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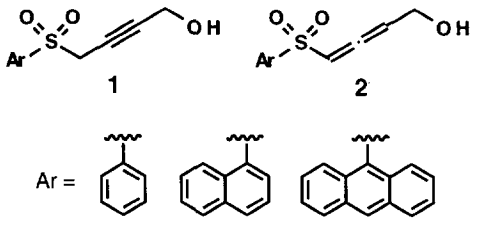
DNA CLEAVAGE OF NOVEL PROPARGYLIC SULFONES. ENHANCEMENT OF POTENCY VIA INTERCALATING INTERACTION

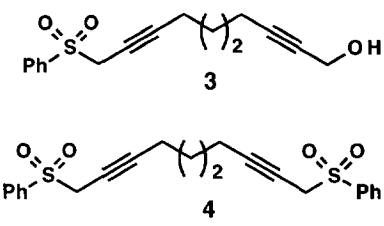
Wei-Min Dai,*[a] Kin Chiu Fong,[a] Hiroshi Danjo,[b] Sei-ichi Nishimoto,*[b]
Michael Solow,[a] Wing Leung Mak,[a] and Mau Lam Yeung[a]

[a]*Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong*
and
[b]*Laboratory of Excited-State Hydrocarbon Chemistry, Division of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*

Abstract: A number of novel hydroxy propargylic sulfones **7-15** were synthesized as DNA cleaving agents. Enhancement of DNA cleavage potency was observed with those compounds which could interact with DNA through intercalation of the extended aromatic rings. Copyright © 1996 Elsevier Science Ltd

DNA strand cleavage initiated by organic molecules is of considerable interests in the development of anticancer agents.[1] Reported first by the Nicolaou's group in 1989, a new class of propargylic and allenic sulfones such as **1** and **2** exhibited DNA cleavage activity in a pH-dependent manner.[2] The allenic sulfones **2**

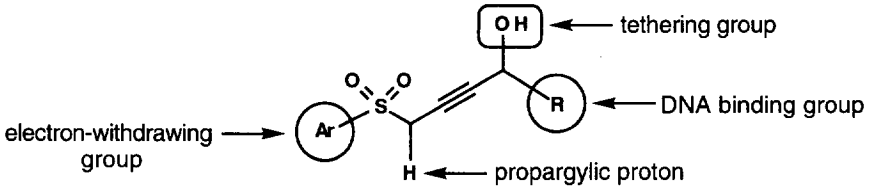




showed higher potency than the corresponding propargylic sulfones **1** under the same assay conditions. A mechanism of action[2b] was proposed featuring a key step of the nucleophilic addition of nucleic base toward the allenic sulfone **2**. The latter species could be derived *in situ* from **1** by base-induced isomerization. Conjugation of **1** with lexitropsins has been achieved[3] with an improved DNA cleavage potency as the result of binding to DNA minor groove. Cytotoxicity of propargylic sulfones **3** and **4** and other related compounds was examined using KB/P cancer cells.[4] Compound **3** exhibited an IC_{50} value of 5.3×10^{-6} M, being as potent as the clinically used 5-FU. In this paper, we disclose our preliminary results on the molecular design, chemical synthesis, and DNA cleavage studies of a novel family of the hydroxy-containing propargylic sulfones **7-15**.

Molecular design. We proposed the parent molecular structure **5** in our studies. The considerations are: (a) to provide a flexible structure with the possibility of structural modifications; and (b) to allow a ready access to the demanded compounds by chemical synthesis. Propargylic sulfone is a pro-drug which exhibits DNA cleavage activity through its allenic isomer. This feature has merit in the molecular design. The acidity of the propargylic protons indicated in **5** should correlate to the DNA cleavage activity. Increase of the acidity can be achieved by incorporating an electron-withdrawing group (Ar) to the sulfonyl moiety. In fact, this point has been demonstrated in several known molecular systems. For example, an aryl group attached to the sulfur atom

Chart 1. Molecular design of novel propargylic sulfones **5**.

