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Photoinduced Cleavage of DNA by Bromofluoroacetophenone–Pyrrolecarboxamide Conjugates

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Abstract—Bromofluoroacetophenone derivatives which produce fluorine substituted phenyl radicals that cleave DNA upon excitation were investigated as a novel photonuclease. Pyrrolecarboxamide-conjugated bromofluoroacetophenones; 4′-bromo-2′-fluoroacetophenone and 2′-bromo-4′-fluoroacetophenone were synthesized and their DNA cleaving activities and sequence selectivities were determined. Bromofluoroacetophenone–pyrrolecarboxamide conjugates were found to be effective DNA cleaving agents upon irradiation in concentration dependent manner based on plasma relaxation assay. The DNA cleaving activities of 2′-bromo-4′-fluoroacetophenone derivatives were larger than those of 4′-bromo-2′-fluoroacetophenone derivatives.

Development of DNA cleaving agents is an important subject in chemistry, biology, and medicine.[1] Therefore considerate effort has been made by chemists and biochemists to identify and characterize new molecules capable of mediating nucleic acids strand scission. Enediynes and other radical progenators are representative and proven to possess highly potent antitumor activity.[2] Especially the design of compounds, which cleave DNA under photo-irradiation,[3] is of great importance not only from a fundamental biological point of view but also in a photodynamic therapeutic approach as an antitumor agent because of their potential ability to target specific sites. In an effort to design simple photoinducible DNA cleaving agents, we recently reported that commercially available benzotriazoles and haloarenes, 4′-bromoacetophenones for DNA cleavage when combined with a DNA recognition subunit can upon photoactivation cleave DNA in a potent and selective fashion.[4]

In the present paper, we extended our previous studies on 4′-bromoacetophenones to fluoro substituted bromoacetophenones as radical progenitors for DNA cleavage and report herein that fluorinated bromoacetophenones upon excitation are highly effective DNA cleaving agents. Our studies are prompted by a series of reports that examined the effect of fluorine substitution of alkyl and aryl radical progenitors on the reactivity of the radicals generated for hydrogen atom abstraction. Enhanced reactivities of perfluoroalkyl and fluoroaryl radicals have proven by several research groups comparing with their non-fluorinated counterparts.[5] In addition, as we examined previously, it was expected that this fluorinated aryl radical generation could be localized to specific sites on DNA by attaching the DNA cleaver to DNA recognition element.[6] In the present studies, we elected to use bromofluoroacetophenones linked to distamycin type of synthetic oligopeptide, pyrrolecarboximide[7] as shown in Figure 1.

As a preliminary study, we investigated the ability of two regioisomers of simple bromofluoroacetophenones

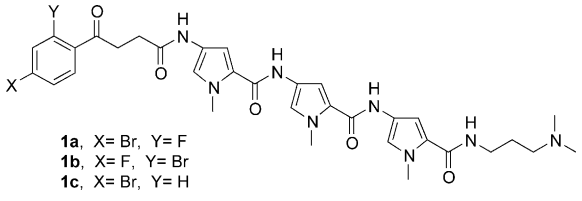

Figure 1. Bromofluoroacetophenone–pyrrolecarboxamide and 4′-bromoacetophenone–pyrrolecarboxamide conjugates as photoinducible DNA cleaving agents.

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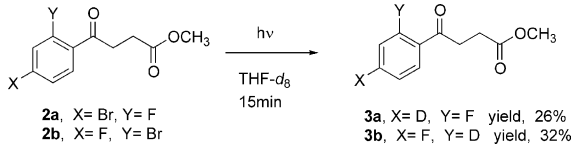
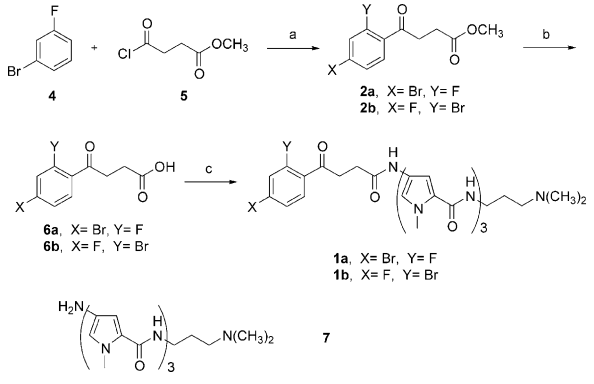
to abstract hydrogen atoms upon excitation, a required event for DNA cleavage. The photolytic behaviors of 4′-bromo-2′-fluoroacetophenone and 2′-bromo-4′-fluoroacetophenone are representative. The photolyses were performed by using a medium pressure mercury arc lamp equipped with a Pyrex filter under anaerobic conditions for 15 min. THF-d_8 was used as solvent and as a DNA surrogate. Deuterium incorporation, which is induced by homolytic cleavage of C–Br bond of methyl succinyl substituted derivatives **2** was examined. In both cases, the debrominated products **3** was the major compound produced. In accord with the intermediacy of an aryl radical, when the photolysis of 4′-bromo-2′-fluoroacetophenone and 2′-bromo-4′-fluoroacetophenone was conducted in THF-d_8, the monodeuterated 2′-fluoroacetophenone analogue and 4′-fluoroacetophenone were obtained in 15 min in 26% and 32% yield, respectively (Scheme 1).

