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Synthesis of a Heterocyclic Aza-Enediyne and Its DNA-Cleavage Properties

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Abstract—The benzimidazolium salt **2**, incorporating an aza-enediyne moiety, has been prepared and is shown to be a very effective DNA-cleavage agent under mild physiological conditions. Its mechanism of action is currently under investigation but may involve the generation of a diradical intermediate, DNA alkylation, or both.

Introduction

The last decade ushered in the investigation of several potent antitumor, antibiotic natural products such as calichemicin,[1] dynemicin,[2] and neocarzinostatin,[3] all of which cleave DNA upon suitable triggering of core diradical-generating structures. These natural products are extremely toxic and there has been much effort directed towards the design of synthetic analogues capable of cleaving DNA in a similar manner.[4] There are, however, very few examples of aza-substituted analogues. Several groups have investigated the ability of aza-substituted enyne allenes to undergo radical-generating cyclizations, but none have demonstrated DNA-cleavage activity.[5]

Our approach utilizes mild, physiologically relevant 'triggering' mechanisms and the effect of heteroatom substitution in enediyne moieties for the design of novel DNA-cleavage warheads with the goal of increasing antitumor selectivity without sacrificing potency.[6] Continuing our efforts in this area, we sought to develop a hydrolytically stable heterocyclic system incorporating an aza-enediyne moiety that would be physiologically viable (Fig. 1).[7] The *N*-propargyl, 2-alkynyl heterocycle shown may be able to undergo pH-triggered isomerization to generate an *N*-allenyl, 2-alkynyl species. The aza-enyne allene moiety may be capable of an aza-Myers type cyclization, thereby generating a diradical persistent enough to perform hydrogen atom abstraction under physiological conditions. Alternatively, electrophilic chemistry may occur via the addition of a DNA nucleophile at the 2-alkyne position. DNA cleavage is expected in either case. The *N*-propargyl, 2-alkynyl heterocyclic system has the potential of being tunable; varying the heterocyclic framework and the propargyl and alkyne substituents may modulate the desired reactivity such that a suitable antitumor drug candidate can be developed.

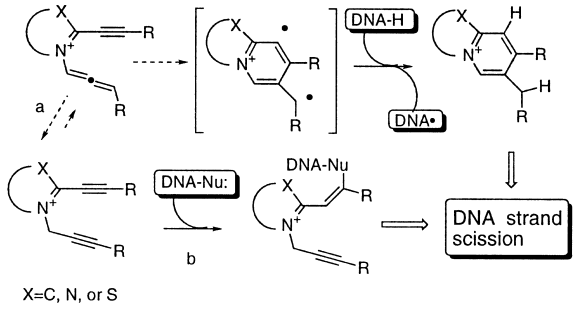

Figure 1. Two possible mechanisms for DNA cleavage arising from an *N*-propargyl-2-alkynyl heterocyclic system: (a) isomerization, followed by Myers cyclization to generate a diradical; (b) nucleophilic attack.

Synthesis and DNA Cleavage

We now report the synthesis and DNA-cleavage activity of 2-phenylethynyl-1-(3-phenyl-prop-2-ynyl)-3-methyl-1*H*-benzimidazolium tetrafluoroborate or trifluoro methanesulfonate salt **2**. Synthesis of **2** is easily accomplished in two steps: condensation of phenylenediamine with phenylpropargyl aldehyde to generate 2-phenylethynyl-1-(3-phenyl-prop-2-ynyl)-1*H*-benzimidazole **1**,[8]

