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Synthesis, DNA Cleavage, and Cytotoxicity of a Series of Bis(propargylic) Sulfone Crown Ethers

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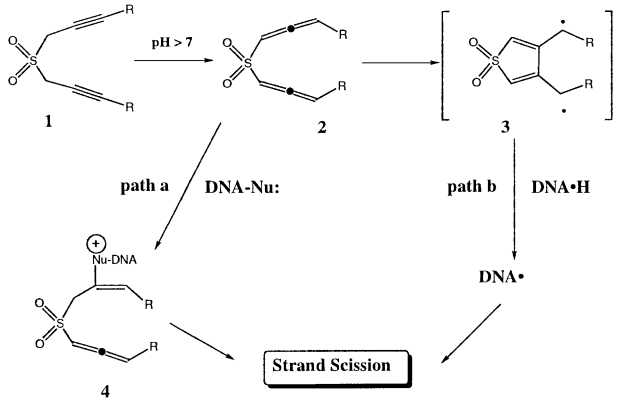
Abstract—Compounds that couple molecular recognition of specific alkali metal ions with DNA damage may display selective cleavage of DNA under conditions of elevated alkali metal ion levels reported to exist in certain cancer cells. We have prepared a homologous series of compounds in which a DNA reactive moiety, a bis(propargylic) sulfone, is incorporated into an alkali metal ion binding crown ether ring. Using the alkali metal ion pricrate extraction assay, the ability of these crown ethers to bind Li^+, Na^+, and K^+ ions was determined. For the series of crown ethers, the association constants for Li^+ ions are generally low ($<2\times10^4\,M^{-1}$). Only two of the bis(propargylic) sulfone crown ethers associate with Na^+ or K^+ ions (K_a 4–$8\times10^4\,M^{-1}$), with little discrimination between Na^+ or K^+ ions. The ability of these compounds to cleave supercoiled DNA at pH 7.4 in the presence of Li^+, Na^+, and K^+ ions was determined. The two crown ethers that bind Na^+ and K^+ display a modest increase in DNA cleavage efficiency in the presence of Na^+ or K^+ ions as compared to Li^+ ions. These two bis(propargylic) sulfone crown ethers are also more cytotoxic against a panel of human cancer cell lines when compared to a non-crown ether macrocyclic bis(propargylic) sulfone.

Introduction

Nicolaou and coworkers have described bis(propargylic) sulfone-containing molecules (e.g., **1**, Scheme 1) as novel DNA-cleaving agents.[1] These agents were designed with the capacity to enter two manifolds in which two putative DNA-cleaving intermediates could be generated (Scheme 1). In both paths, isomerization to a bis(allenic) species, **2**, is required. This reactive intermediate was postulated to act either as an electrophile to alkylate DNA (path a) or as a precursor to a diradical intermediate via a Braverman cyclization reaction[2] (path b). Electrophilic alkylation of DNA can result in DNA strand cleavage through a variety of routes.[3] Diradical intermediates (path b) may be capable of abstracting hydrogen atoms from the DNA backbone, resulting in strand scission.[4] We,[5] along with Nicolaou and coworkers,[6] have shown data that suggests that the alkylation mechanism (path a) rather than the diradical mechanism (path b) is the predominant or exclusive mode of DNA cleavage for these compounds. While this finding is in accord with Braverman's earlier reports of the relatively high temperature required for the cyclization of bis-γ,γ-dimethylallenyl sulfones to the corresponding diradicals,[2] a more recent report by Braverman and coworkers demonstrates that certain bis(propargylic) sulfones (e.g., **1**, R=Ph) do undergo a facile reaction involving the diradical intermediate **3**.[7]

Incorporation of the bis(propargylic) sulfone moiety into a macrocyclic ring might affect the efficiency of DNA cleavage through ring strain effects on the isomerization to the bis(allenylic) sulfone or the partitioning between electrophilic and free radical pathways



Scheme 1. DNA cleavage by bis(propargylic) sulfones.