Having established the utility of bromofluoroacetophenone as an aryl radical progenitor, we next sought to attach this subunit to a distamycin type pyrrolecarboxamide-based DNA minor groove binders. The pyrrolecarboxamide-linked bromofluoroacetophenone conjugates were synthesized as shown in Scheme 2. Preparation of 3-bromofluorobenzoyl propanoic acid **6** was performed by Friedel–Crafts succinoylation of 1-bromo-3-fluorobenzene **4** with succinic anhydride showing very low yield due to the electron withdrawing-fluorine substituent. Friedel–Crafts acylation of 1-bromo-3-fluorobenzene by treating with 3-carbomethoxypropionyl chloride gave bromofluorophenyl methyl succinoates **2** in better yield leading to two regioisomers, 4′-bromo-2′-fluoro **2a** and 2′-bromo-4′-fluoro derivatives **2b** as 1.5 to 1 ratios. The regioisomers were isolated by column chromatography and then hydrolyzed by treating with barium hydroxide to succinoic acids **6**. Small amount of **2a** and **2b** were converted to *ortho*- or *para*-fluorophenyl methyl succinoates by treatment with nBu$_3$SnH to confirm their geometry as well to get debrominated fluoroacetophenone as a standard for photolytic study. Coupling of succinoic acids **6** with aminopyrrole **7** which was prepared by Shibuya's method[8] gave pyrrolecarboxamide-linked bromofluoroacetophenones **1**.



Scheme 1. Photolysis of 4-(bromofluorophenyl)-4-oxo-butyric acid methyl ester. The yields were determined by ^{1}H NMR based on the internal standard.



Scheme 2. Preparation of bromofluroacetophenone–pyrrolecarboxamide conjugates: (a) $AlCl_3$, CS_2, 80–90 °C, 4 h, 84%; (b) $Ba(OH)_2$, CH_3OH/H_2O (2/1), rt, 2 h, 95%; (c) **7**, DCC, DMAP, DMF.

The DNA cleaving activities of bromofluoroacetophenones were determined as shown in Figures 2 and 3 by monitoring their effectiveness in converting circular supercoiled DNA (form I) to circular relaxed DNA (form II) and linear DNA (form III). Simple bromofluoroacetophenones and pyrrolecarboxamide-linked bromofluoroactophenones were irradiated at various concentrations for 30 min in the presence of ϕX174RFI DNA (30 μM/bp) in 1:9 DMSO/Tris buffer (20 mM, pH 7.5) under aerobic conditions. All compounds tested caused DNA cleavage. The cleavage efficiency was substrate and concentration dependent. DNA cleaving activity of 4′-bromo-2′-fluoroacetophenone was as much as same with that of non-fluorinated 4′-bromoacetophenone. On the other hand, 2′-bromo-4′-fluoroacetophenone showed more effective cleavage of DNA comparing with 4′-bromo-2′-fluoroacetophenone in

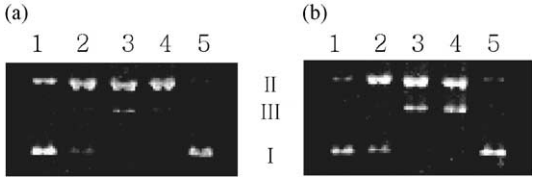


Figure 2. Light induced cleavage of DNA by (a) 4′-bromo-2′-fluoroacetophenone; (b) 2′-bromo-4′-fluoroacetophenone. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 30 min at 25 °C. Lanes 1–4, DNA (30 μM/bp) + haloacetophenone at concentrations of 0.1, 1, 10, and 100 mM, respectively; lane 5, control ϕX174RF DNA + haloacetophenone (100 mM), no hν.

