Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3513–3515

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Thiadiazole: A New Family of Intercalative Photonuclease with Electron Transfer and Radical Mechanisms

Yonggang Li,[a,c] Yufang Xu,[a,c] Xuhong Qian[b,*] and Baoyuan Qu[a,c]

[a]*East China University of Science and Technology, Shanghai 200237, China*
[b]*State Key Lab. Fine Chemicals, Dalian University of Technology, Dalian 116012, China*
[c]*Shanghai Key Lab. Chem. Biology, Shanghai 200237, China*

Received 1 May 2003; accepted 10 July 2003

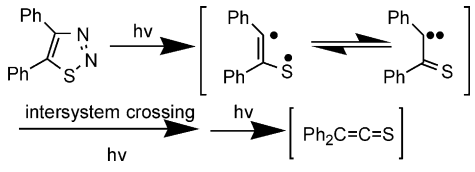
Abstract—A new family of photonuclease, thiadiazole-naphthalimide were synthesized and evaluated. Thiadiazole group was incorporated for the first time. These compounds intercalated into DNA efficiently and damaged DNA as low as 10 μM photochemically. Mechanism experiment showed that electron transfer and radicals were involved.

Photonucleases are of great interest in biology, chemistry and medicine. Triggered by the ultra-violet or visible light, they can initiate significant damage on DNA without external chemical initiators via a wide variety of mechanism such as radicals, electron transfer or singlet oxygen.[1,2] It is an effective strategy to incorporate new functional groups to design novel DNA photocleavers. Our group have reported several series of photonucleases containing hydroperoxide, *N*-aroyloxy or thiono groups, which cleaved DNA via oxygen-centered radicals or electron transfer.[3–6] Herein, a new functional group, thiadiazole, is incorporated into the area of photodamage of DNA for the first time and a new family of photonucleases based on this group are designed and assessed.

Benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), a plant activator, induced accumulation of SAR (Systematic Acquired Resistance) gene and expression of PR-1 (Pathogensis-Related) gene in plant.[7–10] It was possibly implied that thiadiazole compounds had some potential interaction with nucleic acid. Also, 1,2,3-thiadiazoles would produce several active intermediates photochemically, such as thioketocarbene, thiocarbondiradical and thioketene (Scheme 1).[11–13] We expected that these intermediates should be able to react with DNA similarly based on the fact that triazole could be used as a photonuclease.[14–16] Few photocleaver had been reported to possess the 1,2,3-thiadiazole ring as functional group. Thus, it was chosen as a photo-triggered active part.

In our research, naphthalene ring was conjugated to the thiadiazole moiety to shift the absorption bathochromically, which was safe to manipulate and quite matched with the photoirradiation wavelength. Moreover, planar rigid structure would facilitate the drug to intercalate into DNA efficiently as it was known that enhanced DNA affinity was helpful for the photodamage of DNA.[3] *N*,*N*-dimethylaminoethyl or similar groups were also chosen for the enhanced DNA affinity because these groups appeared commonly in clinically useful anti-cancer drugs that interacted with DNA such as amonafide and mitonafide.[17,18] Hence, a new family of thiadiazole photonuclease $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ (Fig. 1) was designed.

These compounds were synthesized from 4-bromo-3-nitronaphthalic anhydride shown in Scheme 2. After separation with column chromatography their structures were confirmed by IR, ^{1}H NMR, HRMS and element analysis.[19]



Scheme 1. Photolysis of typical thiadiazole compound.

*Corresponding author. Tel.: +86-411-367-3466; fax: +86-411-367-3488; e-mail: xhqian@dlut.edu.cn

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00737-6

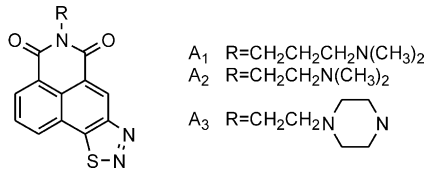
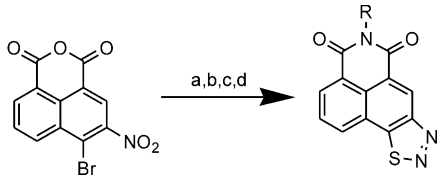


Figure 1. Novel photonucleases $\mathbf{A_1}$–$\mathbf{A_3}$.



