Bioconjugate Chemistry

Communication

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Relationship Between Structure of Conjugated Oxime Esters and Their Ability to Cleave DNA

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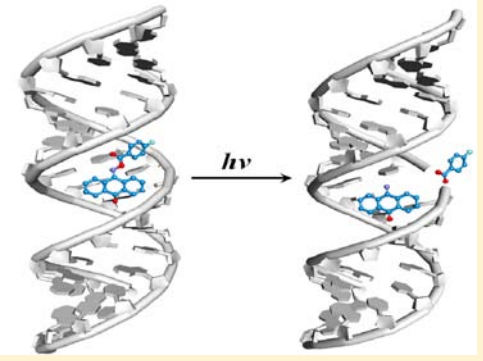
Supporting Information

ABSTRACT: The size and geometry of polycycles are critical to intercalation into DNA. This work involves the establishment of a new compound library that includes 35 *O*-benzoyl oxime esters with intercalators of five types. These conjugated compounds were synthesized by the condensation of substituted benzoyl chlorides (XC_6H_4COCl; X = H, Me, CN, F, and NO_2) or naphthoyl chlorides with oximes of fluoren-9-one, 9,10-anthraquinone, xanthen-9-one, thioxanthen-9-one, and 9*H*-thioxanthen-9-one 10,10-dioxide to give the corresponding esters in 80−99% yields. All of these compounds could cleave DNA when photolyzed by UV light. Of these conjugates, 9,10-anthraquinone-*O*-9-(4-fluorobenzoyl)oxime with a binding constant of 4.49×10^4 M^{-1} cleaved DNA most efficiently. Examination of the structure−activity relationship supports a conclusion that two factors affect DNA-cleaving potency. These are (1) the planarity of the intercalating moiety, and (2) the size and substituents of the benzoyl ring. The DNA-cleaving ability followed the order 9,10-anthraquinone > fluoren-9-one ≥ xanthen-9-one ∼ thioxanthen-9-one > 9*H*-thioxanthen-9-one 10,10-dioxide. The benzoyl-containing oxime ester conjugates were more active than the corresponding naphthoyl-containing conjugates. The potency that was associated with the different substituents on the benzoyl ring followed the order F > CN ≥ NO_2 > Me ∼ H.



INTRODUCTION

Intercalating moieties are commonly incorporated into DNA-cleaving agents to improve their activity.[1] Schuster, Williams, and co-workers[2] used anthraquinone derivatives as intercalators for DNA scission. Fluoren-9-one and thioxanthen-9-one also serve as DNA intercalators, as indicated by Kearns and co-workers.[3] Cushman and co-workers[4] found that the electrostatic attraction between intercalating indeno[1,2-*c*]-isoquinolines and the base pairs of the "DNA−topoisomerase I cleavage complex" plays a role in stabilization. More importantly, some organic intercalators are valuable drugs, as reported by Braña et al.[5] Such drugs have been used to treat ovarian cancer, breast cancer, and acute leukemias. In 2004, Dervan and co-workers[6] synthesized a polyamide−acridine conjugate as a combination of a groove binder and an intercalator for sequence-specific DNA bis-intercalation.

Arylhydrazones can nick DNA when UV light is used as a trigger.[7] In these photochemical reactions, iminyl and aminyl radicals are generated to cleave DNA. Zard and co-workers[8] described elegant methods for producing iminyl radicals by reacting *O*-benzoyl oximes with tributylstannane and 2,2′-azobisisobutyronitrile or with nickel powder and acetic acid.[9] Intramolecular capture of such species can efficiently lead to the formation of heterocycles and alkaloids.[10] Iminyl and benzoyloxy radicals can be formed through the homolytic fission of an *O*-benzoyl oxime by photolysis.[11−13] The weak N−O bond[14] in oxime esters causes conjugates in this class to have high reactivity.

Some oxime esters have recently been reported to exhibit DNA-cleaving ability in a process that is triggered by UV light.[15−17] These esters perform single-strand scission because of the iminyl and carboxyl radical species. Meanwhile, an oxime moiety is incorporated into aromatic nuclei as a chromophore that absorbs UV light. We planned to study systematically the intercalating capacity of the polycyclic moieties including fluoren-9-one, 9,10-anthraquinone, xanthen-9-one, thioxanthen-9-one, and 9*H*-thioxanthen-9-one 10,10-dioxide when conjugated with a benzoyl or a naphthoyl group. These intercalators have similar ring sizes but different degrees of planarity. Our goal is to understand the relationship between their structure and their activity, as doing so will support the design of new agents cleaving DNA efficiently.

