only **12** was shown to cleave DNA, while the acyloxyl adduct **3b** (and similarly **3a**) did not produce any measurable strand scission. The lack of reactivity of the acyloxyl derivatives could be attributed to the facile radical decarboxylation of such species, which generates less reactive carbon-centered radicals.

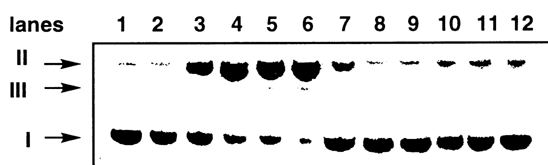


Figure 1. Concentration-dependent photocleavage of DNA using **12** and **2**. The irradiation (all samples except those in lanes 2 and 8) was performed for 1 h at 4 °C with two lamps (GE, 300 W), placed at about 20 cm from the samples. Lane 1: ϕX174 DNA (control); lane 2: DNA and 1.0 mM of **12** (no hν); lanes 3–6: DNA and **12** at concentrations of **12**: 0.5, 1.0, 1.5, and 2.0 mM respectively; lane 7: DNA, 2.0 mM of **12** and 3.0 mM of glutathione. Lane 8: DNA and 1.0 mM of **2** (no hν); lanes 9–12: DNA and **2** at concentrations of **2**: 0.5, 1.0, 1.5, and 2.0 mM respectively.

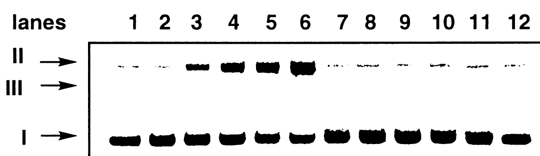


Figure 2. Concentration-dependent photocleavage of DNA using **12** and **3b**. The irradiation (all samples except those in lanes 2 and 7) was performed for 1 h at 4 °C with two lamps (GE, 300 W), placed at about 20 cm from the samples. Lane 1: ϕX174 DNA (control); lane 2: DNA and 1.0 mM of **12** (no hν); lanes 3–6: DNA and **12** at concentrations of **12**: 0.1, 0.3, 0.6, and 1.0 mM repectively. Lane 7: DNA and 1.0 mM of **3b** (no hν); lanes 8–11: DNA and **3b** at concentrations of **3b**: 0.1, 0.3, 0.6, and 1.0 mM respectively; lane 12: ϕX174 DNA (control).

Attachment of the *N*-benzoyloxy-2-thiopyridone to 9-aminoacridine; design, synthesis, and DNA-photocleaving studies

The above experiments confirmed that the *N*-benzoyloxy-2-thiopyridone (**12**) could be used as a photoactivated DNA-cleaving agent. In order to increase the efficiency of the cleavage and test the versatility of our method, we synthesized the acridine–thiopyridone conjugates **15–18** (Fig. 3). The rationale for this design is based on covalently attaching the photocleaving entity to a 'recognition element'. The acridinyl group could assure high affinity for duplex DNA via intercalation,[25] while the thiopyridone entity could account for the strand scission.

The synthesis of compounds **15–17** is depicted in Scheme 4 and began with condensation of commercially available ω-bromocarbonyl chlorides **20** with *p*-amino methyl benzoate (**19**), to produce bromides **21**. Compounds **21** were then converted to azides **22**, which upon saponification of the methylester moiety and reduction of the azido functionality gave rise to amino acid **25**, through intermediates **23** and **24**. Coupling of **25** with 9-chloroacridine (phenol, 110 °C), followed by esterification with 2-mercaptopyridine-*N*-oxide (**2**) (EDC, DMF) afforded conjugate **15–17** in good overall yields (individual yields are given in the experimental section). Conjugate **18** was synthesized from the commercially available acridine-9-carboxylic acid (**26**) as illustrated in Scheme 5, in 79% yield.

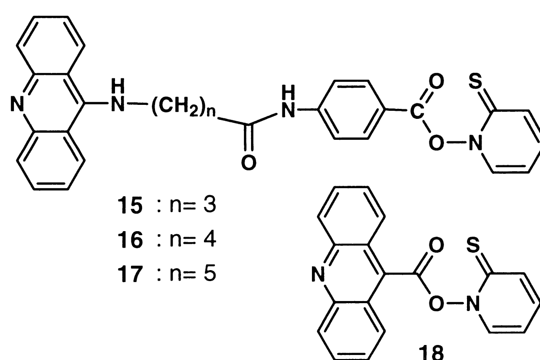
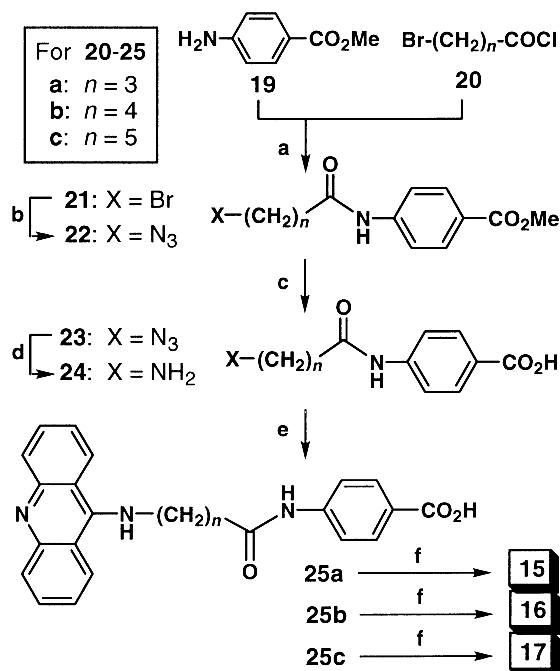
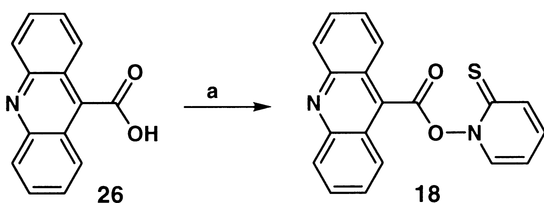


Figure 3. Molecular structure of acridine–thiopyridone ester conjugates **15–18**.

