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N-Propargyl-2-alkynylbenzothiazolium Aza-enediynes: Role of the 2-Alkynylbenzothiazolium Functionality in DNA Cleavage

Dalip Kumar, Wendi M. David and Sean M. Kerwin*

Division of Medicinal Chemistry, College of Pharmacy, University of Texas at Austin, Austin, TX 78712, USA

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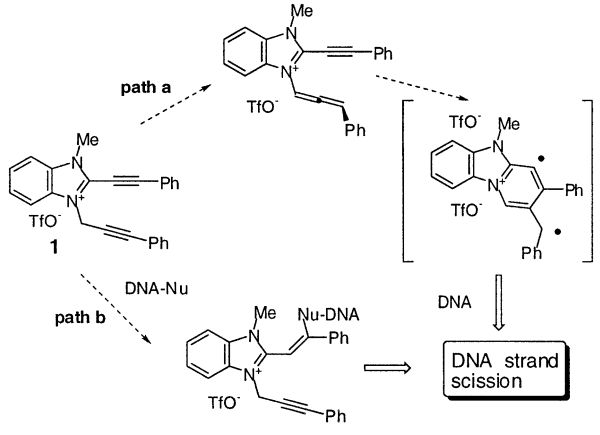
Abstract—The 2-alkynylbenzothiazolium salts **3a–d** incorporating an *N*-propargyl moiety have been prepared as aza-enediyne analogues. While these aza-enediynes are shown to be modest DNA cleavage agents, DNA cleavage was also observed with the *N*-methyl-2-alkynylbenzothiazolium salt **4**, which lacks the aza-enediyne moiety. The structural requirements for DNA cleavage, and the correlation of DNA cleavage efficiency with the propensity of these compounds to undergo nucleophilic addition by methanol support a proposed DNA cleavage mechanism involving DNA alkylation by appropriate 2-alkynyl-substituted benzothiazolium salts.

The design of new DNA cleavage agents has been an area of great interest recently. This interest has been fueled by the discovery 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. While these natural products have proven too toxic for use as anticancer drugs, an antibody–calicheamicin–analogue conjugate has recently been approved for cancer therapy, demonstrating the feasibility of using diradical-based DNA cleavage agents as warheads for selective cytotoxic agents.[4,5] There has been much effort directed towards the design of synthetic enediyne analogues capable of cleaving DNA.[6] There are, however, very few examples of aza-substituted analogues.[7] Several groups have investigated the ability of aza-substituted enyne allenes to undergo radical-generating cyclizations, but none have demonstrated DNA cleavage activity.[8]

We have been exploring the utilization of mild, physiologically relevant 'triggering' mechanisms and the effect of heteroatom substitution in enediynes in the design of novel DNA cleavage warheads with the goal of increasing antitumor selectivity.[6] We recently reported the design of a series of *N*-propargyl-2-alkynyl heterocycles, exemplified by the benzimidazolium compound **1** (Scheme 1), which incorporate an aza-enediyne moiety.[9]

These aza-enediynes may undergo isomerization to generate an aza-enyne allene (Scheme 1, path a). The aza-enyne allene may be capable of an aza-Myers type cyclization, thereby generating a diradical persistent enough to perform DNA cleavage chemistry under physiological conditions. Alternatively, these aza-enediynes may alkylate DNA via the addition of a DNA nucleophile at the 2-alkyne position (Scheme 1, path b). In the case of a DNA base serving as nucleophile, the resulting DNA adduct might undergo spontaneous loss of the base (e.g., depurination) and decomposition of the abasic site to yield a strand break.[10] In preliminary



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

*Corresponding author. Tel.: +1-512-471-5074; fax: +1-512-232-2606; e-mail: skerwin@mail.utexas.edu