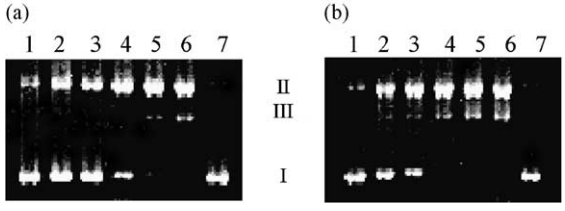


Figure 3. Light induced cleavage of DNA by (a) pyrrolecarboxamide-linked 4′-bromo-2′-fluoroacetophenone **1a** and (b) pyrrolecarboxamide-linked 2′-bromo-4′-fluoroacetophenone **1b**. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 30 min at 25 °C. Lane 1, control ϕX174RF DNA (30 μM/bp), no hν; lanes 2–6, DNA + **1a**, **1b** at concentrations of 3, 7, 15, 20, and 25 μM, respectively; lane 7, DNA + **1** (30 μM), no hν.

correspondence with the photolytic results of bromofluoroacetophenones in the absence of DNA.

In the case of pyrrolecarboxamide-linked bromofluoroacetophenones **1** for DNA cleavage, 3 to 25 μM range of low concentrations were applied. As expected, the cleavage efficiency of the compounds was remarkably increased by attachment of the DNA recognition element, pyrrolecarboxamide and strongly influenced by geometry of the fluorine substituent on the phenyl ring as well as the concentration of the test compound. The effect of the fluorine substitution itself seemed to be minor for DNA cleavage but the different geometry of fluorine substituent led to a significant difference. 2′-Bromo-4′-fluoroacetophenone derivative produced more pronounced cleavage than 4′-Bromo-2′-fluoroacetophenone derivative over the same concentration range. 2′-Bromo-4′-fluoroacetophenone derivative produced form III DNA at a concentration of 15 μM with complete disappearance of form I. The 7 μM reaction of 2′-bromo-4′-fluoroacetophenone derivative gave similar activity with those observed at 2-fold higher concentrations of 4′-bromo-2′-fluoroacetophenone derivative. It was corresponding to the preliminary investigation of photolytic behavior and cleaving activity of bromofluoroacetophenones.

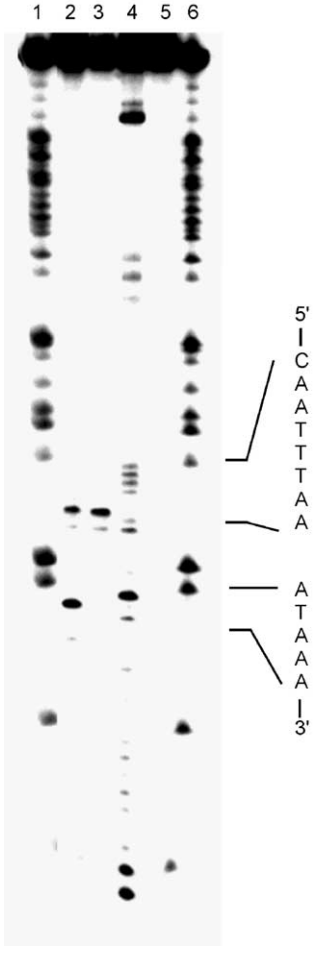


Figure 4. Autoradiogram of 10% denaturing gel polyacrylamide gel showing cleavage of 3′-^{32}P end-labeled 167 base pair restriction fragment (EcoRI/RsaI) from pBR322 by pyrrolecarboxamide-linked 4′-bromo-2′-fluoroacetophenone **1a**, pyrrolecarboxamide-linked 2′-bromo-4′-fluoroacetophenone **1b**, and pyrrolecarboxamide-linked 4′-bromoacetophenone **1c**. All reactions were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 30 min at 25 °C. The cleaving site is shown to the right of the autoradiogram. Lanes 1 and 6, Maxam-Gilbert G reaction; lanes 2–4, DNA + 30 μM of **1c**, **1a**, and **1b**, respectively; lane 5, DNA control.