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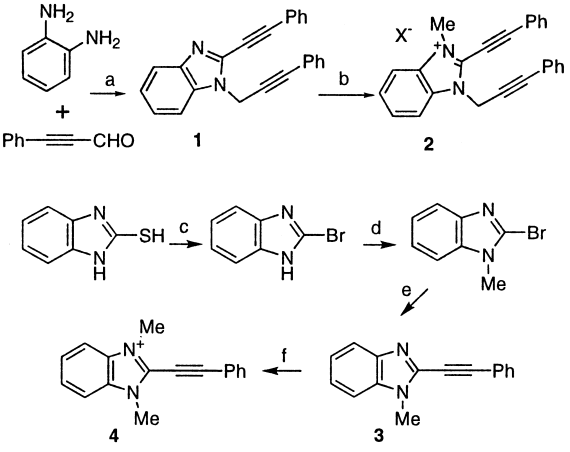
followed by alkylation with methyl triflate or trimethyloxonium tetrafluoroborate[9] (Scheme 1). Synthesis of the 2-phenylethynyl-1,3-dimethyl-1*H*-benzimidazolium trifluoromethanesulfonate salt **4** proceeds through the 2-phenylethynyl benzimidazole **3**. Palladium catalyzed coupling of phenylacetylene to 2-bromo-1-methyl-1*H*-benzimidazole,[10] followed by alkylation with methyl triflate yields **4**[11] (Scheme 1).

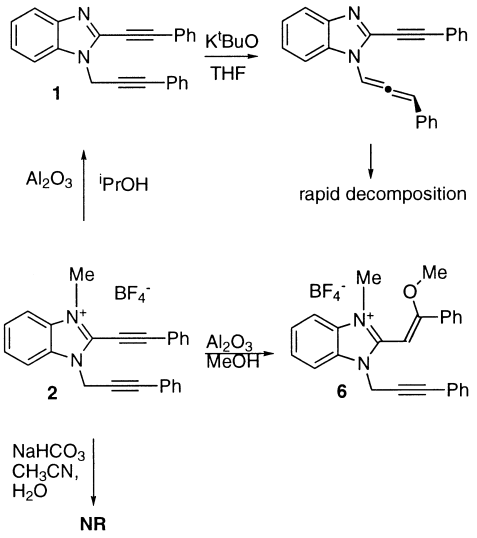
In order to probe the ability of **1** and **2** to undergo an aza-Myers type cyclization, thereby generating diradicals that might be effective DNA-cleavage agents, we investigated conditions capable of promoting an *N*-propargyl to *N*-allenyl isomerization in these benzimidazoles (Scheme 2). Isomerization of the *N*-propargyl substituent of **1** to an *N*-allene occurs only in the presence of strong base, as evidenced by the disappearance in crude reaction mixtures of the ^{13}C NMR alkyne resonances at δ 81.6 and 85.3 ppm and the appearance of new resonances at δ 198.3, 111.6, and 106.3 ppm. However, the allene is too unstable to be isolated and purified; it quickly undergoes decomposition to polymeric products, perhaps through a process involving an aza-Myers cyclization. Alkylation of a benzimidazole nitrogen (as in **2**) renders the 2-alkynyl substituent more reactive to nucleophilic substitution. Treating a methanolic solution of **2** with basic alumina affords the addition product **6** in 35% yield;[12] no isomerization to an allene is observed. In addition, interestingly, the less nucleophilic isopropanol promotes *N*-demethylation of **2** under mildly basic conditions. It would appear that the ability of these molecules to undergo either isomerization or electrophilic chemistry is extremely dependent on both heterocycle substituent effects and external conditions.

Benzimidazole **2** causes extensive cleavage of supercoiled DNA when incubated under mild conditions (Fig. 2). Both the tetrafluoroborate and triflate salts of **2** are equipotent; the counterion has no effect on the cleavage (data not shown). Frank single-strand DNA cleavage is observed at concentrations as low as 1 μM, and the amount of cleavage is unchanged after additional heating of the incubation samples. At pH 8, 100 μM concentration of **2** cleaves approximately 50% of supercoiled DNA. In contrast, compounds **1**, **4**, and **6** do not show any appreciable cleavage of supercoiled DNA, even at concentrations of 1 mM (Fig. 3).