This paper describes the synthesis of 35 oxime ester conjugates that bore various intercalating moieties and aroyl

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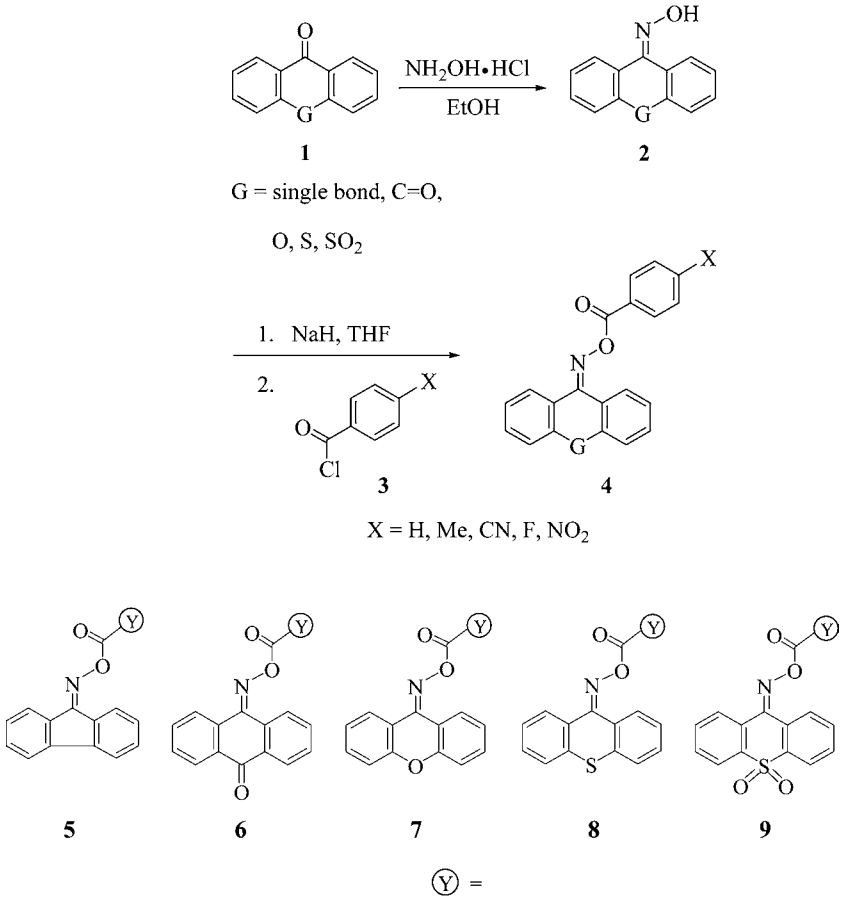
Scheme 1. Synthesis of Conjugated Oxime Esters 4 with Various Intercalating Moieties and Aroyl Groups



groups with different substituents. Their DNA-cleaving ability was analyzed. It depended on the intercalating moiety on one side of the conjugates, as well as the electronic and the steric effects of the substituents on the other side. The relationship between their structure and activity is revealed—the intercalating polycyclic moieties and the substituents in the benzoyl nucleus are ordered in terms of their effect on DNA scission.

RESULTS

Synthesis of Conjugated Oxime Esters. First, the oximation of various ketones **1** with hydroxyamine hydrochloride in ethanol was carried out according to the methods shown in Scheme 1. Second, the corresponding oximes **2** were condensed with 1.3−1.5 equiv of benzoyl chlorides **3** to generate the desired conjugated oxime esters **4**. The intercalating moieties in the final targets **4** came from the starting materials **1**, which were fluoren-9-one, 9,10-anthraquinone, xanthen-9-one, thioxanthen-9-one, and 9*H*-thioxanthen-9-one 10,10-dioxide. At the para position of benzoyl chlorides was a substituent of Me, CN, F, or NO_2. As well as benzoyl chlorides **3**, 1- and 2-naphthoyl chlorides were used for the oximation. Accordingly, conjugated compounds **5**−**9** with purity >98.0% were generated in high yields (80−99%). Their values of absorption maximum (λ_{max}) and molar absorptivity (ε) of UV spectra were summarized in Table 1.