Table 1 presents the extent of DNA photocleavage induced by the above compounds in two different concentrations (10 and 60 μM). Compounds **15, 16**, and **17** were found to induce efficient strand cleavage at low μM concentrations. Among them, conjugate **16** ($n=4$ carbons) was the most efficient and was approximately two orders of magnitude more reactive than the parent *N*-benzoyloxy-2-thiopyridone (**12**).[17] This substantial increase in efficiency of cleavage was attributed to the intercalating properties of 9-aminoacridine.[25] Interestingly, much less cleavage was observed with **18**, (equivalent to **12**), testifying to the importance of a flexible polymethylene linker for our design. Again, the least reactive derivatives were the *N*-acyloxy-2-thiopyridones **3a** and **3b** presumably due to the rapid radical decarboxylation event, which produces relatively stable carbon-centered radicals.



Scheme 4. Reagents and conditions: (a) 1.0 equiv of **19**, 1.0 equiv of **20**, 1.2 equiv of Et_3N, CH_2Cl_2, 0 °C, 1 h; (b) 2.0 equiv of NaN_3, 0.05 equiv of [18-c-6], DMF, 25 °C, 3 h; (c) 2.0 equiv of KOH, THF/H_2O: 1/1, 25 °C, 24 h; (d) 0.1 equiv of 10% Pd/C, H_2, MeOH, 24 h; (e) 1.0 equiv of 9-chloroacridine, PhOH, 110 °C, 1 h; (f) 1.0 equiv of **2**, 1.0 equiv of EDC, DMF, 25 °C, 1 h.



Scheme 5. Synthesis pathway to 1-methyl-4-(3,4-dimethylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**23**). Reagents and conditions: (i) *n*-BuLi, THF; (ii) $ZnCl_2$, THF; (iii) 4-bromopyridine, $Pd(PPh_3)_4$; (v) CH_3I, acetone; (v) $NaBH_4$, MeOH; (vi) $H_2C_2O_4$, Et_2O.

Table 1 Comparison of DNA cleavage (%) with **2, 3a, 3b, 12** and **15–18**[a]

Conc.	Agent							
	2	**3a**	**3b**	**12**	**15**	**16**	**17**	**18**
10 μM	4	0.5	1	9	40	47	45	10
60 μM	8	0.6	0.9	19	81	98	96	18

[a]The reported data refer to % yield of DNA photocleavage and were obtained by densitometry reading of the scission event.

To verify that conjugate **16** intercalates onto duplex DNA we conducted UV–vis and fluorescence spectroscopy studies. The UV–vis studies were performed by titrating a solution of **16** (in 50 mM Tris–HCl) with salmon testes DNA and indicated a hypochromic and bathochromic shift of the UV spectrum of **16**, in accordance with the intercalation models.[26] For the fluorescence experiments the aminoacridine moiety of **16** was selectively excited at 412 nm and its fluorescence emission was monitored at 420–540 nm (Figs 4 and 5). Measurements of the emission spectra of **16** upon titration with salmon testes DNA (Fig. 5), in comparison to

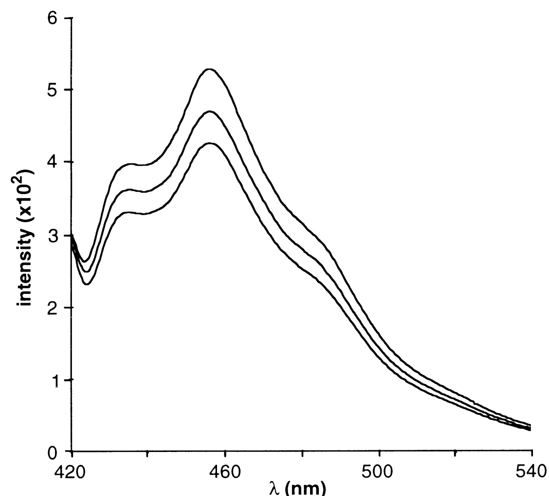


Figure 4. Fluorescence quenching studies of **16** with DNA; titration with buffer.

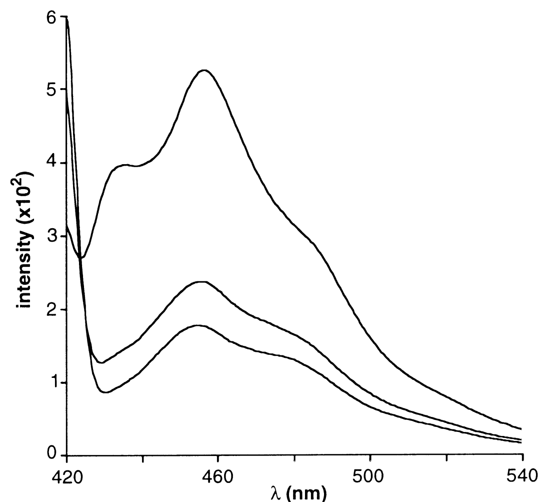


Figure 5. Fluorescence quenching studies of **16** with DNA; titration with salmon testes DNA.

