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BIFUNCTIONAL 2-NAPHTHYL PROPARGYLIC SULFONES EXHIBITING HIGH DNA INTERCALATING AND ALKYLATING ACTIVITY

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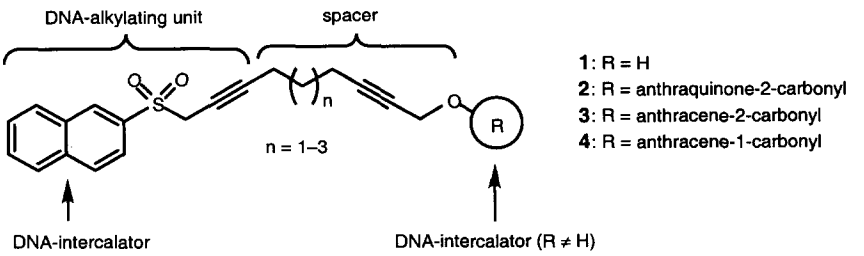
Abstract: A number of novel 2-naphthyl propargylic sulfones were synthesized as nucleic base alkylating agents. Extremely high DNA cleavage activity was observed for the sulfones with a free ω-hydroxyl group in the carbon chain in contrast to the ester conjugates possessing an additional intercalating unit.

Keywords: *DNA; Antitumour compds, Substituent effects.*

Studies on the DNA–interacting agents are essential to understanding the mechanism of action of DNA–damaging agents and to developing novel medicine and biomedical tools with improved efficacy. Nucleic base alkylation is one of the common modes of DNA damage caused by organic anticancer agents.[1] Some well–known examples of DNA alkylating agents are the antibiotics, such as CC-1065 and mitomycin C, and the artificial agents, such as nitrogen mustards and chloroethylnitrosoureas.[2] In our previous studies on DNA cleavage by phenyl propargylic sulfones,[3–6] we noted that remarkably enhanced potency was achieved through formation of conjugates with DNA intercalators.[3c] Such tethering effect is useful in molecular design of anticancer agents. However, we recently found that the ester conjugates **2–4** were significantly less active compared to the parent 2-naphthyl propargylic sulfone **1** (n =1–3) regardless the length of the spacer group. Now, we report on the synthesis and DNA cleavage profiles of **1–4**. These new findings support that 2-naphthyl propargylic sulfones **1** (n =1–3) act as bifunctional DNA intercalating and alkylating agents.



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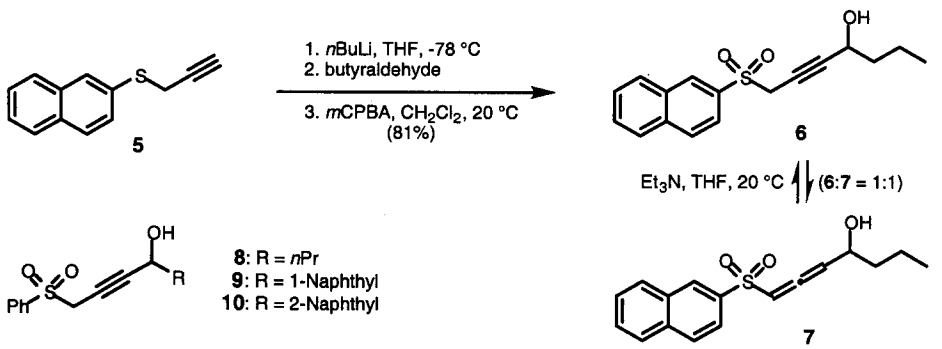
In 1989, Nicolaou and co-workers[4a] reported the pH-dependent DNA cleavage of synthetic propargylic sulfones and proposed the nucleic base alkylation mechansim.[4b] They found that 1-naphthyl and 9-anthracene propargylic sulfones exerted higher DNA cleavage potency compared to the phenyl and alkyl analogs. Control experiments using a known DNA intercalator, ethidium bromide (EB), suggested that intercalation of 1-naphthyl group occurs prior to base alkylation.[4b] In 1993, Lown et al. reported the synthesis of a number of hybrids of naphthyl propargylic sulfones with minor groove binding lexitropsins.[5a] High DNA cleavage activity was observed for the 1-naphthyl propargylic sulfones with the minor groove binder attached to the C_5 position of the naphthalene nucleus, whereas diminished potency was noted for the 2-naphthyl propargylic sulfones possessing a C_3 minor groove binder.[5] Because the unsubstituted 2-naphthyl propargylic sulfones have not been studied so far, we examined the DNA cleavage activity of **6** (Scheme 1 and Figure 1). Deprotonation of 2-naphthyl propargyl sulfide **5**[7] with 1 equiv of *n*BuLi in THF at -78 °C gave the lithium acetylide that reacted with butyraldehyde followed by oxidation to furnish **6** in 81% overall yield. Treatment of **6** in THF with excess Et_3N at room temperature for over 24 h resulted in an inseparable 1:1 mixture of the propargylic sulfone **6** with the allenic sulfone **7**. DNA cleavage by **6** and **7** was then assayed together with the phenyl propargylic sulfones **8**–**10**[3b] using ΦX174 RFI DNA. As shown in Figure 1a, sulfone **6** is generally >100–fold more potent than **8–10**