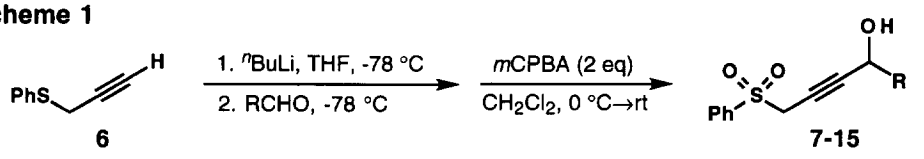


5

is better than an alkyl group in the DNA cleavage of **1** (Ar = aryl *vs.* -$CH_2C{\equiv}CCH_2OH$); the difference was estimated to be over at least 100 times.[2a-c] The Ar group in **5** can be used as the position for DNA recognition. Enhanced potency was obtained with compound **1** possessing 1-naphthyl and 9-anthryl groups through intercalation with DNA.[2a,b] The group R in **5** offers another site for engineering DNA intercalating moiety. Considering the synthetic sequence outlined below, the position R is more suitable for structural modifications. Finally, the hydroxyl group in **5** serves two purposes: to improve the aqueous solubility and to provide the point for conjugating with DNA recognizing molecules. The latter function can be attained with a wide range of molecular diversity such as DNA intercalating agents,[5] pyrrole/imidazole-containing polypeptides as DNA minor groove binders,[3,5b,6] crown ethers,[7] and oligosaccharides.[8] The chiral center at the hydroxy-bearing carbon atom allows the investigation of stereochemical requirement for the interaction with DNA.

Synthesis of novel hydroxy propargylic sulfones. In the current study we focused on the racemic propargylic sulfones **5** (Ar = Ph) with a different R group. Scheme 1 showed the synthesis of the hydroxy-containing propargylic sulfones **7-15** by the two-step sequence developed by the HKUST team.

Scheme 1

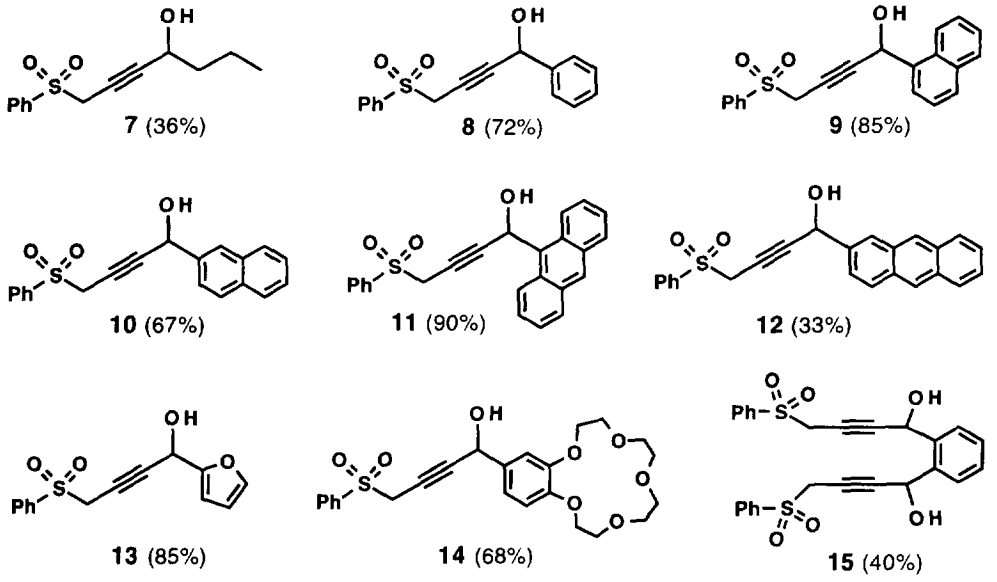


6 **7-15**

Deprotonation of phenyl propargylic sulfide (**6**)[9] with one mole equivalent of ^{n}BuLi in THF at -78 °C formed the corresponding lithium acetylide which reacted with an aldehyde to provide the hydroxy sulfide. Oxidation with 2 mole equivalents of *meta*-chloroperbenzoic acid (*m*CPBA) converted the sulfide into the sulfone. The molecular structures of **7-15**[10] are illustrated in Table 1 together with the overall chemical yields given in the parentheses. The aldehydes RCHO in Scheme 1 were selected to examine the effects on DNA cleavage via

intercalating interaction, the metal-regulation, and the action of bifunctional compound.