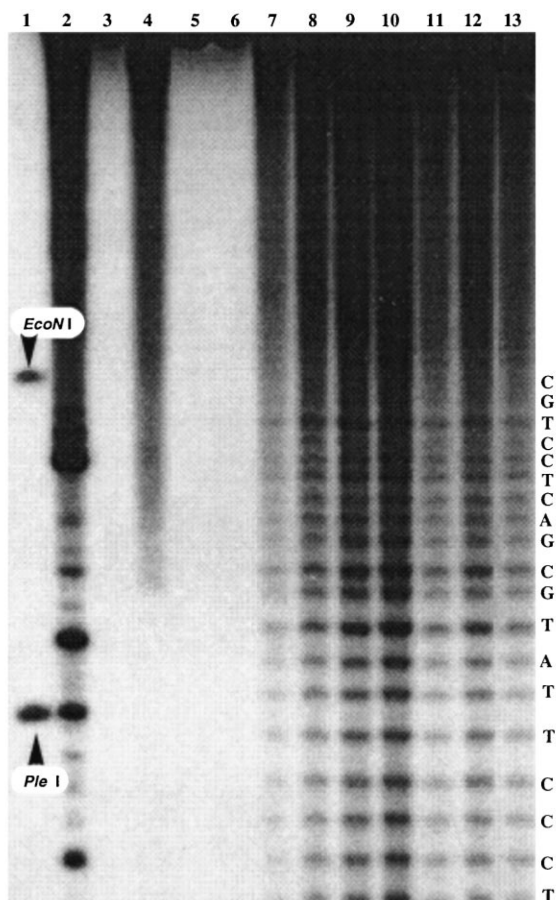


Figure 6. Autoradiogram of a 10% denaturing polyacrylamide gel showing photocleavage of 5′-^{32}P end-labeled *Sal* I/*Sph* I restriction fragment of pBR322 duplex DNA (93 mer), induced by **16** and **12**. DNA was incubated for 1 h at 25 °C with compounds **16** or **12** in buffered solution (30 mM Tris–HCl, 20 mM NaCl) and then irradiated (with two GE 300 W lamps placed at 20 cm from the samples) for another 2 h at 4 °C (lanes 3, 4 and 7–13). The resulting solution was treated with piperidine (1 M) at 90 °C for 30 min, followed by ethanol precipitation (lanes 8–13). Lane 1: DNA cut by *PIe* I (14 base pairs) and *EcoN* I (28 base pairs); lane 2: DNase footprinting; lane 3: DNA (control); lane 4: DNA irradiated and piperidine treated without **12** nor **16**; lane 5: DNA and 200 μM of **12** (no hv); lane 6: DNA and 60 μM of **16** (no hv); lane 7: DNA and 5 μM of **16** (no piperidine treatment); lanes 8–10: DNA and **16** at concentrations of **16**: 5, 10, 20 μM respectively; lanes 11, 12: DNA and **12** at concentrations of 40, 60 μM respectively; lane 13: DNA, **12** (60 μM) and 9-aminoacridine (30 μM).

those of the control experiments (Fig. 4) indicated a significant quenching of fluorescence, supporting the intercalation event.[27]

The base-selectivity of the DNA cleavage induced upon irradiation of **12** and **16** was evaluated using 'single-hit' conditions (Fig. 6).[17] These experiments were performed with the 5′-^{32}P labeled *Sal* I/*Sph* I restriction fragment of pBR322 duplex DNA (93-mer). Our data (Figs 6 and 7) show that both **12** and **16** generate identical DNA ladders and the photocleavage is indisputably neither base- nor sequence-specific. In addition, comparison of lanes 8–12 of Figure 6 indicate that **16** is more efficient than **12** in cleaving DNA and can accurately cut the duplex at concentrations as low as 5 μM. The possibility that the 9-aminoacridinyl group enhances cleavage by altering

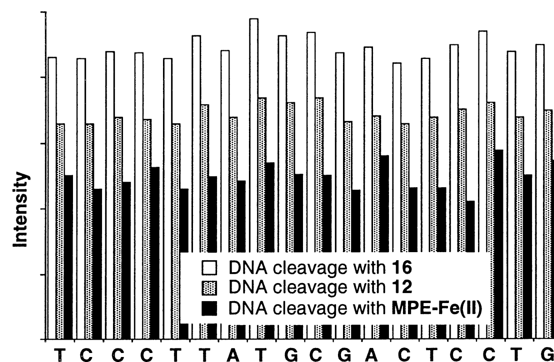


Figure 7. Relative intensity of strand scission induced by compounds **16, 12** and **MPE-Fe(II)** in a 18 base pairs region of the *Sal* I/*Sph* I fragment of pBR322 duplex DNA (93 mer). Quantification was performed using ImageQuant software.

the conformation of the DNA was ruled out, since less cleavage was detected when 9-aminoacridine was added as an external intercalator (compare lines 12,13). Furthermore, the cleavage is more enhanced upon subsequent treatment with piperidine at 90 °C for 30 min without any change in sequence or base specificity (lines 7,8). Based on the above data we believe that in the case of **16** the DNA cleavage is performed by the intercalation complex and is probably mediated by aroyloxyl radicals.

Conclusion

It is evident from the above studies that the *N*-aroyloxy-2-thiopyridones (such as **12** or **16**) can induce single strand nicks in duplex DNA in a light-dependent reaction. These compounds are potentially interesting nucleic acid-cleaving agents since they possess the following characteristics: a purely organic structure, facile preparation and prolonged stability in the absence of light. Furthermore, they undergo an efficient photoexcitation, with very good quantum yields (&0.5)[22] using visible light (λ&350 nm). The DNA cleavage occurs simply by irradiation, without the need of external additives and is concentration- and time-dependent. In addition, the strand scission is neither base- nor sequence-specific and is probably mediated by aroyloxyl radicals. The efficiency and/or selectivity of the cleavage could be tuned by the proper attachment of the DNA-recognition element. In addition, the light intensity that is responsible for the photoactivation could be tuned by structurally modifying the thiopyridone core. Thus, the *N*-aroyloxy-2-thiopyridones can be used for the design of new DNA photocleaving reagents with potential use as 'photofootprinting reagents' or as 'site-directed photonucleases'.