Scheme 1. Synthesis of 2-naphthyl sulfones **6** and **7** and the structures of phenyl sulfones **8–10**.

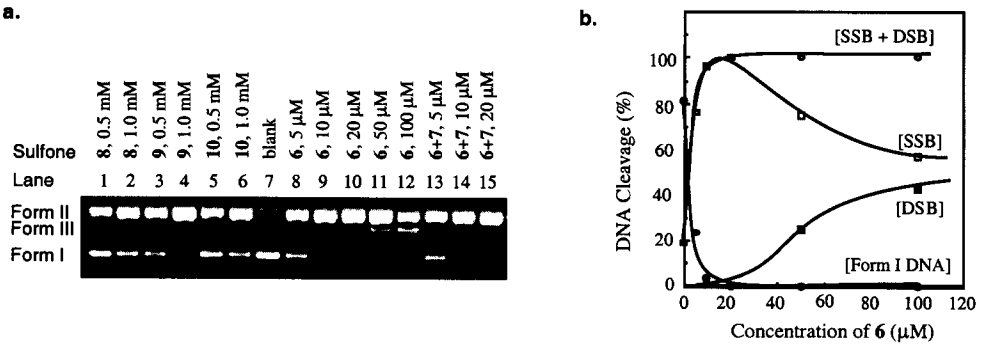
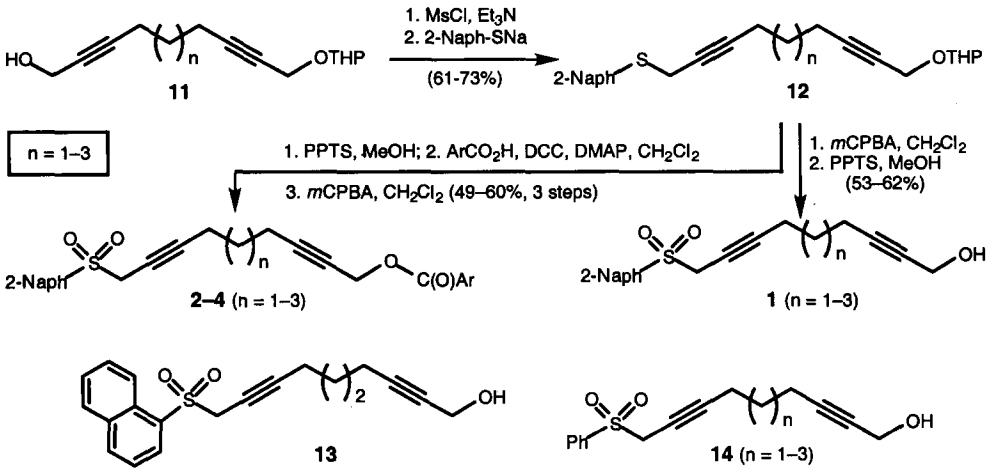


Figure 1. Results of DNA cleavage by sulfones **6–10**. (a) 1% agarose gel electrophoresis. ΦX174 RFI DNA (54.3 μM/bp) was incubated with **6–10** at various concentrations in 20% DMSO containing TEA buffer solution (pH 8.5) at 37 °C for 72 h and then analyzed by gel electrophoresis and ethidium bromide stain. (b) The scanning densitometry results of lanes 8–12 in the gel picture a. See note 8 for the definition of % DNA cleavage.