The DNA sequence specificities of pyrrolecarboxamide-linked bromofluoroacetophenones were examined by DNA sequencing techniques based on electrophoretic procedures using high resolution denaturing polyacrylamide sequencing gels. The 3′-^{32}P end labeled 167 base pair restriction enzyme fragment from pBR322 was used.[9] The cleaving sites were interpreted by comparing with the Maxam-Gilbert G marker which indicates the G sites of the DNA sequence. Pyrrolecarboxamide-linked 4′-bromoacetophenone **1c** was also loaded and compared it's sequence selectivity and reactivity with those of bromofluoroacetophenone derivatives. The results in Figure 4 show that 2′-bromo-4′-fluoroacetophenone derivative **1b** has the highest activity to DNA in accord with the previous results from agars gel electrophoresis. As expected for a cleaving agent bound to pyrrolecarboxamide known as a distamycin or netropsin analogue, the cleavage intensities are the highest in AT-rich regions of the DNA as marked on the autoradiogram.

An autoradiogram (data not shown) obtained was quantified by densitometry and this data was used to

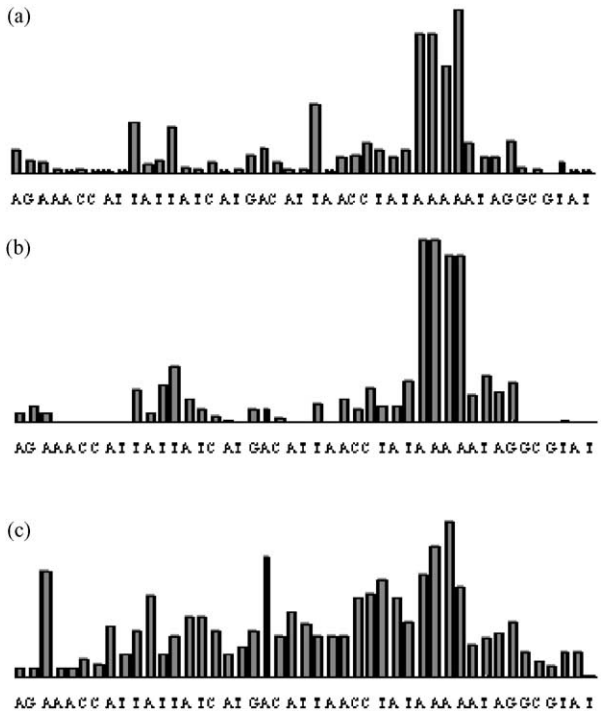


Figure 5. Histograms of DNA cleavage sites by (a) pyrrolecarboxamide-linked 4′-bromoacetophenone **1c**, (b) pyrrolecarboxamide-linked 4′-bromo-2′-fluoroacetophenone **1a**, and (c) pyrrolecarboxamide-linked 2′-bromo-4′-fluoroacetophenone **1b**. The relative extent of cleavage was estimated from the densitometric scans of the autoradiograms shown in Figure 4 and the height of the bar represents relative cleavage intensity. (a–c) 50 μM of compound **1c**, **1a** and **1b**, respectively.

construct histograms for the DNA cleavage observed. The histograms shown in Figure 5 clearly show that 4′-bromoactophenone derivative and 4′-bromo-2′-fluoroacetophenone derivative produce the similar cleavage sites and patterns leading to the cleavage within and adjacent to sites of multiple contiguous AT base pairs. On the other hand, 2′-bomo-4′-fluoroacetophenone derivative seemed to lose the selectivity although the affinity to AT base sequence still remained at the applied concentration. It is supposed that the higher reactivity of the phenyl radical species from the 2′-bromo-4′-fluoroacetophenone derivative led to the higher reactivity for the cleavage of DNA with decreasing selectivity. The cleavage assay and sequencing of the tested compounds showed prominent correlation between reactivity of the phenyl radical and DNA cleaving activities with the highest activity of 2′-bromo-4′-fluoroacetophenone derivative.

In summary, 4′-bromo-2′-fluoroacetophenone and 2′-bromo-4′-fluoroacetophenone derivatives upon irradiation can function as DNA cleaving agents putatively through the generation and reaction of phenyl radicals. 2′-Bromo-4′-fluoroacetophenones revealed higher DNA cleaving activity than 4′-bromo-2′-fluoroacetophenone derivatives supposedly induced by the higher reactivity of the phenyl radical generated from 2′-bromo-4′-fluoroacetophenones. The attachment of a minor groove binding moiety to bromofluoroacetophenone results in increased strand scission of DNA mainly in sequence selective manner. This work provides a new class of DNA cleaving agents that are easy to prepare and verify.