Scheme 2. Synthesis of target compounds: (a) benzyl mercaptan, K_2CO_3, DMF, Ar, 8 h, 93% yield; (b) $SnCl_2$, HCl, 2 h, 98% yield; (c) $NaNO_2$, HCl, 0 °C, 3.5 h, 86% yield; (d) RNH_2, ethanol, 2.5 h, yield: $\mathbf{A_1}$: 65%; $\mathbf{A_2}$: 70%; $\mathbf{A_3}$: 58%.

It was found that the absorption wavelengths of compounds $\mathbf{A_1}$–$\mathbf{A_3}$ were at 362 nm, which was quite matched with the photoirradiation wavelength of transluminator (366 nm). Also, their weak fluorescence ($\Phi < 0.0003$) implied that their excitation energy was easily transferred from the excited singlet state to lead to the cleavage of the N–S bond,[11,13] besides the dissipation in internal transfer (data not shown). Meanwhile, with $\mathbf{A_2}$ as an example, a study of the intercalation of $\mathbf{A_2}$ to calf thymus DNA was carried out using UV–vis spectra technique instead of the fluorescence quenching method,[4,20] as $\mathbf{A_1}$–$\mathbf{A_3}$ had very weak fluorescence. By titration a solution of $\mathbf{A_2}$ (in 30 mM Tris–HCl buffer, pH 7.5) with an increased CT–DNA it appeared hypochromic and bathochromic shift in the UV spectrum of $\mathbf{A_2}$ (Fig. 2), in accordance with the intercalation model.[21]

The cleavage activities of compounds $\mathbf{A_{1-3}}$ were evaluated using closed supercoiled pBR322 DNA under photoirradiation with a transluminator (366 nm) at a distance of 20 cm at 0 °C for 2 h and analyzed on a 1% agarose gel. The photocleavage efficiency was defined by the degree of the relaxation of supercoiled DNA, relaxed circular DNA (single-strand cleavage) as form II. All of these compounds were found to cleave DNA efficiently into form II as shown in Figure 3a and $\mathbf{A_2}$ acted more efficiently than the other two under this condition. Further experiment showed that $\mathbf{A_2}$ could damage the supercoiled DNA into the relaxed circular form as low as 10 μM (Fig. 3b). Its DNA photodamage ability was sensitive to pH value. With the elongation of irradiation time, it photo-nicked DNA more intensely. However, no DNA damage was observed in the absence of light.

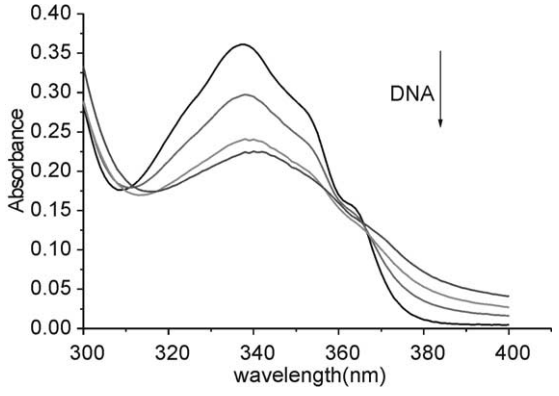


Figure 2. Interaction of $\mathbf{A_2}$ and calf thymus DNA. Absorption changes of $\mathbf{A_2}$ during addition of calf thymus DNA (0, 50, 100, 200 μM) in DMSO–10 mM Tris–HCl (pH 7.5) solution (1:4, v/v).