(lanes 2, 4, and 6 versus lane 9), and the potency of the mixture of **6** and **7** is on the same level as that of the pure **6** (lanes 8–10 versus lanes 13–15). These results confirm again that the propargylic sulfone undergoes a base-induced isomerization to the allenic sulfone prior to the DNA alkylation occurs.[4b] Figure1b shows the scanning densitometry results of lanes 8–12 in the gel picture (Figure 1a) for DNA cleavage of **6** at different concentrations. At ca. 20 μM of **6**, the Form I DNA was completely consumed to form the single strand breakage [SSB] product (Form II DNA), whereas at >20 μM concentrations of **6**, the double strand breakage [DSB] product (Form III DNA) gradually increased. The C_{70} values[9] for DNA cleavage of **6** and **8** were determined as 5 μM and 800 μM, respectively, i.e. **6** is 160-fold more potent than **8**. We also determined the inhibitory binding constant (K') of **6** and **8** against EB using ΦX174 RFI DNA according the known fluorescence measurements.[10] The K' values are 7.0×10^4 and 2.1×10^3 M^{-1} for **6** and **8**, respectively. It indicates that the 2-naphthyl nucleus is a much better DNA intercalator than the phenyl group and the intercalating capacity with DNA correlates with the DNA cleavage potency.

Encouraged by the high DNA cleaving ability of **6**, we synthesized a number of 2-naphthyl propargylic sulfones and the ester conjugates **1–4** bearing different chain length (Scheme 2). Mesylation of the known alcohols **11** (n = 1–3)[3a,c] followed by reacting with sodium 2-naphthalenethiolate provided the sulfides **12** in 61–73% yield. Oxidation of **12** by 2 equiv of *m*-chloroperoxybenzoic acid (*m*CPBA) at room temperature gave



Scheme 2. Synthesis of 2-naphthyl sulfones **1–4** and the structures of sulfones **13** and **14**.

the sulfones that were converted into the hydroxyl sulfones **1** (53–62% overall yield) upon removing the THP ether by treating with *p*-toluenesulfonic acid (PPTS) in MeOH at room temperature. Alternatively, the THP ether in **12** was removed first to give the alcohols, condensation with the aromatic carboxylic acids ($ArCO_2H$) under the DCC–DMAP condition formed the esters, and finally, *m*CPBA oxidation afforded the 2-naphthyl sulfone–ester conjugates **2–4** in 49-60% overall yield. The 1-naphthyl propargylic sulfone **13** was also prepared in a similar manner in 67% yield from **11** (n = 2).

DNA cleavage by the sulfones **1** (n = 1–3) was then examined in comparison with the phenyl analogs **14**[3c] and the results are shown in Figure 2. In general, the sulfones **1** at 10 μM produced much more Form II DNA than **14** at 1000 μM (Figure 2a), it means that the 2-naphthyl propargylic sulfones **1** are >100-fold more

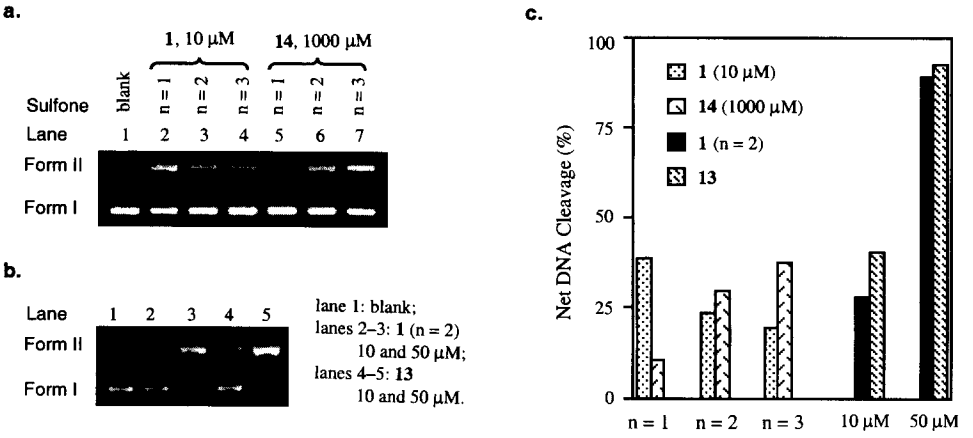
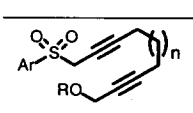
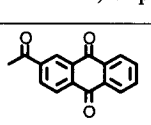
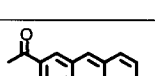
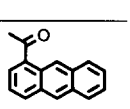