DNA Cleavage by Conjugated Oxime Esters Upon UV Irradiation. To a sodium phosphate buffer solution (Na_2HPO_4 and NaH_2PO_4; 0.10 M, pH = 6.0) that contained supercoiled circular *ϕX174* RFI DNA (form I, 50 μM/base pair) was added an oxime ester (**5**−**9**) at a concentration of 500 μM. The solution under aerobic conditions was irradiated with UV light (312 nm, 16 W) at room temperature for 2.0 h. The results of gel electrophoresis revealed that relaxed circular DNA (form II) was generated in all of these experiments. The ratios of (form II)/(form I) for **5**−**9** ranged from 0.37 to 44 (Table 2). Moreover, our results shown in Figure 1 indicate that anthraquinone oxime ester **6c** also performed double strand cleavage of DNA.

Measurements of the doses of conjugates **6c**−**e** (0−50 μM) presented in Figure 2 show that the cleavage of DNA depended on their concentrations. These three oxime ester conjugates that bore a 9,10-anthraquinone moiety nicked DNA with (form II)/(form I) = 1.0 at concentrations even as low as 12, 26, and 2.5 μM, respectively. This figure illustrates a two-phase process for DNA scission by oxime esters: a fast one at low oxime ester concentration and a slower one at concentration above 2.5 μM.

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Table 1. Values of Absorption Maximum (λ_{max}) and Molar Absorptivity (ε) of UV Spectra for Conjugated Oxime Esters

compound	UV (CH_2Cl_2) λ_{max} (ε)
5a	304 (ε 9,600), 259 (ε 48,000), 251 (ε 35,000), 225 (ε 19,385)
5b	304 (ε 13,543), 259 (ε 60,547), 250 (ε 47,109), 221 (ε 25,469)
5c	310 (ε 11,097), 259 (ε 49,321), 250 (ε 42,037), 217 (ε 21,296)
5d	310 (ε 14,159), 259 (ε 54,458), 250 (ε 42,320), 221 (ε 23,631)
5e	304 (ε 17,104), 259 (ε 68,476), 219 (ε 27,429), 214 (ε 26,000)
5f	314 (ε 12,595), 259 (ε 53,971), 251 (ε 43,197), 224 (ε 55,235)
5g	314 (ε 12,782), 259 (ε 54,774), 251 (ε 43,840), 224 (ε 56,057)
6a	310 (ε 7,315), 279 (ε 20,444), 252 (ε 29,259), 218 (ε 26,444)
6b	310 (ε 9,131), 280 (ε 20,325), 252 (ε 29,351), 218 (ε 20,994)
6c	310 (ε 19,243), 259 (ε 79,109), 250 (ε 67,426), 217 (ε 34,158)
6d	310 (ε 10,351), 279 (ε 21,839), 253 (ε 33,124), 231 (ε 26,228)
6e	310 (ε 12,759), 278 (ε 18,416), 253 (ε 28,614), 218 (ε 18,911)
6f	321 (ε 12,161), 283 (ε 11,390), 221 (ε 51,358)
6g	304 (ε 10,504), 282 (ε 15,228), 236 (ε 60,020)
7a	340 (ε 12,240), 221 (ε 45,217)
7b	340 (ε 12,265), 222 (ε 42,904)
7c	343 (ε 7,815), 238 (ε 21,346), 220 (ε 24,674), 212 (ε 14,182)
7d	340 (ε 10,109), 221 (ε 37,470)
7e	347 (ε 10,987), 239 (ε 16,760), 220 (ε 33,582)
7f	341 (ε 13,583), 222 (ε 65,585), 211 (ε 22,909)
7g	340 (ε 14,604), 235 (ε 56,126), 222 (ε 61,634)
8a	357 (ε 4,192), 233 (ε 26,420), 216 (ε 17,761)
8b	357 (ε 4,159), 234 (ε 22,826)
8c	360 (ε 2,181), 246 (ε 32,933)
8d	357 (ε 5,025), 233 (ε 27,771), 212 (ε 19,179)
8e	360 (ε 3,534), 257 (ε 15,714), 233 (ε 13,835)
8f	356 (ε 3,614), 224 (ε 40,079), 207 (ε 19,382)
8g	357 (ε 15,506), 238 (ε 57,950)
9a	304 (ε 6,768), 264 (ε 16,694), 229 (ε 32,149), 208 (ε 20,579)
9b	304 (ε 12,652), 269 (ε 20,603), 231 (ε 35,427), 203 (ε 23,744)
9c	304 (ε 7,184), 264 (ε 16,746), 235 (ε 27,937), 218 (ε 22,302)
9d	304 (ε 14,376), 268 (ε 18,198), 230 (ε 34,142), 220 (ε 30,676)
9e	304 (ε 12,399), 268 (ε 23,100), 232 (ε 26,500), 217 (ε 27,100)
9f	320 (ε 8,788), 222 (ε 57,279)
9g	304 (ε 10,220), 234 (ε 69,510)

At low dose with a low compound base pair ratio, **6e** was more potent than **6c**. The outcome of the two-phase process indicates that two different binding modes may exist, including intercalation and groove binding.