Experimental

General techniques

All reactions were carried out under an argon atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Tetrahydrofuran

(THF) and diethyl ether (Et_2O) were distilled from sodium/benzophenone; dichloromethane (CH_2Cl_2) from calcium hydride; DMF from calcium chloride. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at highest commercial quality and used without further purification unless otherwise stated. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F–254) using UV light as visualizing agent and 7% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash chromatography. Preparative thin-layer chromatography separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F–254). NMR spectra were recorded on a Varian 500 instrument and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were recorded on a Perkin–Elmer Model 781 spectrometer. UV and fluorescence spectra were recorded on a Hewlett–Packard 8452A Diode Array Spectrophotometer and a Perkin–Elmer LS50B luminescence spectrometer respectively. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under chemical ionization (CI) conditions or on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions.

***N*-Benzoyloxy-2-thiopyridone 12.** To a solution of *N*-hydroxy-2-thiopyridone (**2**) (12.7 g, 100 mmol) in methylene chloride (200 mL) was added, at 0 °C and in the dark, pyridine (10.5 mL, 130 mmol) followed by benzoyl chloride (12.8 mL, 110 mmol). The mixture was stirred for 3 h, and then diluted with methylene chloride (300 mL) and washed twice with aqueous saturated sodium bicarbonate (2×300 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure at 20 °C in the dark. Rapid filtration over silica gel (60% ethyl ether in hexane) followed by recrystallization from methylene chloride–hexane afforded the benzoyloxyl derivative **12** (21.25 g, 92 mmol, 92%). **12**: yellow solid; ^1H NMR (500 MHz, $CDCl_3$) δ 8.25–8.15 (d, 2H, J = 8 Hz), 7.72–7.62 (m, 3H), 7.58–7.48 (m, 2H), 7.26–7.18 (m, 1H), 6.72–6.65 (dt, 1H, J = 1.8, 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5, 162.4, 137.8, 137.0, 134.8, 133.6, 130.4, 128.7, 125.4, 112.7.

Photolysis of 12 in the presence of *tert*-butylthiol. The photolysis was performed under argon, using degassed, dry methylene chloride as solvent. A solution of **12** (0.462 g, 2.0 mmol) and *tert*-butylthiol (1.1 mL, 10 mmol) in methylene chloride (4 mL) at 0 °C was irradiated with one tungsten lamp (GE, 300 W) in a Pyrex flask from a distance of about 20 cm. The consumption of **12** was followed by TLC and completed after 20 min. The solvent was then removed under reduced pressure and the residue subjected to flash chromatography (silica, 5→60% ethyl ether in hexane) to give **14** (0.217 g, 1.84 mmol, 92%) and **10** (0.350 g, 1.78 mmol, 89%). **10**: colorless liquid; ^1H NMR (500 MHz, $CDCl_3$) δ 8.48–8.31 (m, 1H), 7.65–7.50 (m, 2H), 6.91–7.02 (m, 1H), 1.35 (s, 9H, *tert* Bu).

Bromide 21b. To a solution of methyl 4-aminobenzoate (**19**) (14.02 g, 92.74 mmol) and triethylamine (13.9 mL, 99.7 mmol) in methylene chloride (100 mL) at 0 °C was added 5-bromovaleryl chloride (**20b**) (14.61 mL, 99.7 mmol). The reaction mixture was stirred at 0 °C for 30 min and at 25 °C for an additional 1 h. The mixture was then diluted with methylene chloride and washed with three portions of aqueous saturated sodium bicarbonate (3×200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give compound **21b** (28.56 g, 90.89 mmol, 98%). **21b**: white solid; R_f = 0.20 (silica, 50% ethyl acetate in hexanes); IR (KBr plate) ν_{max} 3319 (s), 3199 (m), 2951 (m), 1721 (s), 1676 (s), 1602 (s), 1537 (s), 1509 (m), 1407 (m), 1279 (s), 1169 (s), 1099 (m), 771 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, 2H, J = 9.0 Hz), 7.60 (d, 2H, J = 9.0 Hz), 7.56 (s, 1H), 3.89 (s, 3H), 3.42 (t, 2H, J = 6.5 Hz), 2.42 (t, 2H, J = 6.5 Hz), 1.91 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 166.8, 142.2, 130.8, 125.5, 119.0, 52.0, 36.4, 33.0, 31.8, 23.7; HRMS calcd for $C_{13}H_{16}NO_3Br$, $(M + Cs^+)$ 445.9368, found 445.9387.

Bromide 21a. Preparation of this compound was accomplished following the procedure described for bromide **21b**. **21**a: (92%); white solid; R_f = 0.25 (silica, 50% EtOAc in hexanes); IR (film) ν_{max} 3306 (s), 1716 (s), 1602 (s), 1539 (s), 1411 (s), 1279 (s), 1173 (m), 1102 (m), 953 (m), 857 (m), 771 (s), 697 (m), 512 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, 2H, J = 9.0 Hz), 7.86 (d, 2H, J = 8.5 Hz), 7.32 (s, 1H), 3.80 (s, 3H), 3.54 (t, 2H, J = 6.5 Hz), 2.60 (t, 2H, J = 7.0 Hz), 2.28 (t, 2H, J = 6. 5 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.3, 166.8, 142.5, 130.8, 125.5, 118.9, 51.9, 35.2, 33.1, 27.7; HRMS, calcd for $C_{12}H_{14}NO_3Br$ $(M + H^+)$ 300.0235, found 300.0245.