The DNA cleavage exhibited by **2** is remarkably sensitive to temperature, pH, and the length of incubation with supercoiled DNA (Figs. 4 and 5). At 37 °C, incubation for at least 8 h is required before any appreciable cleavage is observed; nearly complete cleavage is not observed until 24 h. There is hardly any detectable cleavage at 25 °C, but at physiological temperature cleavage is substantial. The pH dependence of the observed DNA cleavage is most striking (Fig. 5). At pH 9 complete cleavage is effected by 100 μM of **2**, and complete degradation of the supercoiled DNA is observed at 1 mM of **2**. Diminished cleavage still occurs at pH 7. This pH dependence is commensurate with a mechanism in which *N*-propargyl to *N*-allenyl isomerization, followed by cyclization and diradical formation, is operative. The ease of isomerization would increase with increasing pH. The pH dependence is not inconsistent with a nucleophilic mechanism whereby



Scheme 1. Synthesis of benzimidazoles. Reagents and conditions: (a) cat. HCO_2H, toluene, 33%; (b) $(Me)_3OBF_4$ (1.3 equiv), $ClCH_2CH_2Cl$, >99%, X = BF_4^- or MeOTf (1.6 equiv), ether, 86%, X = TfO^-; (c) 48% HBr (1.3 equiv)/HOAc, Br_2 (4 equiv), 80%; (d) $(MeO)_2OSO_2$ (1.8 equiv), 1 N NaOH (2.3 equiv), 61%; (e) $Pd(OAc)_2$ (10 mol%), $P(Ph)_3$ (20 mol%), CuI (15 mol%), Et_3N, 60%; (f) MeOTf (1.6 equiv), ether, 69%.



Scheme 2.

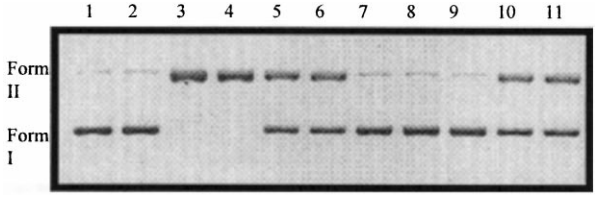


Figure 2. DNA cleavage by **2**. pBR322 plasmid DNA (50 μM base pairs) was incubated with various concentrations of **2** (pH 8, 50 mM Tris buffer, 13% v/v DMSO, 20 h at 37 °C) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain). Lanes 1, 2, and 9, DNA without drug; lanes 3–4, 1 mM **2**; lanes 5–6 and 10–11, 100 μM **2**; lane 7, 10 μM **2**; lane 8, 1 μM **2**; lanes 9, 10, and 11 were heated at 70 °C for 90 s after incubation.

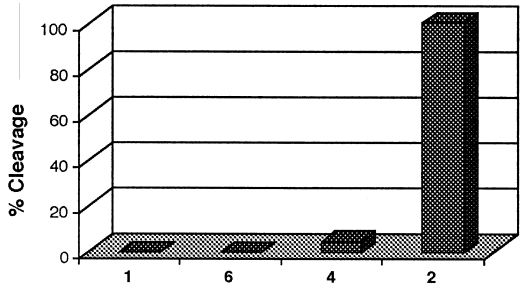


Figure 3. DNA cleavage by **1**, **6**, **4**, and **2**. ΦX174 (RF-1) phage DNA (50 μM base pairs) was incubated with 1 mM **1**, **6**, **4**, or **2** (pH 8, 50 mM Tris buffer, 13% v/v DMSO, 20 h at 37 °C) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain).

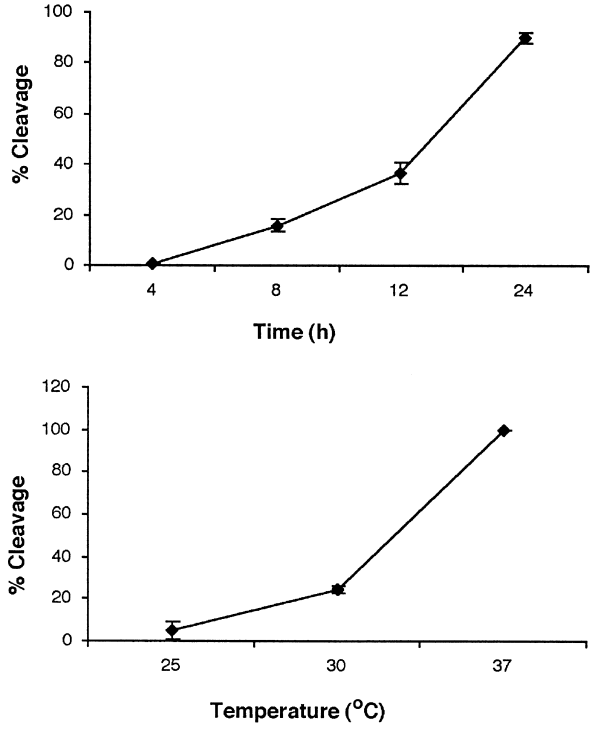


Figure 4. Time and temperature dependence of DNA cleavage by **2**. ΦX174 (RF-1) phage DNA (50 μM base pairs) was incubated with 1 mM **2** at various temperatures and for various time spans (pH 8, 50 mM Tris buffer, 13% v/v DMSO) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain).