Figure 2. Results of DNA cleavage by sulfones **1**, **13**, and **14**. (a and b) 1% agarose gel electrophoresis. ΦX174 RFI DNA (54.3 μM/bp) was incubated with **1**, **13**, and **14** at various concentrations in 20% DMSO containing TEA buffer solution (pH 8.5) at 37 °C for 72 h and then analyzed by gel electrophoresis and ethidium bromide stain. (c) The scanning densitometry results of the gel pictures in a and b. See note 8 for the definition of % net DNA cleavage.

potent than **14**. The quantitative DNA cleavage results of **1** and **14** are illustrated in Figure 2c. The 2-naphthyl sulfone **1** (n = 1) is 350–fold more active than the phenyl sulfone **14** (n = 1). Similarly, the 1-naphthyl sulfone **13** exerted high DNA cleavage activity and is slightly more potent than its isomer **1** (n = 2) (Figure 2b,c). The DNA binding constants K' of **1** (n = 2) and **13** are of the same order of magnitude [7.0 x 10^4 for **1** (n = 2) and 2.3 x 10^4 for **13**].[10] In contrast to the DNA cleavage activity, the 1-naphthyl sulfone **13** is a relatively weaker DNA binder than the 2-naphthyl sulfone **1** (n = 2).

We reported a remarkable tethering effect on the DNA cleavage by the ester conjugates **15–17**.[3c] Table 1 and Figure 3 show the DNA binding constants K' and cleavage potency of sulfones **14–17** and **1–4**. Generally, the esters **15–17** possessing an intercalating moiety exhibit higher DNA affinity (ca. 3~12–fold) than the parent alcohols **14**. Therefore, the esters **15–17** (n = 1, 2) are much more effective in causing DNA strand breakage (Figre 3a). The best DNA cleaver **17** (n = 2) is ca. 10–fold more potent than the alcohol **14** (n = 2) in parallel with the higher DNA affinity (12–fold). The exceptionally low activity of **15–17** (n = 3) compared to **14** (n = 3)

Table 1. DNA binding constants K' (in units of 10^4 M^{-1}) of propargylic sulfones **1–4** and **14–17**.[a]

	R = H	R = 	R = 	R = 
Ar = 	**14** (n =1): 0.94	**15** (n =1): 1.7	**16** (n =1): 3.1	**17** (n =1): 1.3
	14 (n =2): 0.21	**15** (n =2): 1.8	**16** (n =2): 2.6	**17** (n =2): 1.9
	14 (n =3): 0.38	**15** (n =3): 1.8	**16** (n =3): 1.5	**17** (n =3): 2.1
Ar = 	**1** (n =1): 3.2	**2** (n =1): 3.7	**3** (n =1): 4.6	**4** (n =1): 5.4
	1 (n =2): 7.0	**2** (n =2): 4.8	**3** (n =2): 3.1	**4** (n =2): 3.8
	1 (n =3): 5.2	**2** (n =3): 3.7	**3** (n =3): 6.0	**4** (n =3): 6.1

[a] All measurements were done in the buffer solution containing 5% DMSO using ΦX174 RFI DNA.[10]

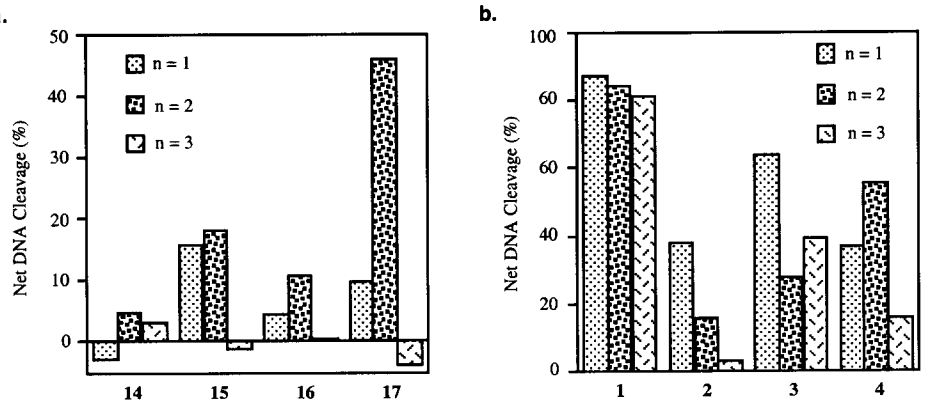


Figure 3. The scanning densitometry results of DNA cleavage by propargylic sulfones. ΦX174 RFI DNA (54.3 μM/bp) was incubated with the samples in 20% DMSO containing TEA buffer solution (pH 8.5) at 37 °C for 72h and then analyzed by gel electrophoresis and ethidium bromide stain (gel pictures not shown). (a) The results of phenyl propargylic sulfones **14–17** at 200 μM. The negative values are the result of DNA decomposition in the control. (b) The results of 2-naphthyl propargylic sulfones **1–4** at 100 μM. See note 8 for the definition of % net DNA cleavage.

indicates that the spacer group is another important factor that influences the overall biological profiles of the conjugates.