Bromide 21c. Preparation of this compound was accomplished following the procedure described for bromide **21b**. **21c**: (95%); white solid; R_f = 0.25 (silica, 50% EtOAc in hexanes); IR (film) ν_{max} 3340 (s), 2948 (m), 1691 (s), 1596 (s), 1532 (s), 1432 (s), 1258 (s), 1161 (s), 861 (m), 772 (s), 702 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, 2H, J = 9.0 Hz), 7.60 (d, 2H, J = 9.0 Hz), 7.56 (s, 1H), 3.88 (s, 3H), 3.40 (t, 2H, J = 7.0 Hz), 2.39 (t, 2H, J = 7.0 Hz), 1.88 (m, 2H), 1.75 (m, 2H), 1.51 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.5, 166.8, 142.2, 130.8, 125.5, 118.9, 51.9, 37.3, 33.4, 32.3, 27.6, 24.3; HRMS, calcd for $C_{14}H_{18}NO_3Br$ $(M + H^+)$ 328.0548, found 328.0540.

Azide 22b. A solution of bromide **21b** (28.43 g, 90.5 mmol), sodium azide (11.76 g, 181 mmol) and 18-crown-6 (100 mg) in dry DMF (180 mL) was stirred at 25 °C for 3 h. The reaction mixture was then diluted with ethyl ether and washed with three portions of aqueous saturated sodium bicarbonate (3×200 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and chromatographed (silica, 20→50% ethyl acetate in hexanes) to give **22b** (22.84 g, 82.67 mmol,

91%). **22b**: white solid; R_f = 0.45 (silica, 50% ethyl acetate in hexanes); IR(KBr plate) ν_{max} 3312 (s), 3192 (m), 3123 (m), 2950 (s), 2872 (m), 2091 (s), 1721 (s), 1674 (m), 1602 (s), 1543 (s), 1431 (s), 1410 (s), 1251 (s), 1174 (s), 1100 (s), 867 (m), 770 (s), 697 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, 2H, J = 9.0 Hz), 7.61 (s, 1H), 7.60 (d, 2H, J = 9.0 Hz), 3.89 (s, 3H), 3.31 (t, 2H, J = 7.0 Hz), 2.42 (t, 2H, J = 7.0 Hz), 1.81 (m, 2H), 1.66 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 166.9, 142.4, 130.8, 125.4, 119.1, 52.0, 51.0, 36.7, 28.2, 22.5; HRMS, calcd for $C_{13}H_{16}N_4O_3$ ($M+H^+$) 277.1301, found 277.1304.

Azide 22a. Preparation of this compound was accomplished following the procedure described for azide **22b**. **22a**: (93%); white solid; R_f = 0.3 (silica, 50% ethyl acetate in hexanes); IR (KBr plate) ν_{max} 3312 (m), 2957 (m), 2098 (s), 1720 (s), 1674 (s), 1596 (s), 1432 (s), 1279 (s), 1172 (s), 1101 (m), 770 (m), 697 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, 2H, J = 7.0 Hz), 7.59 (d, 2H, J = 7.5 Hz), 7.35 (s, 1H), 3.90 (s, 3H), 3.43 (t, 2H, J = 7.0 Hz), 2.50 (t, 2H, J = 7.5 Hz), 2.02 (t, 2H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 166.8, 142.2, 130.8, 125.5, 118.9, 51.9, 50.5, 34.0, 24.3; HRMS, calcd for $C_{12}H_{14}N_4O_3$ ($M+H^+$) 263.1144, found 263.1150.

Azide 22c. Preparation of this compound was accomplished following the procedure described for azide **22b**. **22c**: (89%); white solid; R_f = 0.5 (silica, 50% ethyl acetate in hexanes); IR (KBr plate) ν_{max} 3306 (m), 2947 (m), 2094 (s), 1720 (s), 1674 (s), 1538 (s), 1407 (m), 1280 (m), 1175 (m), 1103 (m), 852 (m), 770 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, 2H, J = 8.5 Hz), 7.60 (d, 2H, J = 8.5 Hz), 7.37 (s, 1H), 3.89 (s, 3H), 3.28 (t, 2H, J = 7.0 Hz), 2.40 (t, 2H, J = 7.0 Hz), 1.76 (m, 2H), 1.63 (m, 2H), 1.46 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 166.8, 142.4, 130.7, 125.3, 118.9, 51.9, 51.0, 37.2, 28.4, 26.1, 24.7; HRMS, calcd for $C_{14}H_{18}N_4O_3$ ($M+Na^+$) 313.1277, found 313.1286.

Acid 23b. To a solution of the azide **22b** (20.94 g, 75.78 mmol) in tetrahydrofuran (200 mL) was added a solution of potassium hydroxide (10 g, 178 mmol) in H_2O (200 mL). The resulting milky mixture was stirred vigorously at 25 °C for 24 h until it turned clear. Acidification with concentrated hydrochloric acid to pH = 1 and filtration afforded compound **23b** (19.49 g, 74.30 mmol, 98%). **23b**: white solid; R_f = 0.12 (silica, 50% ethyl acetate in hexane); IR (KBr plate) ν_{max} 3313 (s), 2947 (m), 2862 (m), 2669 (m), 2550 (m), 2093 (s), 1674 (s), 1609 (s), 1525 (s), 1427 (s), 1299 (s), 1182 (s), 938 (m), 868 (m), 769 (s), 693 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.27 (s, 1H), 7.88 (d, 2H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.0 Hz), 3.33 (t, 2H J = 6.5 Hz), 2.38 (t, 2H, J = 6.5 Hz), 1.62 (m, 2H), 1.55 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.7, 167.2, 143.5, 130.5, 125.1, 118.4, 50.5, 35.9, 27.9, 22.2; HRMS, calcd for $C_{12}H_{14}N_4O_3$ ($M+Na^+$)285.0984, found 285.0989.