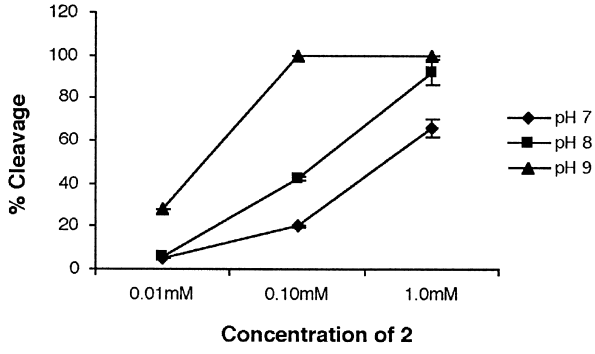


Figure 5. pH dependence of DNA cleavage by **2**. ΦX174 (RF-1) phage DNA (50 μM base pairs) was incubated with various concentrations of **2** at pH 7, 8, and 9 (50 mM Tris buffer, 13% v/v DMSO, 20 h at 37 °C) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain).

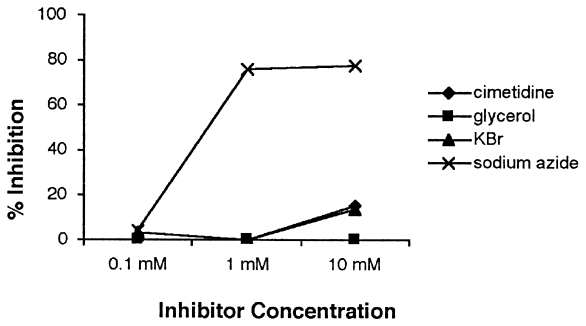


Figure 6. Effect of inhibitors on the DNA cleavage by **2**. ΦX174 (RF-1) phage DNA (50 μM base pairs) was incubated with 1 mM **2** and various concentrations of inhibitors (pH 8, 50 mM Tris buffer, 13% v/v DMSO, 20 h at 37 °C) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain).

DNA alkylation is responsible for cleavage; however, the absence of any increase in the observed DNA cleavage after heat treatment of the drug–DNA incubation samples would seem to argue against DNA alkylation.

We investigated the ability of cimetidine, glycerol, potassium bromide, and sodium azide to inhibit cleavage of DNA by **2** (Fig. 6). Cimetidine and glycerol are both radical inhibitors, while KBr and NaN_3 could function as sacrificial nucleophiles. A comparison of the cleavage inhibition efficiencies of these four inhibitors demonstrates that NaN_3 is most effective, causing over 70% inhibition at equimolar concentration with **2**. Potassium bromide and cimetidine each show ~10% inhibition at 10 times the concentration of **2**. Since sodium azide is also an inhibitor of singlet oxygen we were curious whether some oxygen-dependent species may be involved in the DNA cleavage caused by **2**. Preliminary results, however, show no difference in cleavage between anaerobic and aerobic conditions.

Conclusion

Clearly, based on its DNA-cleaving ability, compound **2** has the potential to function as an effective antitumor agent and we are currently investigating its efficacy. The mechanism of DNA cleavage effected by **2** remains to be elucidated. If a simple electrophilic mechanism is involved, it is curious that **2** is so much more potent than the dimethyl benzimidazole **4**. Additionally, evidence of frank single-strand breakage by **2** and the steep pH-dependence of its DNA cleavage indicate something unique about the mechanism of action of **2** compared to the other benzimidazoles. We are actively pursuing elucidation of the mechanism of action of **2** and related heterocycles.