Mechanism experiment was performed with the addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide radical scavenger), superoxide dismutase (SOD, superoxide radical killer) and DMSO (radical quencher) (Fig. 4). Obviously, singlet oxygen was not involved in the DNA damage. However, DMSO inhibited its reactivity a little and DTT did greatly (Fig. 4, lanes 1, 2 and 7). Thus, it was believed that electron transfer from nucleobase to compound and then to oxygen to form superoxide played a major role in the photodamage of DNA besides radicals produced upon photoirradiation. As for the similar triazole derivatives, Wender group proposed the electron transfer mechanism.[15,16] However, Armitage figured that the spontaneous nature of the cleavage and a distinct preference for cleavage at the 3′-G of the GG steps by the triazole argued against cleavage by an electron transfer pathway,[1] and Manfredini reported the possible radical mechanism for the pyrazolo-trizoles DNA

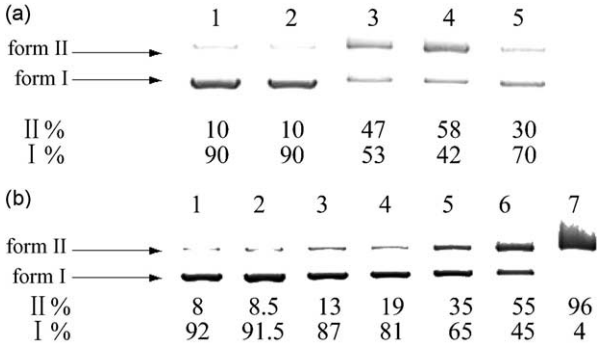


Figure 3. Photocleavage of closed supercoiled DNA by photonucleases in 10% acetonitrile in Tris–HCl buffer. (a) photoirradiation: 2 h; lane1: DNA alone (no hν); lane 2: DNA alone; lanes 3–5: DNA and compound $\mathbf{A_1}$–$\mathbf{A_3}$ at concentration of 100 μM. (b) lane 1: DNA alone (no hν); lane 2: DNA alone (hν, 120 min); lanes 3–7: DNA and $\mathbf{A_2}$ at concentration of 10, 20, 50, 100, 200 μM, respectively.

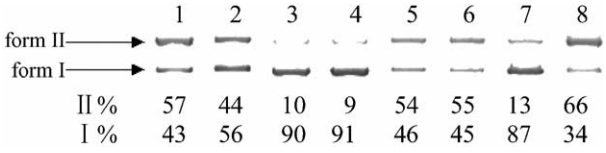


Figure 4. Effect of additives on the photocleavage of DNA in 10% acetonitrile in Tris–HCl buffer. photoirradiation: 2 h; lane 3: DNA alone (no hν); lane 4: DNA alone; lanes 1 and 5: DNA and compound $\mathbf{A_2}$ at concentration of 50 μM; lane 6: DNA and $\mathbf{A_2}$ in the presence of histidine (6 mM); lane 7: DNA and $\mathbf{A_2}$ in the presence of dithiothreitol (DTT, 30 mM); lane 8: DNA and $\mathbf{A_2}$ in the presence of superoxide dismutase (SOD, 100 μg/mL); lane 2: DNA and $\mathbf{A_2}$ with addition of DMSO (1.4 M).

cleaving agents.[14] Here, thiadiazole-naphthalimide photocleaved DNA via combined electron transfer and radical mechamism. (The triplet state of naphthalimide was also possibly involved in the photocleavage of DNA.[22,23]) It should be pointed out that SOD did not slow the rate of DNA-cleaving reaction because the hydrogen peroxide produced by SOD from superoxide could lead to DNA damage photochemically.

In summary, the present work demonstrated the design and evaluation of novel intercalative photonucleases, thiadiazole fused naphthalimides $\mathbf{A_1}$–$\mathbf{A_3}$. Thiadiazole is incorporated as a photoactive moiety in the photonuclease study for the first time. These compounds could cleave circular supercoiled pBR322 efficiently under the irradiation of long-wavelength UV light (366 nm) via electron transfer and radical mechanism. The anti-tumor research of these compounds are in progress.