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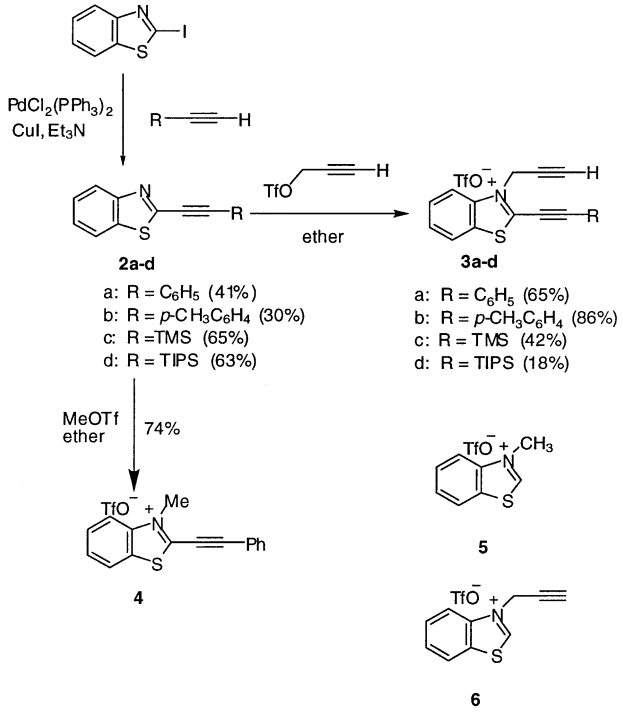
examination of the DNA cleavage chemistry of **1**, evidence for both diradical and electrophillic DNA cleavage pathways was found.[9,11] Here, we report the synthesis and DNA cleavage ability of a series of the corresponding benzothiazolium aza-enediynes **3a–d** (Scheme 2). The benzothiazolium compounds reported here appear to cleave DNA by a pathway that is independent of the ability to form diradical intermediates.

A series of *N*-propargyl-2-alkynyl substituted benzothiazolium salts **3a–d** in which the alkynyl substituent was varied were prepared by alkylation of the corresponding 2-alkynyl substituted benzothiazoles **2a–d**, which were prepared by palladium-catalyzed coupling using the method of Schlegel and Maas[12] (Scheme 2). Alkylation of these benzothiazoles with propargyl triflate[13] afforded the desired benzothiazolium salts **3a–d**.[14] Less reactive propargylating reagents did not afford good yields of the desired salts. A model *N*-methyl benzothiazolium salt was prepared by alkylation of 2-(2-phenylethynyl)benzothiazole **2a** with methyl triflate to afford methyl triflate salt **4** in good yield. The 2-unsubstituted *N*-methyl benzothiazolium triflate **5** and the corresponding *N*-propargyl salt **6** were also prepared by the alkylation of benzothiazole with methyl triflate and propargyl triflate, respectively.

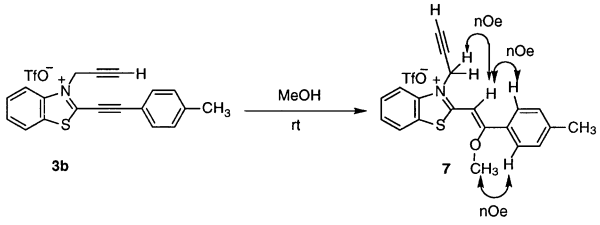
Some of the benzothiazolium salts are unstable in solvents like methanol and DMSO. In the case of methanolic solutions of salts **3a** and **3b**, standing at room temperature for 30 min results in complete disappearance of the starting material along with the formation of one major product. Benzothiazolium salts **3c** and **4** also undergo conversion in methanolic solution. In contrast, the benzothiazolium **3d**, in which the alkynyl substituent is the sterically demanding TIPS group, is stable in methanolic solution. Similarly, the benzothiazolium salts **5** and **6** are stable in methanol.



Scheme 2. Synthesis of benzothiazolium salts.

Isolation of the product formed from methanolic solutions of **3b** and characterization by COSEY, NOSEY, and HMQC NMR demonstrate that it is the methanol addition product (*Z*)-2-(2-methoxy-2-*p*-tolylethylene)-3-pro-2-ynyl benzothiazolium triflate (**7**, Scheme 3). The benzimidazolium salt **1** was also observed to form a structurally related methanol addition product; however, in the case of **1** the methanol addition product was only formed in the presence of base.[9] Apparently, the benzothiazolium salts **3a–c** are more susceptible to nucleophilic attack at the 2-alkynyl substitutent than the corresponding benzimidazolium salts, and as a result would be expected to serve as pH-independent electrophillic DNA alkylating/cleavage agents.