Measurement of Binding Constants for Conjugated Oxime Esters with an Intercalating Moiety. For the measurement of the apparent equilibrium binding constants (K_{app}), the oxime esters were used to inhibit the binding of ethidium bromide to calf thymus DNA.[18] The solubility, however, was poor in phosphate buffers for most oxime esters except the anthraquinone derivatives. The K_{app} values of anthraquinone oxime ester conjugates **6c–e** were obtained as 3.31×10^4, 4.49×10^4, and 3.64×10^4, respectively (Figure 3).

DISCUSSION

Intercalation occurs when ligands (such as polycycles and arenes) with a suitable size fit well in between base pairs in DNA.[19−21] The planarity of the nuclei generally influences the ability of the molecules to intercalate with DNA.[22−24] The results in Table 2 reveal that anthraquinone–benzoyloxime conjugates **6c–e** exhibited higher potencies with values of 11–44 than most others, as determined by the ratio of (form II)/(form I). Conjugate **5c** with the fluoren-9-one nucleus also exhibited a significant potency of 24. Therefore, 9,10-anthraquinone and fluoren-9-one have been proven to be better DNA intercalators than xanthen-9-one, thioxanthen-9-one, and 9*H*-thioxanthen-9-one 10,10-dioxide.[3,5] These results emerge clearly from the columns in Figure 4. *Overall, potencies followed the order 9,10-anthraquinone > fluoren-9-one ≥ xanthen-9-one ∼ thioxanthen-9-one > 9H-thioxanthen-9-one 10,10-dioxide.* This order matches the order of planarity of these polycyclic moieties.

Table 2. Ratios of (Form II)/(Form I) in DNA Cleavage Obtained by Use of Conjugated Oxime Esters 5–9 with Various Intercalating Moieties and Aroyl Groups[a,b]

	intercalating moiety				
aroyl group	fluoren-9-one (cf. **5**)	9,10-anthraquinone (cf. **6**)	xanthen-9-one (cf. **7**)	thioxanthen-9-one (cf. **8**)	thioxanthen-9-one 10,10-dioxide (cf. **9**)
C_6H_5CO (cf. **a**)	1.8	5.7	2.1	2.7	0.42
p-Me-C_6H_4CO (cf. **b**)	2.1	5.2	2.5	1.6	0.37
p-CN-C_6H_4CO (cf. **c**)	24	31	1.4	1.4	1.9
p-F-C_6H_4CO (cf. **d**)	6.1	44	4.9	1.9	2.1
p-NO_2–C_6H_4CO (cf. **e**)	1.3	11	1.8	1.2	1.0
1-$C_{10}H_7CO$ (cf. **f**)	0.82	1.2	1.3	0.85	1.1
2-$C_{10}H_7CO$ (cf. **g**)	0.92	1.0	1.1	0.78	0.92

[a]Cleavage of supercoiled circular $\phi X174$ RFI DNA (form I; 50 μM/base pair, molecular weight 3.50×10^6, 5386 base pairs in length) to form relaxed circular DNA (form II) under aerobic conditions and photolysis under 312 nm UV light at room temperature for 2.0 h. [b]Analyzed by gel electrophoresis with 1.0% agarose gel and ethidium bromide staining.

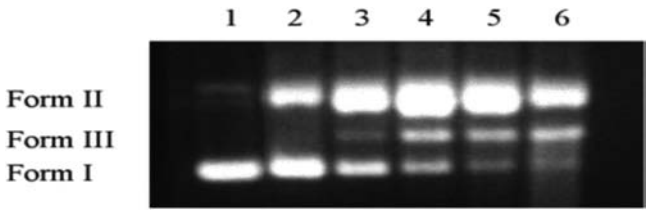

Figure 1. Dose measurement of oxime ester **6c** for its DNA cleaving ability in sodium phosphate buffer (pH 6.0, 0.10 M) upon irradiation with 312 nm UV light at 25 °C for 2.0 h; Lane 1, DNA with **6c**, 500 μM, in the dark; Lanes 2–6, 25, 50, 100, 250, 500 μM, individually.