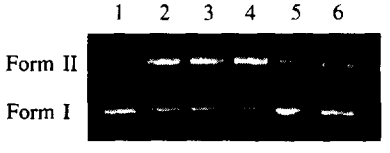
Table 1. Molecular structures and chemical yields of propargylic sulfones **7-15**.[a]



[a]The given yield is the sum of the two steps and was not optimized.

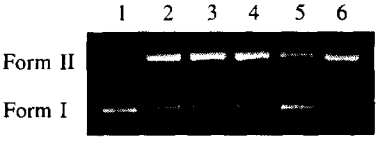
DNA cleavage studies. DNA cleavage by propargylic sulfones **7-15** was investigated using supercoiled ΦX174 Form I DNA and analyzed by agarose gel electrophoresis. Form II and Form III bands represent the single and double strand cleaved DNA products.[11] Figures 1 and 2 showed the DNA cleavage profiles of compounds **7-15** at 1 mM concentration incubated at 37 °C for 48-72 h. The potency of these agents can be divided into two groups. The propargylic sulfones **7-10**, **12**, and **15** produced more Form II DNA product than the propargylic sulfones **11**, **13**, and **14**. The electron-rich furan and benzo-15-crown-5 residues

Figure 1



ΦX174 Form I DNA (50 μM/bp) was incubated for 48 h at 37 °C with propargylic sulfones in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: DNA+1 mM **7**; Lane 3: DNA+1 mM **8**; Lane 4: DNA+1 mM **9**; Lane 5: DNA+1 mM **11**; Lane 6: DNA+1 mM **13**.

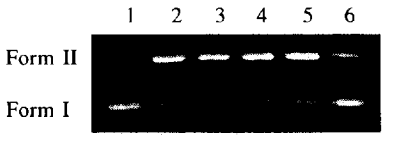
Figure 2



ΦX174 Form I DNA (50 μM/bp) was incubated for 72 h at 37 °C with propargylic sulfones in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: DNA+1 mM **8**; Lane 3: DNA+1 mM **10**; Lane 4: DNA+1 mM **12**; Lane 5: DNA+1 mM **14**; Lane 6: DNA+1 mM **15**.

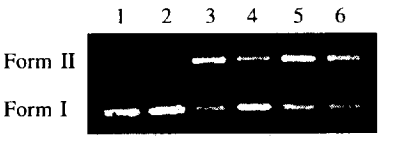
in **13** and **14** might prevent the interaction with DNA substrate and reduced the DNA cleavage efficiency (Lane 6 in Figure 1 and Lane 5 in Figure 2). The diminished potency of the 9-anthryl-containing agent **11** (Lane 5 in Figure 1) is interesting compared with compound **1** (Ar = 9-anthryl). The distance between the 9-anthryl group and the electrophilic center is shorter in **11** by one C-S single bond than that of **1**. This might handicap the alkylation of nucleic bases by the intercalated **11** due to the unfavorable orientation of the electrophile. When the attaching point of the propargylic sulfone unit was moved from 9-anthracene to 2-anthracene as in compound **12**, the DNA cleavage activity was resumed (Lane 4 in Figure 2). Figure 3 compared the DNA cleaving potency of **8**-**12** at 1 mM concentration. It is clearly demonstrated that the propargylic sulfones **9** and **10** possessing an intercalating naphthalene ring exhibited higher efficiency (Lanes 3 and 4 in Figure 3). Ethidium bromide (EB) is

Figure 3



ΦX174 Form I DNA (50 μM/bp) was incubated for 72 h at 37 °C with propargylic sulfones in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: DNA+1 mM **8**; Lane 3: DNA+1 mM **10**; Lane 4: DNA+1 mM **9**; Lane 5: DNA+1 mM **12**; Lane 6: DNA+1 mM **11**.

Figure 4



ΦX174 Form I DNA (50 μM/bp) was incubated for 72 h at 37 °C with propargylic sulfones in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: 50 μM EB pretreated DNA; Lane 3: DNA +1 mM **7**; Lane 4: 50 μM EB pretreated DNA+1 mM **7**; Lane 5: DNA+1 mM **10**; Lane 6: 50 μM EB pretreated DNA+1 mM **10**.
(EB = ethidium bromide).