We have studied the DNA cleavage ability of the benzothiazolium salts **3a–d**, **4**, **5**, and **6** by measuring the amount of circular relaxed (Form II) and linear (Form III) DNA produced when fixed concentrations (100 μM and 1 mM) of these compounds were incubated with supercoiled ΦX174 DNA in 20 mM TRIS buffer (pH 8 or 9) for 20 h at 37 °C. In no case was any significant amount of linear DNA produced, indicating that the DNA cleavage reactions afforded only single-strand DNA breaks (Fig. 1). The DNA cleavage products were also formed from reactions run in the dark (data not shown), precluding the role of photochemical reactions in the DNA cleavage process.



Scheme 3. Facile addition of methanol to benzothiazolium salt **3b**.

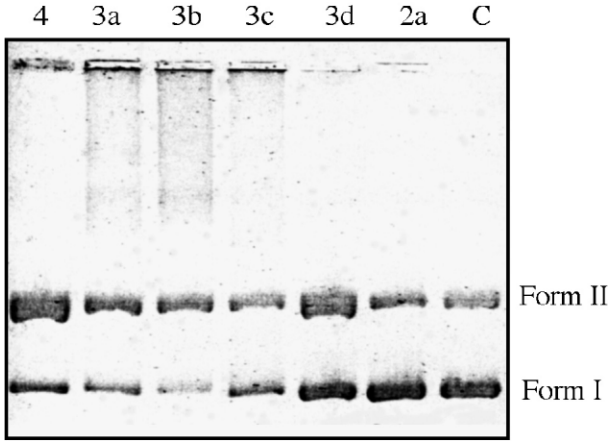


Figure 1. DNA cleavage by compounds **3a–d**, **4**, and **2a**. Supercoiled ΦX174 phage DNA (50 μM base pairs) was incubated with 100 μM of compounds **4**, **3a–d**, and **2a** or alone (C) (pH 8, 20 mM Tris buffer, 13% v/v DMSO, 20 h at 37 °C) and analyzed by gel electrophoresis (0.8% agarose, ethidium bromide stain).

Table 1. Cleavage of supercoiled DNA by some benzothiazolium salts

Compd	Normalized percent DNA cleavage[a]	
	pH 8.0	pH 9.0
3a	30±19 (6)	40±13 (4)
3b	33±18 (6)	42±1 (2)
3c	20±4 (6)	33±5 (2)
3d	7±4 (6)	7±1 (2)
4	39±15 (6)	48±15 (2)
5	2.7±0.7 (2)	nd[b]
6	16±0.2 (2)	nd[b]
2a	0±0 (2)	0±0 (2)

[a]Normalized percent cleavage of DNA from cleavage reactions (20 h) containing 100 μM compound and 50 μM (bp) supercoiled DNA at the indicated pH in 20 mM TRIS buffer. The values reported are mean±standard deviation for the number of separate determinations indicated in parentheses.
[b]Not determined.

Agarose gels of DNA cleavage reaction mixtures containing the benzothiazolium salts **3a**–**c** and **4** showed evidence of low mobility DNA products that remained in or near the loading wells of the gels (Fig. 1). These low mobility products made quantification of the DNA cleavage somewhat imprecise, as evidenced by the large standard deviations reported in Table 1. In the case of reaction mixtures containing 1 mM concentration of these compounds, all of the DNA remained in the loading wells. This abnormally low mobility of the DNA was not prevented by chloroform/phenol extraction of the reaction mixtures prior to gel electrophoresis.

As shown in Table 1, aza-enediyne benzothiazolium salts **3a**–**b** cleave DNA to approximately the same extent at pH 8. However, both the simple *N*-methyl-2-alkynylbenzothiazolium salt **4** and the *N*-propargylbenzothiazolium salt **6** cleave DNA as well. Thus, in contrast to the aza-enediyne benzimidazolium analogues,[9] the DNA cleavage by these aza-enediyne benzothiazolium salts does not require the presence of the aza-enediyne functionality. Neither the *N*-methyl-2-unsubstituted benzothiazolium salt **5** nor the aza-enediyne benzothiazole **2a** cleave DNA to any appreciable extent. Based on these results, it appears that either 2-alkynyl substituted benzothiazolium salts (e.g., **4**) or *N*-propargyl benzothiazolium salts (e.g., **6**) can give rise to DNA cleavage. In the aza-enediyne benzothiazolium salts containing both of these functionalities, the importance of the 2-alkynyl functionality is demonstrated by the manner in which the 2-alkynyl substituent affects the DNA cleavage potential. The aryl-substituted compounds **3a**–**b** cleave DNA much better than the silyl-substituted compounds **3c**–**d**, which cleave DNA with about the same efficiency as the *N*-propargyl-2-unsubstituted derivative **6**.