The aroyl group on the other terminal of oxime ester conjugates influenced their DNA-cleaving capacity. In these conjugates, almost all of the phenyl-containing compounds were more potent than the naphthyl-containing compounds (Figure 4 and Table 2). None of the columns that are associated with the conjugates containing a naphthyl group (1- or 2-$C_{10}H_7$) had a (form II)/(form I) ratio of over 1.5. Accordingly, the size of the aroyl groups also affected the capacity of DNA scission. In the series of anthraquinone–benzoyloxime conjugates, *the effectiveness of the substituents on the benzoyl nuclei in DNA scission followed the order F > CN > NO_2 > Me ∼ H.* The substituents are therefore concluded to contribute substantially to DAN cleavage.

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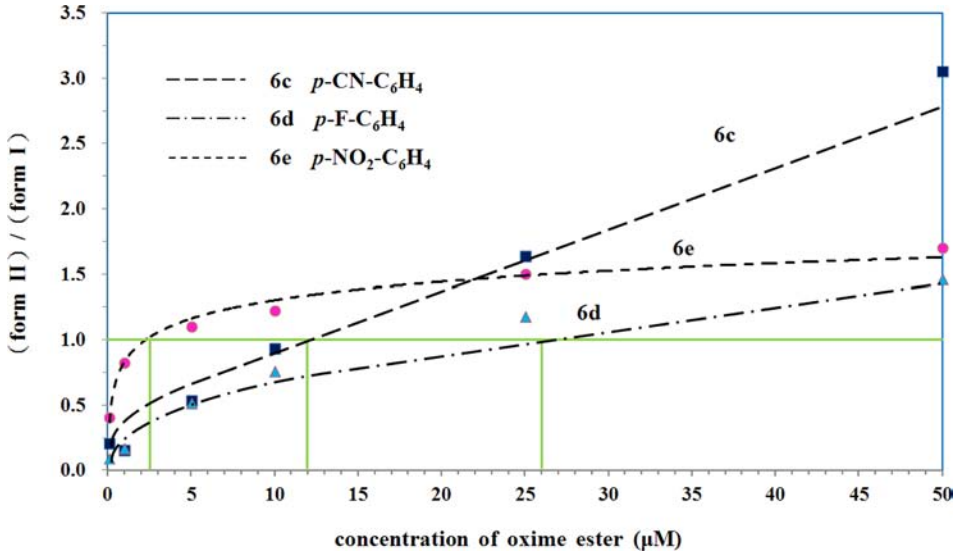

Figure 2. Measured doses of conjugated oxime esters **6c−e** and their ability to cleave DNA in a sodium phosphate buffer (pH 6.0, 0.10 M) under irradiation with 312 nm UV light.

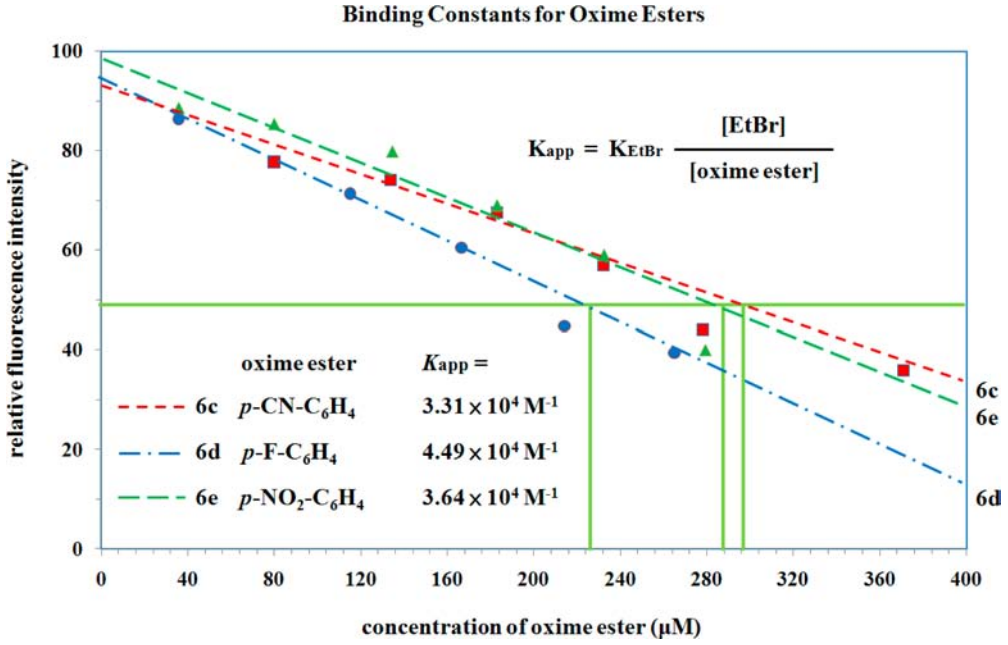

Figure 3. Apparent equilibrium binding constants of conjugated oxime ester **6c−e** determined by inhibition of ethidium bromide binding to calf thymus DNA in a phosphate buffer (pH = 6.0, 0.10 M).