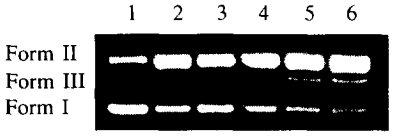
known as DNA intercalator. The effect of EB on action of the propargylic sulfones **7** and **10** were evaluated (Figure 4). Compound **7** lacking the intercalating ability lost some of its activity with the EB-pretreated DNA substrate[12] (Lane 4 compared with Lane 3). This result indicated that the propargylic sulfone **7** cannot alkylate the EB-bound DNA. In contrast to **7**, compound **10** suffered a little inhibitory effect imposed by EB (Lane 6 compared with Lane 5). It is suggested that the naphthalene residue in **10** could replace the DNA-bound EB and initiated the alkylation process. This phenomenon is different from the previous observation.[2b,13]

The bifunctional electrophile **15** has the potential to react with double-helical DNA across the strands and lead to interstrand crosslinkage formation.[14,15] Figure 5 illustrated the agarose gel electrophoresis picture of the reaction products of **8** and **15** with DNA. Due to precipitation of the sulfones at higher concentrations the incubation time with DNA was extended to 1 week. It was revealed that the DNA cleaving potency of **15** was roughly two times of that for **8** (Lanes 2 and 3). Moreover, compound **15** showed double strand cleavage (formation of Form III DNA product) at >2 mM concentrations. The Form III band is clearly seen in Lanes 5 and 6 of Figure 5.

It has been known that the positively charged complex of crown ether with cation can bind and enhance the interaction with DNA.[7] The effect of metal complex of **14** on DNA breakage was examined. The acetates of Na^+, K^+, Sr^{+2}, and Ba^{+2} alone caused no DNA cleavage at 10 mM concentration (data not showed). The metal ions Na^+, K^+, Sr^{+2} did not modify the potency of **14** (Lanes 2-5 in Figure 6). However, an inhibition on DNA

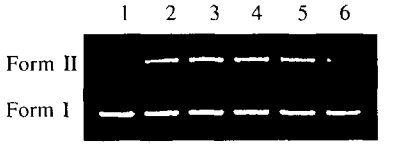
cleavage by **14** in the presence of 1 mM $Ba(Ac)_2$ was observed in Lane 6. The cause of this phenomenon is not known and deserves further experimentation.

Figure 5



ΦX174 Form I DNA (50 μM/bp) was incubated for 168 h at 37 °C with propargylic sulfones in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: DNA+2 mM **8**; Lane 3: DNA+1 mM **15**; Lane 4: DNA+2 mM **15**; Lane 5: DNA+3 mM **15**; Lane 6: DNA+3.5 mM **15**.

Figure 6



ΦX174 Form I DNA (50 μM/bp) was incubated for 72 h at 37 °C with propargylic sulfone **14** in TEA buffer (pH 8.5) containing 20% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA only; Lane 2: DNA+1 mM **14**; Lane 3: DNA+1 mM **14** +1 mM NaAc; Lane 4: DNA + 1 mM **14** + 1 mM KAc; Lane 5: DNA + 1 mM **14** + 1 mM $Sr(Ac)_2$; Lane 6: DNA+1 mM **14**+1 mM $Ba(Ac)_2$.

In summary, a number of novel hydroxy-containing propargylic sulfones were synthesized and their DNA cleavage activities were examined. Higher potency was observed with compounds **9** and **10** which possess a DNA-intercalating naphthalene residue. Orientation of the DNA-binding moiety is crucial in DNA cleavage by the anthracene-containing propargylic sulfones **11** and **12**. Preliminary experiments on the bifunctional agent **15** and the metal-regulatory effect on the benzo-15-crown-5-derived compound **14** were performed. The results described above should spark new ideas on the molecular design of the propargylic sulfone-based DNA cleaving and anticancer agents.

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10. All new compounds gave satisfactory ^{1}H and ^{13}C NMR spectra and MS data.

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12. The DNA substrate was treated with 50 μM ethidium bromide for 2 h before incubating with the propargylic sulfones.

13. In contrast to the naphthalene-derived propargylic sulfone **10**, incubation of the anthryl analogs **11** and **12** (1 mM) with the EB-pretreated DNA (50 μM) at 37 °C for 72 h or 192 h under the conditions indicated in Figure 4 showed a complete loss of DNA cleavage activity. It gives a qualitative order of intercalating ability with DNA: naphthalene = ethidium bromide > anthracene.

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