The difference in DNA cleavage ability of the benzothiaolium salts **3a**–**d** and **4** at pH 8 and 9 was not statistically significant, indicating that these compounds, unlike the benzimidazolium salt **1**, do not undergo base-promoted isomerization to aza-enyne allene species in order to cleave DNA.

The DNA cleavage results for the 2-alkynylbenzothiazolium salts **3a**–**b** and **4** may be explained by a cleavage mechanism involving alkylation of DNA by the electrophilic 2-alkynyl benzothiazolium substituent, followed by DNA scission at the alkylated site (Scheme 1, path b). Similar Michael-type addition of DNA bases to electrophiles have been reported to provide labile DNA adducts, particularly those involving the N7 and C8 site of purine bases.[15] Support for this proposed mechanism comes from DNA cleavage reactions performed on singly 5′-[^{32}P]-labeled oligodeoxynucleotides. These cleavage reactions reveal that the DNA cleavage due to **4** is specific for guanosine residues.[11] The observation of low-mobility products from both the oligodeoxynucleotide and supercoiled DNA cleavage reactions is commensurate with DNA alkylation or crosslinking by these 2-alkynyl benzothiazolium salts. Additional support for this electrophilic DNA cleavage mechanism comes from studies on the effect of inhibitors on the DNA cleavage reaction by **4**. Addition of 10 mM KBr as a sacrificial nucleophile to DNA cleavage reactions involving **4** results in complete inhibition of the DNA cleavage (data not shown).

For the benzothiazolium salts examined here that cleave DNA but lack the 2-alkynyl substitutent (**6**), or have a sterically and electronically blocked 2-alkynyl substituent (**3c**–**d**), a different DNA cleavage mechanism must be operating. We propose that the DNA cleavage observed with these compounds is due to the *N*-propargyl substituent. Benzothiazolium salt **5**, which lacks both the *N*-propargyl and 2-alkynyl substituents, does not cleave DNA to any significant extent. However, the simple *N*-propargyl benzothiazolium salt **6** cleaves DNA about as well as compounds **3c**–**d**, in which nucleophilic attack at the 2-alkynyl substituent is both sterically and electronically disfavored. The mechanism of this alternative DNA cleavage reaction is currently under investigation.

We have prepared a series of *N*-propargyl-2-alkynyl substituted benzothiazolium aza-enediynes. These compounds are moderately potent DNA cleavage agents. We present a DNA cleavage structure–activity relationship that demonstrates the aza-enediyne functionality is not required for DNA cleavage activity. We find that these 2-alkynyl substituted benzothiazolium salts undergo facile nucleophilic addition of methanol under neutral conditions. These results support a mechanism for DNA cleavage involving DNA alkylation by the electrophilic 2-alkynyl substituent. Thus, simple 2-alkynyl-substituted benzothiazolium salts may serve as a new class of DNA-directed electrophiles. We are currently in the process of further defining the mechanism of covalent DNA modification and evaluating the biological activity of these novel DNA cleavage agents.