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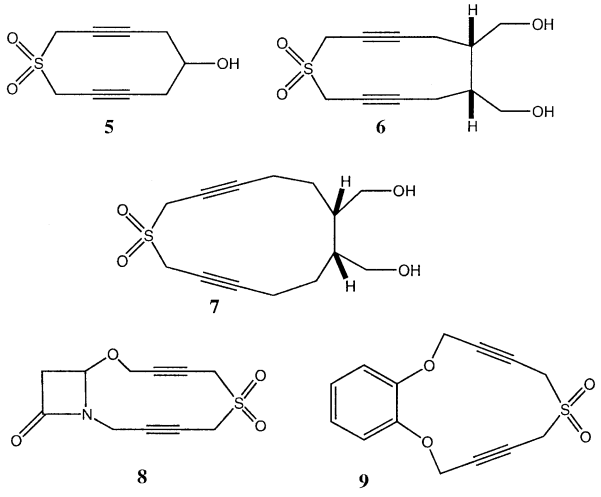
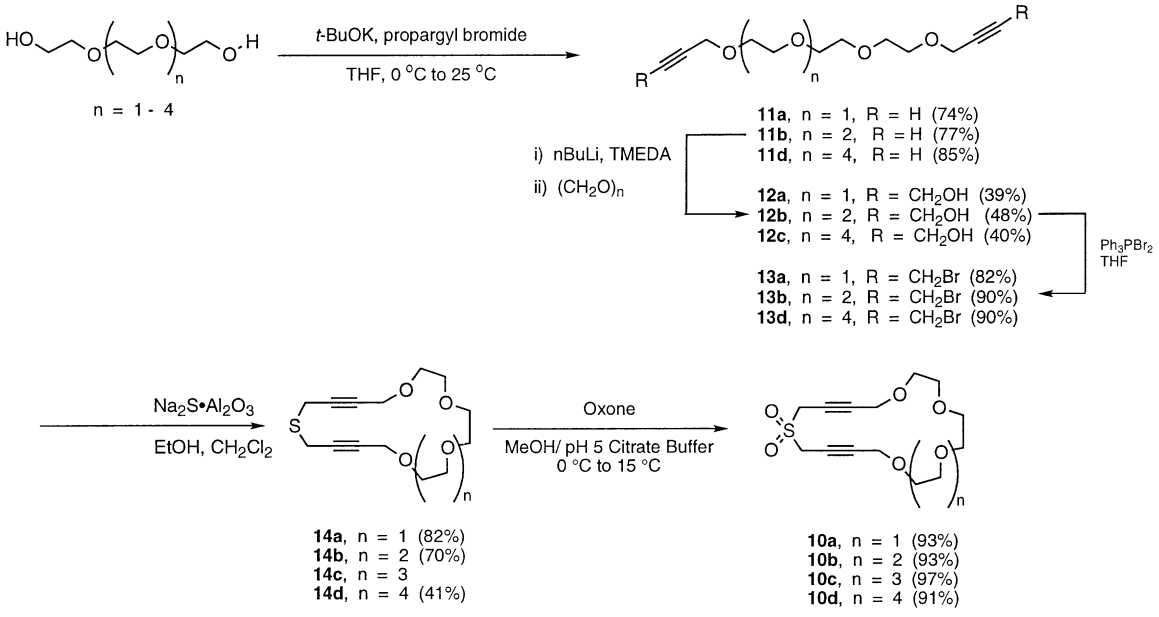
(Scheme 1). The original DNA cleavage studies of Nicoloau revealed that the cyclic bis(propargylic) sulfones display an interesting relationship between ring size and DNA cleavage potency. The 11-membered ring compound **6** (Scheme 2) was found to be slightly more potent than either the 10-membered ring compound **5** or the 13-membered ring compound **7**.[1] Later, Basak and Khamrai reported the 'locking' effect of the β-lactam moiety in macrocyclic bis(propargylic)sulfone **8** (Scheme 2), that, unlike the catecol-derived compound **9**, does not undergo isomerization to the bis(allenylic) sulfone.[8]

We have attempted to modulate the DNA cleavage of bis(propargylic) sulfones by incorporating this moiety into crown ether rings. In addition to inherent differences in DNA cleavage efficiency dictated by the crown ether ring size, we hypothesized that complexation of the crown by an appropriate metal ion might affect the facility of DNA cleavage by virtue of the conformational changes brought about by such complexation or by increased affinity of the cationic metal ion complexes with the polyanionic backbone of DNA.[9]



Scheme 2. Structures of cyclic bis(proparglic) sulfones.

Dai and coworkers have reported the synthesis, DNA cleavage, and cytotoxicity of a number of propargylic sulfone-armed lariat benzo-15-crown-5 crown ethers.[10,11] In this series of compounds, there was no evidence of metal-ion enhanced DNA cleavage or increased cancer cell cytotoxicity relative to non-crown-containing analogues; however, the metal ion binding ability of these lariat crown ethers was not reported. We have recently reported the synthesis and alkali metal ion-regulated DNA cleavage by a distinct series of propargylic sulfone-armed lariat crown ethers.[12] These compounds cleave DNA more readily in the presence of K^+ than Li^+ ions, with selectivities ranging from 2- to 10-fold. While these results are encouraging, the need for higher degrees of selectivity and Na^+/K^+ ion descrimination have led us to examine compounds in which the bis(proparglyic) sulfone DNA cleavage moiety incorporated within a metal ion binding crown ether ring. In this paper, we report the synthesis, alkali metal ion binding, DNA cleavage studies, and cancer cell cytotoxicity of a series of macrocyclic bis(propargylic) sulfone crown ethers **10a–d** (Scheme 3). For those compounds in this series that bind sodium and potassium ions, there is a modest enhancement of the DNA cleavage ability in the presence of these alkali metal ions. However, even in the presence of the cognate alkali metal ions, the DNA cleavage ability of these compounds is in general worse than a simple macrocyclic bis(propopargylic) sulfone that is unable to bind metal ions. The cytotoxicity of the sodium and potassium binding bis(propargylic) sulfone crown ethers is greater than a macrocyclic bis(propargylic) sulfone that does not incorporate a crown ether ring.