Acknowledgements

National Natural Science Foundation of China, The Ministry of Education of China and Shanghai Foundation of Science and Technology financially supported this study.

References and Notes

1. Amitage, B. *Chem. Rev*. **1998**, *98*, 1171.
2. Saito, I.; Nakatani, K. *Bull. Chem. Soc. Jpn*. **1996**, *69*, 3007.
3. Yao, W.; Qian, X.; Hu, Q. *Tetrahedron Lett*. **2000**, *41*, 7711.
4. Qian, X.; Yao, W.; Chen, G.; Huang, X.; Mao, P. *Tetrahedron Lett*. **2001**, *42*, 6175.
5. Qian, X.; Mao, P.; Yao, W.; Guo, X. *Tetrahedron Lett*. **2002**, *43*, 2995.
6. Qian, X.; Huang, T.; Wei, D.; Zhu, D.; Fan, M.; Yao, W. *J. Chem. Soc., Perkin Trans. 2* **2000**, 715.
7. Kunz, W.; Schurter, R.; Maetzke, T. *Pestic. Sci*. **1997**, *50*, 275.
8. Gorlach, J.; Volrath, S.; Knauf-Breiter, G.; Henry, G.; Beckhovec, U.; Kogel, K.-H.; Ryals, J. *Plant Cell* **1996**, *8*, 629.
9. Friedrich, L.; Lawton, K.; Ruess, W.; Masner, P.; Specker, N.; Gut Rella, M.; Meier, B.; Dincher, S.; Staub, T.; Ukness, S.; Metraux, J.-P.; Kessmann, H.; Ryals, J. *Plant J*. **1996**, *10*, 61.
10. Lawton, K.; Friedrich, L.; Hunt, M.; Weymann, K.; Delaney, T.; Kessmann, H.; Staub, T.; Ryals, J. *Plant J*. **1996**, *10*, 71.
11. Murial, H.; Torres, M.; Strausz, O. P. *J. Am. Chem. Soc*. **1979**, *101*, 3976.
12. Murai, H.; Torres, M.; Strausz, O. P. *J. Am. Chem. Soc*. **1980**, *102*, 1421.
13. Krauss, P.; Zeller, K.-P.; Meier, H.; Muller, E. *Tetrahedron* **1971**, *27*, 5953.
14. Manfredini, S.; Vicentini, C. B.; Manfrini, M.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *Bioorg. Med. Chem*. **2000**, *8*, 2343.
15. Wender, P. A.; Touami, S. M.; Alayrac, C.; Philipp, U. C. *J. Am. Chem. Soc*. **1996**, *118*, 6522.
16. Touami, S. M.; Poon, C. C.; Wender, P. A. *J. Am. Chem. Soc*. **1997**, *119*, 7611.
17. Malviya, V. K.; Liu, P. Y.; Alberts, D. S.; Surwit, E. A.; Craig, J. B.; Hanningan, E. V. *Am. J. Clin. Oncol*. **1992**, *15*, 41.
18. Rosell, R.; Carles, J.; Abad, A.; Ribelles, N.; Barnadas, A.; Benavides, A.; Martin, M. *Invest. New Drugs* **1992**, *10*, 171.