Acknowledgements

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14. Selected data for compounds: **3a**: ^{1}H NMR (CD_2Cl_2) δ 2.75 (t, 1H, J = 2.6 Hz, acetylenic proton), 5.78 (d, 2H, J = 2.6 Hz, CH_2), 7.56–7.59 (m, 2H, $C_{3'}$–H and $C_{5'}$–H), 7.69 (dd, 1H, J = 1.3, 8.1 Hz, $C_{4'}$–H), 7.85–7.92 (m, 3H, $C_{2'}$–H, $C_{6'}$–H and $C_{7'}$–H), 7.99 (dd, 1H, J = 1.2, 8.4 Hz, C_7–H), 8.25 (dd, 2H, J = 7.9, 8.4 Hz, C_5–H and C_6–H); ^{13}C NMR (CD_2Cl_2) δ 41.74, 73.18, 76.32, 78.11, 117.70, 118.08, 119.27, 124.61, 129.70, 130.11, 130.58, 131.61, 133.88, 139.51, 149.45, 153.76. Anal. ($C_{19}H_{12}F_3NO_3S_2$) C, H, N, S. **3b**: ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3), 2.80 (t, 1H, CH, J = 2.5 Hz, acetylenic proton), 5.77 (d, 2H, J = 2.5 Hz, CH_2), 7.40 (d, 2H, J = 7.9 Hz, $C_{3'}$–H and $C_{5'}$–H), 7.77 (d, 2H, $C_{2'}$–H and $C_{6'}$–H, J = 8.2 Hz), 7.80–8.01 (m, 2H, C_5–H and C_6–H), 8.02–8.31 (dd, 2H, J = 8.2, 8.6 Hz, C_4–H and C_7–H); ^{13}C NMR ($CDCl_3$) δ 22.24, 41.48, 73.20, 76.49, 78.02, 114.90, 117.47, 120.24, 124.70, 129.97, 130.32, 130.47, 131.44, 133.86, 139.35, 145.69, 153.78. Anal. ($C_{20}H_{14}F_3NO_3S_2$) C, H, N, S. **3c**: ^{1}H NMR ($CDCl_3$) δ 0.413 (s, 9H, TMS), 2.64 (t, 1H, J = 2.5 Hz, acetylenic proton), 5.71 (d, 2H, J = 2.3 Hz, CH_2), 7.78–7.89 (m, 2H, C_5–H and C_6–H), 8.18 (d, 1H, J = 8.4 Hz, C_4–H), 8.27 (d, 1H, J = 8.2 Hz, C_7–H); ^{13}C NMR ($CDCl_3$) δ −1.23, 41.26, 72.85, 77.40, 87.99, 117.49, 124.67, 129.50, 129.65, 130.16, 131.16, 138.84, 152.37**.** **3d**: ^{1}H NMR (CD_2Cl_2) δ 1.02–1.49 (m, 21H, TIPS), 2.76 (t, 1H, J = 2.5 Hz), 5.74 (d, 2H, J = 2.5 Hz, CH_2), 7.90–8.05 (m, 2H, C_5–H and C_6–H), 8.39 (dd, 2H, J = 8.2, 8.6 Hz, C_4–H and C_7–H); ^{13}C NMR (CD_2Cl_2) δ 11.42, 18.63, 41.63, 72.86, 78.00, 90.37, 117.79, 124.84, 129.21, 129.91, 130.84, 131.83, 139.29,152.60. Anal. ($C_{22}H_{28}F_3NO_3S_2Si$) C, H, N, S. **4**: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 4.24 (s, 3H, CH_3), 7.21–7.69 (m, 7H, Ar–H), 7.82–7.95 (m, 1H, C_7–H), 8.04–8.18 (m, 1H, C_4–H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 37.26, 75.02, 116.08, 116.66, 123.61, 128.16, 128.73 (2C), 129.34, 129.82, 132.15 (2C), 139.25, 151.82. Anal. ($C_{17}H_{12}F_3NO_3S_2$) C, H, N, S. **7**: ^{1}H NMR (CD_2Cl_2) δ 2.49 (s, 3H, CH_3), 2.65 (t, 1H, J = 2.6, acetylenic proton), 4.71 (s, 3H, OCH_3), 5.50 (d, 2H, J = 2.6 Hz), 6.69 (s, 1H, olefinic proton), 7.43 (d, 2H, J = 8.4 Hz, $C_{3'}$–H and $C_{5'}$–H), 7.60 (d, 2H, J = 8.2 Hz, $C_{2'}$–H and C_6–H), 7.68–7.71 (m, 1H, C_5–H), 7.80–7.84 (m, 1H, C_6–H), 7.95 (dd, 1H, J = 1.8, 8.6 Hz, C_7–H), 8.10 (dd, 1H, J = 1.7, 8.6 Hz, C_4–H); ^{13}C NMR (CD_2Cl_2) δ 21.80, 38.55, 61.26, 73.87, 76.83, 97.23, 115.33, 123.82, 128.31, 128.42, 128.59, 129.11, 130.10, 130.44, 139.20, 144.49, 166.92, 177.62. HRMS (CI) calcd for $C_{20}H_{18}NOS$ (M^+) m/z 320.1118, found 320.1109.
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