All of the new compounds exhibited favorable solubility in DMSO and acetone. The solubility of the conjugates other than anthraquinone derivatives in methanol or ethanol was poor. When solutions of either DMSO or acetone were added to buffers, the salting-out effect[25] was evident. Given the issue of solubility, the oxime esters of anthraquinone are considered to be the best candidate DNA cleavers. The apparent equilibrium binding constants of anthraquinone derivatives **6c−e** were on the order of 10^4 M^{-1} in a phosphate buffer at pH 6.0.

Among the 35 oxime ester conjugates in the new compound library,[26] *para*-fluoro-*O*-benzoyl oxime (**6d**), bearing an anthraquinone moiety, bound DNA (K_{app} = 4.49 × 10^4 M^{-1}) efficiently and had the greatest DNA-cleaving capacity. Hence, an electron-withdrawing group in *O*-benzoyl oximes with minimal steric congestion is an ideal candidate for increasing their potency in DNA cleavage.

CONCLUSIONS

O-Benzoyl oxime ester conjugates **5−9** with five polycyclic and aromatic intercalators were synthesized. When irradiated under UV light, all of these conjugates nicked DNA. Of these oxime esters, 9,10-anthraquinone *O*-9-(4-fluorobenzoyl)oxime (**6d**) was the most potent cleaver. Investigations of the relationship between their structures and activities reveal the involvement and functions of the two adjacent moieties in the conjugated molecules. The nuclei are crucial to intercalation and the attachment of an electron-withdrawing group to the benzoyl nucleus increases the potency of oxime ester conjugates.

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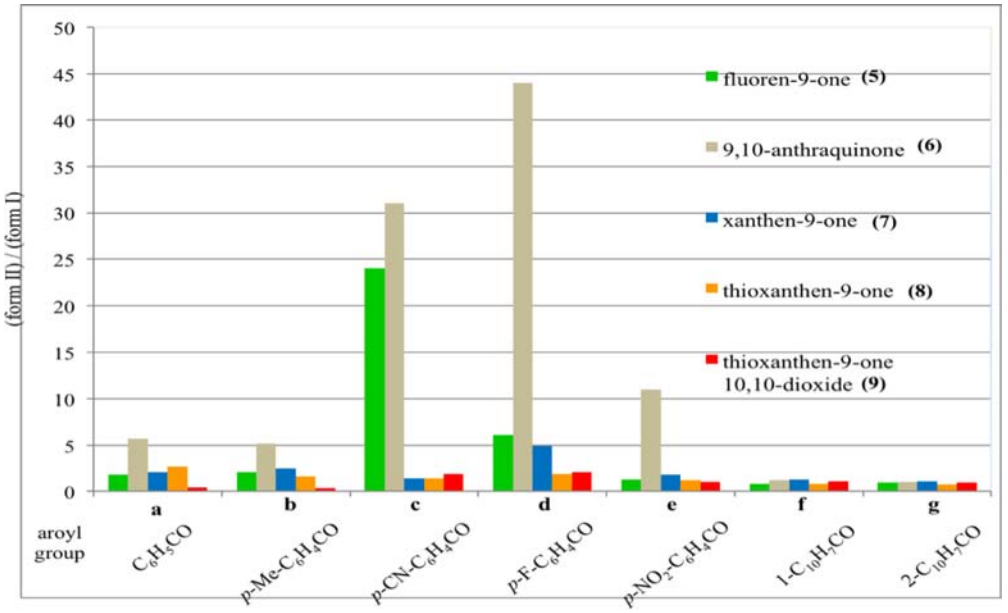

Figure 4. Differences in DNA-cleaving capacity are evident from the column representation of conjugated oxime ester conjugates **5**–**9**, which bore different intercalating moieties and aroyl groups.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, IR, UV, HRMS, and elemental analysis data for oxime esters and their UV spectra as well as experimental details about the synthetic procedures, DNA cleaving procedure, and measurements of apparent equilibrium binding constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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