19. **A1**: mp 101–102 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.9 (m, 2H, NCH_2), 2.22 (s, 6H, NCH_3), 2.43 (t, $J_1=7.16$ Hz, $J_2=7.27$ Hz, 2H, CH_2), 4.23 (t, $J_1=7.49$ Hz, $J_2=7.66$ Hz, 2H, $CONCH_2$), 7.91 (t, $J_1=7.74$ Hz, $J_2=7.75$ Hz, 1H, 2-H), 8.4 (t, $J_1=7.86$, $J_2=0.86$, 1H, 1-H), 8.73 (t, $J_1=7.16$, $J_2=0.81$, 1H, 3-H), 9.58(s, 1H, 7-H). HRMS: $C_{17}H_{16}N_4O_2S$ calculated: 340.0994; found: 340.0991. *m*/*z* (%): 340.0991 (M^+) (10.41), 240.0145 (2.96), 210.0064 (10.85), 157.0130 (5.39), 84.0832 (71.88), 58.0655 (100). IR (KBr): 2960, 2870, 1710, 1670, 1300 cm^{-1}. element analysis: $C_{17}H_{16}N_4O_2S$ calculated: C59.98, H4.74, N16.46; found: C59.74, H4.48, N16.65. **A2**: mp 178–179 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.25 (s, 6H, NCH_3), 2.57 (t, $J_1=6.87$ Hz, $J_2=6.97$ Hz, 2H, NCH_2), 4.19 (t, $J_1=6.86$ Hz, $J_2=6.99$ Hz, 2H, $CONCH_2$), 8.06 (t, $J_1=8.06$ Hz, $J_2=7.72$ Hz, 1H, 2-H), 8.64 (d, $J=7.44$ Hz, 1H, 1-H), 8.85(d, $J=8.04$ Hz, 1H, 3-H), 9.38 (d, $J=1.52$ Hz, 1H, 7-H). HRMS: $C_{16}H_{14}N_4O_2S$ calculated: 326.0837; found: 326.0788. *m*/*z* (%): 326.0788 (M^+) (25.29), 254.0261 (14.92), 209.9968 (39.54), 182.0024 (45.43), 155.9992 (42.73), 71.0683 (84.29), 58.0493 (82.70). IR (KBr): 2940, 2870, 1700, 1660, 1300 cm^{-1}. element analysis: $C_{16}H_{14}N_4O_2S$ calculated: C58.88, H4.32, N17.17; found: C58.69, H4.17, N17.05. **A3**: mp 145–146 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.54 (br, s, 4H, $NHCH_2$ (cyclo)), 2.67 (t, $J_1=6.95$ Hz, $J_2=6.94$ Hz, 2H, CH_2), 2.81 (t, $J_1=4.76$ Hz, $J_2=4.79$ Hz, 4H, NCH_2(cyclo)), 4.34 (t, $J_1=6.93$ Hz, $J_2=7.01$ Hz, 2H, $CONCH_2$), 7.92 (t, $J_1=7.8$ Hz, $J_2=7.840$ Hz, 1H, 2-H), 8.41 (d, $J=7.96$ Hz, 1H, 1-H), 8.73 (d, $J=7.35$, 1H, 3-H), 9.58(s, 1H, 7-H). HRMS: $C_{18}H_{17}N_5O_2S$ calculated: 367.1103; found: 367.1095. *m*/*z* (%): 367.1095 (M^+) (5.33), 325.0751 (6.87), 282.0327 (4.27), 254.0289 (8.85), 182.0091 (4.53), 157.0128 (2.36), 99.0940 (100). IR (KBr): 3310, 2960, 2870, 1710, 1670, 1300 cm^{-1}. element analysis: $C_{18}H_{17}N_5O_2S$ calculated: C 58.84, H 4.66, N 19.06; found: C58.75, H4.92, N18.86.
20. Stevenson, K. A.; Yen, S.-F.; Yang, N.-C.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem*. **1984**, *27*, 1677.
21. Bilik, P.; Tanious, F.; Kumar, A.; Wilson, W. D.; Boykin, D. W.; Colson, P.; Houssier, C.; Facompre, M.; Tardy, C.; Bailly, C. *ChemBioChem*. **2001**, *2*, 559.
22. Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K. *J. Am. Chem. Soc*. **1995**, *117*, 6406.
23. Rogers, J. E.; Kelly, L. A. *J. Am. Chem. Soc*. **1999**, *121*, 3854.