Acknowledgements

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References and Notes

1. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464.
2. Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H.; VanDuyne, G. D.; Clardy, J. *J. Antibiot.* **1989**, *42*, 1449.
3. (a) Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68. (b) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331.
4. For leading references, see (a) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172. (b) Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497. (c) Grissom, J. W.; Gunawardena, G. U.; Klingberg, D.; Huang, D. *Tetrahedron* **1996**, *52*, 6453. (d) Schmittel, M.; Maywald, M.; Strittmatter, M. *Synlett* **1997**, 165. (e) Sakai, Y.; Bando, Y.; Shishido, K.; Shibuya, M. *Tetrahedron Lett.* **1992**, *33*, 957. (f) Sullivan, R. N.; Coghlan, V. M.; Munk, S. A.; Reed, M. W.; Moore, H. W. *J. Org. Chem.* **1994**, *59*, 2276.
5. (a) Gillman, T.; Heckhoff, S. *Tetrahedron Lett.* **1996**, *37*, 839. (b) Wang, K. K.; Wang, Z. *J. Org. Chem.* **1996**, *61*, 1516. (c) Shi, C.; Wang, K. K. *J. Org. Chem.* **1998**, *63*, 3517. (d) Shi, C.; Zhang, Q.; Wang, K. K. *J. Org. Chem.* **1999**, *64*, 925. (e) Schmittel, M.; Steffen, J.-P.; Engels, B.; Lennartz, C.; Hanrath, M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2371.
6. (a) Kerwin, S. M. *Tetrahedron Lett.* **1994**, *35*, 1023. (b) McPhee, M. M.; Kerwin, S. M. *J. Org. Chem.* **1996**, *61*, 9385. (c) David, W. M.; Kerwin, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 1464.
7. David, W. M.; Kerwin, S. M. *Abstracts of Papers*, 219th National Meeting of the American Chemical Society, San Francisco, CA, March 26–30, 2000; American Chemical Society: Washington, DC, 2000; MEDI 55.
8. Muller, E.; Zountsas, G. *Chem. Ber.* **1972**, *105*, 2529.
9. Spectral data for **2b·BF$_4$**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.22 (s, 3), 5.94 (s, 2), 7.34–7.42 (m, 5), 7.59–7.82 (m, 5), 7.99–8.02 (m, 2), 8.10–8.13 (m, 1), 8.21–8.24 (m, 1); ^{13}C NMR (63 MHz, DMSO-d_6) δ 33.37, 37.11, 71.16, 81.45, 86.10, 109.40, 113.40, 113.80, 117.78, 120.77, 127.60, 127.81, 128.80, 129.26, 129.51, 130.32, 131.58, 131.68, 132.58, 133.08, 133.87; CI-HRMS *m*/*e* calcd for: $C_{25}H_{19}N_2$ 347.154824, found 347.153912.
10. Ellingboe, J. W.; Spinelli, W.; Winkley, M. W.; Nguyen, T. T.; Parsons, R. W.; Moubarak, I. F.; Kitzen, J. M.; Von Engen, D.; Bagli, J. F. *J. Med. Chem.* **1992**, *35*, 705.
11. Spectral data for **4**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.17 (s, 6), 7.55–7.82 (m, 5), 7.95–8.13 (m, 4); ^{13}C NMR (63 MHz, DMSO-d_6) δ 33.07, 71.26, 108.18, 113.35, 117.85, 127.34, 129.25, 131.47, 132.40, 132.96, 134.10; ^{19}F NMR (300 MHz, DMSO-d_6) δ −77.64; CI-HRMS *m*/*e* calcd for: $C_{17}H_{15}N_2$ 247.123524, found 247.124027.
12. Spectral data for **6**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.42 (s, 3), 4.19 (s, 3), 5.44 (s, 2), 6.47 (s, 1), 7.18–7.39 (m, 9), 7.41–7.67 (m, 4), 7.76–7.84 (m, 1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 32.43, 37.06, 58.65, 79.62, 81.61, 87.17, 112.36, 112.91, 121.10, 126.73, 126.89, 127.75, 128.39, 129.20, 129.24, 130.66, 131.45, 131.72, 133.73, 150.60, 170.00; CI-HRMS *m*/*e* calcd for: $C_{25}H_{21}N_2O$ 365.165388, found 365.164856.