Acknowledgements

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References and Notes

1. (a) *Nucleic Acid Targeted Drug Design*; Propst, C. L., Perun, T. J., Eds. Marcel Dekker: New York, 1992. *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed. JAI: London, 1992; Vol. 1, p 217. (b) Fukuhara, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3187. (c) Roelfes, G.; Branum, M. E.; Wang, L.; Que, L., Jr.; Feringa, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 11517. (d) Breydo, L.; Gates, K. S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 885. (e) Warner, P. M.; Qi, J.; Meng, B.; Li, G.; Xie, L.; El-Shafey, A.; Jones, G. B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1.

2. (a) *Enediyne Antibiotics As Antitumor Agents*; Border, D. B.; Doyle, T. W., Eds. Marcel Dekker: New York, 1995. (b) Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103. (c) Kerwin, S. M.; David, W. M.; Kumar, D. PCT Int., WO 0170217, 2001. (d) Suzuki, I.; Tsuchiya, Y.; Shigenaga, A.; Nemoto, H.; Shibuya, M. *Tetrahedron Lett.* **2002**, *43*, 6779.

3. (a) Chen, T.; Voelk, E.; Platz, M. S.; Goodrich, R. P. *Photochem. Photobiol.* **1996**, *64*, 622. (b) Theodorakis, E. A.; Xiang, X.; Lee, M.; Gibson, T. *Tetrahedron Lett.* **1998**, *39*, 3383. (c) Mohler, D. L.; Dain, D. R.; Kerekes, A. D.; Nadler, W. R.; Scott, T. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 871. (d) Toshima, K.; Takano, R.; Maeda, Y.; Suzuki, M.; Asai, A.; Matsumura, S. *Angew. Chem. Int. Ed.* **1999**, *38*, 3733. (e) Manfredini, S.; Beatrice Vicentini, C.; Manfrini, M.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *Bioorg. Med. Chem. Lett.* **2000**, *8*, 2343.

4. (a) Wender, P. A.; Touami, S. M.; Alayrac, C.; Philipp, U. C. *J. Am. Chem. Soc.* **1996**, *118*, 6522. (b) Touami, S. M.; Poon, C. C.; Wender, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 7611. (c) Wender, P. A.; Jeon, R. *Org. Lett.* **1999**, *1*, 2117.

5. (a) Dolbier, W. R., Jr. *Chem. Rev.* **1996**, *96*, 1557. (b) Li, R.; Smith, R. L.; Kenttamaa, H. *J. Am. Chem. Soc.* **1996**, *118*, 5056. (c) Shtarev, A. B.; Tian, F.; Dolbier, W. R., Jr.; Smart, B. E. *J. Am. Chem. Soc.* **1999**, *121*, 7335.

6. (a) Nakatani, K.; Shirai, J.; Sando, S.; Saito, I. *J. Am. Chem. Soc.* **1997**, *119*, 7626. (b) Minnock, A.; Lin, L.-S.; Morgan, J.; Crow, S. D. G.; Waring, M. J.; Sheh, L. *Bioconjugate Chem.* **2001**, *12*, 870. (c) Arimondo, P. B.; Boutorine, A.; Baldeyrou, B.; Bailly, C.; Kuwahara, M.; Hecht, S. M.; Sun, J.-S.; Garestier, T.; Helene, C. *J. Biol. Chem.* **2002**, *277*, 3132. (d) Weyermann, P.; Dervan, P. B. *J. Am. Chem. Soc.* **2002**, *124*, 6872.

7. (a) Matsumoto, T.; Toyooka, K.; Nishiwaki, E.; Shibuya, M. *Heterocycles* **1990**, *31*, 1629. (b) Semmelhack, M. F.; Gallagher, J. J.; Ding, W. D.; Krishnamurthy, G.; Babine, R.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4357. (c) Hurley, A. L.; Maddox, M. P., III; Scott, T. L.; Flood, M. R.; Mohler, D. L. *Org. Lett.* **2001**, *3*, 2761.

8. Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945.

9. Pichersky, E. Methods in Molecular Biology 23. In *DNA Sequencing Protocols*. Griffin, H. G., Griffin, A. M. Eds.; Humana: Totowa, NJ, 1993; Chapter 31.