Comparison of DNA binding constants K' between **1–4** and **14–17** reveals that the difference is larger between the alcohols **1** and **14** (3.4~33 folds) compared to that between the esters **2–4** and **15–17** (1.2~4.2 folds). It implies that the conjugate effect is significantly reduced in contrast to the previous funding in the phenyl sulfone series.[3c] Generally, the ester conjugates **2–4** are much more potent in DNA cleaving activity than the phenyl sulfones **15–17**, it is attributed to the 2-naphthyl group. Within the 2-naphthyl sulfone series **1–4**, the ester conjugates **2–4** possess similar DNA binding constants as those of the parent alcohols **1** (Table 1), whereas **2–4** exhibit remarkably diminished DNA cleavage activity (Figure 3b). For example, the esters **2–4** (n = 3) give only 3.3%, 48%, and 19% of the damage caused by the alcohol **1** (n = 3), respectively. These outcomes perhaps result from the interference of the additional intercalating group in the ester conjugates **2–4**, and suggest the importance of the 2-naphthyl nucleus as the bifunctional group for intercalation and alkylation. Furthermore, we found that sulfones **1–4** at 10 μM were inactive on the EB–pre-treated DNA.[11] It confirms that intercalation of the 2-naphthyl sulfone with DNA is essential for the nucleic base alkylation.

Preliminary screening on the cytotoxicity of sulfones **1–4** (n = 1) was performed on P388 cell line (mouse T cell leukemia). The IC_{50} are 1.0 x 10^{-5} M for **1** (n = 1), 4.1 x 10^{-5} M for **2** (n = 1), 1.7 x 10^{-5} M for **3** (n = 1), and 4.2 x 10^{-5} M for **4** (n = 1). They correlate with the DNA cleaving activity.

In summary, we have synthesized a number of novel 2-naphthyl propargylic sulfones **1–4** and examined their DNA binding affinity and cleavage activity. In contrast to the phenyl propargylic sulfones **14**, the 2-naphthyl analogs **1** (n = 1–3) are generally >100–fold more potent in causing DNA cleavage. A significantly reduced tethering effect is noted for the ester conjugates **2–4** as the result of interference of the aromatic ester moiety with the intercalation of the 2-naphthalene nucleus. Thus, we have confirmed that the 2-naphthyl sulfones **1** (n = 1–3) act efficiently as both the DNA intercalators and the DNA cleavers.

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7. 2-Naphthyl propargyl sulfide **5** was synthesized from 2-naphthalenethiol and the mesylate of propargyl alcohol in 89% yield (NaH, THF, 0–20 °C, 1 h).
8. The percentage of DNA cleavage was calculated by the following equations: [SSB] = $(\text{Form II})_s/[(\text{Form I})_s + (\text{Form II})_s + 2 \times (\text{Form III})_s] \times 100$; [DSB] = $[2 \times (\text{Form III})_s]/[(\text{Form I})_s + (\text{Form II})_s + 2 \times (\text{Form III})_s] \times 100$. The percentage of net DNA cleavage was calculated by the following equation: $\{[(\text{Form II})_s + 2 \times (\text{Form III})_s]/[(\text{Form I})_s + (\text{Form II})_s + 2 \times (\text{Form III})_s] \times 100\} - \{(\text{Form II})_c/[(\text{Form I})_c + (\text{Form II})_c] \times 100\}$. The subscripts "s" and "c" refer to as the sample and control, respectively.
9. The C_{70} value is defined as the concentration that causes cleavage of 70% Form I DNA.
10. Strothkamp, K. G.; Strothkamp, R. E. *J. Che. Edu.* **1994**, *71*, 77. All measurements were done in the buffer solution containing 5% DMSO.
11. ΦX174 RFI DNA (54.3 μM/bp) was treated with 10 μM ethidium bromide for 2 h before incubation. Conditions for experiments using the EB–pre-treated DNA are the same as those given in Figure 3.