Acid 23a. Preparation of this compound was accomplished following the procedure described for acid **23b. 23a**: (85%); white solid; R_f = 0.15 (silica, 50% ethyl acetate in hexane); IR (KBr plate) ν_{max} 3311 (m), 2944 (m), 2094 (s), 1655 (s), 1524 (s), 1427 (m), 1302 (m), 1182 (m), 942 (m), 861 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.87 (d, 2H, J = 9.0 Hz), 7.70 (d, 2H, J = 9.0 Hz), 3.38 (t, 2H, J = 7.0 Hz), 2.44 (t, 2H, J = 7.0 Hz), 1.82 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.3, 167.2, 143.5, 130.5, 125.1, 118.5, 50.3, 33.4, 24.2; HRMS, calcd for $C_{11}H_{12}N_4O_3$ ($M+Na^+$) 271.0807, found 271.0809.

Acid 23c. Preparation of this compound was accomplished following the procedure described for acid **23b. 23c**: (93%); white solid; R_f = 0.15 (silica, 50% ethyl acetate in hexane); IR (KBr plate) ν_{max} 3315 (s), 2950 (m), 2094 (s), 1662 (s), 1520 (s), 1300 (m), 1181 (m), 769 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.19 (s, 1H), 7.86 (d, 2H, J = 8.5 Hz), 7.69 (d, 2H, J = 8.5 Hz), 3.32 (t, 2H, J = 7.0 Hz), 2.34 (t, 2H, J = 7.5 Hz), 1.60 (m, 2H), 1.55 (m, 2H), 1.34 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.2, 167.5, 143.9, 130.8, 125.4, 118.7, 50.9, 36.7, 28.4, 26.1, 24.8; HRMS, calcd for $C_{13}H_{16}N_4O_3$ ($M+Na^+$) 299.1120, found 299.1124.

Amino acid 24b. To a solution of the acid **23b** (18.98 g, 72.36 mmol) in methanol (500 mL) was added 10% Pd/C (200 mg) and the mixture was stirred under H_2 atmosphere at 25 °C for 24 h. The Pd/C was then removed by filtration, and the methanol was concentrated to afford crude amino acid **24b**, which was used in the next step without any further purification (15.2 g, 64.41 mmol, 89%). **24b**: white solid; R_f = 0.10 (silica, 40% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3313 (m), 3040 (m), 2951 (m), 1668 (s), 1609 (m), 1528 (m), 1379 (s), 1311 (m), 1173 (m), 786 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.80 (d, 2H, J = 8.0 Hz), 7.60 (d, 2H, J = 8.5 Hz), 2.79 (t, 2H, J = 7.0 Hz), 2.34 (t, 2H, J = 6.5 Hz), 1.62 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.4, 169.1, 141.7, 130.0, 127.0, 118.1, 38.4, 35.8, 26.8, 22.1; HRMS, calcd for $C_{12}H_{16}N_2O_3$ ($M+Na^+$) 259.1059, found 259.1078.

Amino acid 24a. Preparation of this compound was accomplished following the procedure described for amino acid **24b. 24a**: (81%); white solid; R_f = 0.10 (silica, 40% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3151 (m), 3040 (m), 1691 (s), 1596 (s), 1531 (s), 1408 (m), 1314 (s), 1171 (s), 965 (m), 870 (m), 774 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.49 (s, 1H), 7.96 (s, 1H), 7.87 (d, 2H, J = 9.0 Hz), 7.73 (d, 2H, J = 8.5 Hz), 2.82 (m, 2H), 2.47 (m, 2H), 1.86 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.1, 167.1, 143.4, 130.4, 125.1, 118.4, 38.2, 33.1, 22.9; HRMS, calcd for $C_{11}H_{14}N_2O_3$ ($M+H^+$) 223.1083, found 223.1085.

Amino acid 24c. Preparation of this compound was accomplished following the procedure described for amino acid **24b. 24c**: (88%); white solid; R_f = 0.15 (silica, 40% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3331 (m), 2953 (m), 1686 (s), 1596 (s), 1531 (s), 1416 (m), 1292 (s), 1163 (s), 864 (m), 772 (m), 700 (m), 546 (m) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.22 (s, 1H), 7.87 (d, 2H, J = 9.0 Hz), 7.68 (d, 2H, J = 7.0 Hz),

2.52 (t, 2H, $J=6.5$ Hz), 2.49 (m, 2H), 2.38 (t, 2H, $J=7.5$ Hz), 1.66 (m, 2H), 1.59 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.4, 167.3, 143.2, 130.5, 120.7, 118.4, 39.5, 35.5, 24.4, 24.0, 16.0; HRMS, calcd for $C_{13}H_{18}N_2O_3$ (M + H$^+$) 251.1396, found 251.1403.

Acid 25b. To a solution of the amino acid **24b** (2.43 g, 10.3 mmol) in phenol (15 g) at 40 °C was added the 9-chloroacridine (2.2 g, 10.3 mmol) and the mixture was stirred at 110 °C for 1 h. The phenol was then removed under reduced pressure and the residue purified by chromatography on silica (0→30% methanol in methylene chloride) to afford acid **25b** (3.80 g, 9.07 mmol, 90%). **25b**: yellow solid; $R_f=0.15$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3416 (s), 1655 (m), 1603 (m), 1527 (m), 1408 (m), 863 (m), 790 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.28 (m, 2H), 7.85 (d, 2H, $J=8.5$ Hz), 7.64 (m, 6H), 7.25 (m, 2H), 3.83 (t, 2H, $J=6.5$ Hz), 2.35 (t, 2H, $J=6.5$ Hz), 1.76 (m, 2H), 1.69 (m, 2H); HRMS, calcd for $C_{25}H_{23}N_3O_3$ (M + Na$^+$) 436.1637, found 436.1671; UV (DMF, c = 2.0×10^{-4} M) λ_{max} = 253, 270, 379 nm.

Acid 25a. Preparation of this compound was accomplished following the procedure described for acid **25b. 25a**: (87%); yellow solid; $R_f=0.15$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3397 (m), 1698 (m), 1648 (s), 1603 (s), 1533 (s), 1373 (s), 1309 (m), 1164 (s), 789 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.31 (d, 2H, $J=7.5$ Hz), 7.79 (d, 2H, $J=8.0$ Hz), 7.55 (m, 6H), 7.23 (m, 2H), 3.86 (t, 2H, $J=7.0$ Hz), 2.45 (t, 2H, $J=7.0$ Hz), 1.22 (m, 2H); HRMS calcd for $C_{24}H_{21}N_3O_3$ (M + Na$^+$) 422.1481, found 422.1488; UV (DMF, c = 8.0×10^{-5} M) λ_{max} = 254, 270, 348, 360, 390, 419 nm.

Acid 25c. Preparation of this compound was accomplished following the procedure described above, for the synthesis of acid **25b. 25c**: (76%); yellow solid; $R_f=0.10$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3402 (s), 1685 (m), 1596 (m), 1527 (m), 1376 (m), 1254 (m), 1165 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.55 (m, 2H), 7.86 (m, 6H), 7.68 (d, 2H, $J=8.5$ Hz), 7.47 (m, 2H), 4.04 (t, 2H, $J=7.0$ Hz), 2.35 (t, 2H, $J=7.0$ Hz), 1.9 (m, 2H), 1.63 (m, 2H), 1.41 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.3, 157.4, 143.7, 140.6, 134.9, 130.6, 126.5, 126.3, 123.5, 118.7, 118.7, 48.9, 48.9, 36.5, 29.0, 26.1, 24.9; HRMS, calcd for $C_{26}H_{25}N_3O_3$ (M + H$^+$) 428.1974, found 428.1991; UV (DMF, c = 9.75×10^{-5} M) λ_{max} = 269, 358, 395, 430 nm.

Aminoacridine 16. To a solution of the acid **25b** (2.0 g, 4.84 mmol) in dry DMF (20 mL) was added EDC (927 mg, 4.84 mmol) and the mixture was stirred at 25 °C for 30 min. To this solution was added 2-mercaptopyridine-*N*-oxide (**2**) (615 mg, 4.84 mmol), the flask was covered with alumina foil and the mixture was stirred at 25 °C for 12 h. The DMF was then removed under reduced pressure and the residue chromatographed (silica, 0→20% methanol in methylene chloride) to give compound **22** (1.09 g, 2.08 mmol, 43%). **22**: yellow solid; $R_f=0.60$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3255 (m), 3098 (m), 2940 (m), 1772 (m), 1595 (s), 1528 (s), 1411 (m), 1249 (m), 1171 (m), 978 (m), 748 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (s, 1H), 9.85 (m, 1H), 8.61 (m, 2H), 8.48 (d, 1H, $J=6.5$ Hz), 8.08 (d, 2H, $J=9.0$ Hz), 7.96 (t, 2H, $J=8.0$ Hz), 7.90 (d, 2H, $J=9.0$ Hz), 7.84 (d, 2H, $J=8.5$ Hz), 7.58 (d, 1H, $J=9.0$ Hz), 7.50 (m, 3H), 6.92 (t, 1H, $J=6.5$ Hz), 4.12 (m, 2H), 2.44 (m, 2H), 1.95 (m, 2H), 1.74 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.8, 171.8, 167.1, 161.8, 157.4, 147.5, 145.5, 143.5, 140.1, 138.7, 135.9, 134.9, 131.6, 130.4, 127.2, 118.7, 118.5, 113.4, 48.3, 35.8, 28.4, 22.2; HRMS, calcd for $C_{30}H_{26}N_4O_3S$ (M + H$^+$) 523.1804, found 523.1786; UV (DMF, c = 1.9×10^{-4} M) λ_{max} = 250, 271, 347, 390, 428 nm.

Aminoacridine 15. Preparation of this compound was accomplished following the procedure described for aminoacridine **16. 15**: (61%); yellow solid; $R_f=0.60$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3240 (m), 1752 (m), 1697 (m), 1636 (s), 1595 (s), 1528 (s), 1445 (m), 1251 (s), 1173 (m), 987 (m), 746 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1H), 9.86 (s, 1H), 8.62 (m, 2H), 8.49 (d, 1H, $J=6.5$ Hz), 8.05 (d, 2H, $J=8.5$ Hz), 7.95 (t, 2H, $J=7.5$ Hz), 7.87 (d, 2H, $J=8.5$ Hz), 7.76 (d, 2H, $J=8.0$ Hz), 7.59 (d, 1H, $J=8.5$ Hz), 7.53 (m, 3H), 6.93 (t, 1H, $J=6.5$ Hz), 4.19 (t, 2H, $J=6.5$ Hz), 2.60 (t, 2H, $J=6.0$ Hz), 2.25 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.0, 175.2, 172.3, 170.2, 162.2, 158.2, 156.4, 145.5, 140.5, 136.3, 135.4, 135.4, 132.0, 130.1, 127.4, 119.7, 119.0, 113.8, 48.9,3 4.0, 24.7; HRMS calcd for $C_{29}H_{24}N_4O_3S$ (M + H$^+$) 509.1647, found 509.1633; UV (DMF, c = 4.0×10^{-4} M) λ_{max} = 270, 286, 349, 390 nm.

Aminoacridine 17. Preparation of this compound was accomplished following the procedure described above, for the synthesis of aminoacridine **16. 17**: (55%); yellow solid; $R_f=0.60$ (silica, 20% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3386 (m), 1772 (s), 1591 (s), 1530 (s), 1408 (m), 1253 (m), 1169 (m), 987 (m), 750 (m) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.05 (m, 1H), 8.65 (m, 2H), 8.49 (d, 1H, $J=7.0$ Hz), 8.06 (d, 2H, $J=8.5$ Hz), 8.01 (d, 2H, $J=8.0$ Hz), 7.95 (t, 2H, $J=7.5$ Hz), 7.87 (d, 2H, $J=9.0$ Hz), 7.58 (d, 1H, $J=9.0$ Hz), 7.49 (m, 3H), 6.92 (t, 1H, $J=6.5$ Hz), 4.10 (m, 2H), 2.42 (t, 2H, $J=7.5$ Hz), 1.95 (m, 2H), 1.66 (m, 2H), 1.43 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.0, 161.8, 157.4, 145.6, 140.2, 135.8, 134.5, 134.2, 131.5, 127.0, 118.7, 118.6, 118.5, 115.8, 113.4, 111.1, 99.2, 94.4, 48.3, 36.2, 28.5, 25.7, 24.4; HRMS calcd for $C_{31}H_{28}N_4O_3S$ (M + H$^+$) 537.1961, found 537.1992; UV (DMF, c = 1.9×10^{-4} M) λ_{max} = 253, 271, 295, 340, 392, 410, 434 nm.

Acridine 18. A suspension of the 9-aminoacridine carboxylic acid (**26**) (780 mg, 3.5 mmol) in thionyl chloride (20 mL) was heated under stirring at 50 °C for 3 h. The excess thionyl chloride was then removed under reduced pressure, the residue was dissolved in dry methylene chloride (5 mL) and transferred dropwise via syringe to a solution of 2-mercaptopyridine-*N*-oxide (**2**) (490 mg, 3.8 mmol) and pyridine (0.62 mL, 7.0 mmol) in methylene chloride (20 mL). The reaction flask was covered

with alumina foil and the mixture was stirred at 25 °C for 2 h. The reaction mixture was then diluted with methylene chloride (30 mL) and washed with saturated aqueous sodium bicarbonate (3×30 mL). The organic layer was extracted, dried ($MgSO_4$), filtered, concentrated and the residue was crystallized (methylene chloride/hexanes) to give **18** (920 mg, 2.76 mmol, 79%). **18**: yellow solid; R_f = 0.75 (silica, 10% MeOH in CH_2Cl_2); IR (KBr plate) ν_{max} 3412 (m), 1792 (s), 1609 (m), 1525 (s), 1449 (s), 1405 (m), 1226 (m), 1103 (s), 933 (s), 755 (s) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.71 (m, 2H), 8.34 (d, 2H, J = 8.0 Hz), 7.88 (t, 4H, J = 7.0 Hz), 7.73 (t, 2H, J = 7.0 Hz), 7.33 (t, 1H, J = 7.5 Hz), 6.79 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 148.5, 138.0, 137.5, 133.6, 130.6, 130.1, 128.6, 125.3, 123.1, 113.1, 113.0, 105.1, 84.4; HRMS calcd for $C_{19}H_{12}N_2O_2S$ ($M + Cs^+$) 464.9674, found 464.9659; UV (DMF, c = 2.5×10^{-4} M) λ_{max} = 217, 254, 292, 350, 364, 388 nm.

Photocleavage of supercoiled DNA using *N*-aroyloxy-2-thiopyridones. ϕX174 (50 μM per base pair) was incubated for 1 h at 25 °C with the appropriate *N*-aroyloxy-2-thiopyridones and then irradiated for 1 h at 4 °C. The irradiation was performed with two GE 300W lamps placed at approximately 20 cm from the samples. Intact ϕX174 DNA runs at the position noted as I, relaxed circular DNA (single-stranded cleavage) at position II, and linear DNA (double-stranded cleavage) at position III. The results were analyzed on 1% agarose gel (Tris-acetate buffer) stained with ethidium bromide and are displayed in Figures 1–3, 7 and Table 1.

UV–vis and fluorescence studies of conjugate 16. For the UV–vis experiments compound **16** (9.1 mg) was dissolved in 1 mL of DMSO and diluted 60 times with 50 mmol Tris•HCl buffer to give a final concentration of 0.29 mmol. A 2.4 mL of this solution was titrated with 50 μL aliquots of salmon testes DNA (2.25 mmol) dissolved in 50 mmol Tris•HCl buffer and the spectra was recorded after each titration. Control tests were performed in the same fashion by replacing the DNA solution with plain buffer. For the fluorescence quenching studies, conjugate **16** was selectively excited at 412 nm and its fluorescence was recorded at 420–580 nm. Compound **16** (10.2 mg) was dissolved in 10 mL of DMSO and diluted 100 times with 50 mmol Tris•HCl buffer to give a final concentration of 19 μmol. A 3 mL of this solution was titrated with 200 μL aliquots of salmon testes DNA (2.25 mmol) dissolved in 50 mmol Tris•HCl buffer and the spectra was recorded after each titration. Control tests were performed in the same fashion by replacing the DNA solution with plain buffer.

Isolation of the 5′ ^{32}P end-labeled *Sal* I/*Sph* I restriction fragment of the pBR322 duplex DNA. BR322 DNA was cleaved at the unique *Sal* I site, dephosphorylated using calf intestine phosphatase, 5′-^{32}P-phosphorylated using T4 polynucleotide kinase and [γ-^{32}P] ATP (4.5 μCi/pmol) and cleaved again at the unique *Sph* I site. This generated a 93-base-pair double-stranded fragment, 5′-^{32}P-labeled at the *Sal* I end, which was purified by gel electrophores is in a nondenaturing 10% polyacrylamide gel and subsequent column chromatography on Sephadex G-25.

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