Scheme 3. Synthesis of crown ethers.

Results

Synthesis of crown ethers

Our previously published route[5] to the bis(propargylic) sulfone crown ether **10a** involved addition of 4-bromobut-2-yn-4-ol to triethylene glycol. Efforts to produce the homologous series of crown ethers **10b–d** by this route were hampered by difficulties encountered in the alkylation of higher ethylene glycol homologues. Based upon our successful route to the thiocrown ether **14c**, an intermediate in the syntheses of the corresponding enediyne crown ether,[13] we adopted an alternative route to the bis(propargylic) sulfone crown ethers **10a–d**, which is shown in Scheme 3. The appropriate ethylene glycols were first alkylated with propargyl bromide. The resultant bis(propargylic) ethers **11a–d** were subjected to deprotonation with nBuLi in the presence of TMEDA, followed by quenching with paraformaldehyde to afford the diols **12a–d** in modest yield. Treatment of diols **12a–d** with in situ generated PPh_3Br_2 afforded the desired dibromides **13a–d.** Dibromides **13a–c** underwent macrocyclization in the presence of alumina-supported Na_2S to afford the thiocrown ethers **14a–d.** Oxidation with Oxone®[14] afforded excellent yields of the desired bis(propargylic) sulfone crown ethers, which, in most cases, were analytically pure after workup. We also prepared the previously reported[8] compound **9** (Scheme 2) as a control bis(propargylic) sulfone lacking a crown ether ring. Compound **9** (Scheme 2) had been prepared as a mixture along with the corresponding bis(allenylic) sulfone by mCPBA oxidation of the corresponding sulfide. We found that Oxone® oxidation of the sulfide affords the catecol-derived bis(propargylic) sulfone **9** free from any contaminating bis(allenylic) material.

Metal ion binding

The complex association constants, K_a, for the alkali metal ion–host complexes were determined by picrate extraction techniques as described previously,[15] and the average values of these determinations are presented in Table 1. For hosts that did not display measurable affinity for a particular metal ion, the lower limit of the K_a value that could be determined is presented.

Inspection of Table 1 reveals that bis(propargylic) sulfone crown ether **10a** displays a weak affinity for Li^+ ions. This is not surprising considering that this agent has the smallest ring size and thus might accommodate the small Li^+ cation best within the ion-binding cavity. Affinity for Na^+ and K^+ ions follow similar trends in the bis(propargylic) sulfone crown ether series; as the crown ether ring size increases, so too does the affinity for these metal ions. Only the two largest crown ethers, **10c** and **10d,** bind Na^+ and K^+ ions well. In general, these crowns do not distinguish well between Na^+ and K^+ ions. As expected, the model bis(propargylic) sulfone **9**, which lacks a crown ether metal ion recognition moiety, does not bind alkali metal ions to any appreciable extent.

Table 1. Complex association constants, K_a, for Li^+, Na^+, and K^+ ions with bis(propargylic) sulfone hosts

Host	Initial concn (mM)[a]	K_a ($\times 10^{-3}$ M^{-1})		
		Li^+	Na^+	K^+
10a	15	15±4	<3	<3
10b	30	<2	6±2	14±1
10c	13	4±4	38±4	44±1
10d	10	<5	40±10	80±10
9	30	<2	<2	<2

[a]Initial concentration of the host in the organic phase. The initial concentration of alkali metal ion picrate in the aqueous phase was 3 mM.

DNA cleavage studies

Initial DNA cleavage studies utilizing supercoiled, Form I ΦX174 phage DNA at pH 8.5 demonstrated the ability of bis(propargylic) sulfone crown ether **10a** to produce frank strand breakage.[5] However, we were most interested in the ability of alkali metal ions to influence the degree of DNA cleavage exhibited by these compounds at physiological pH. Stock solutions of supercoiled pGAD424 plasmid, isolated using a QIAprep Miniprep kit (QIAGEN Inc.), were diluted with sterile, pH 7.4 lithium, sodium, or potassium phosphate buffers (20 mM alkali metal ions) to afford DNA solutions that contained primarily a single alkali metal cation species. These alkali metal ion-enriched DNA preparations are referred to as M^+DNAs (e.g., Li^+DNA, Na^+DNA, K^+DNA). DNA cleavage experiments were performed by adding freshly prepared solutions of the compounds in DMSO to solutions of M^+DNA followed by incubation at 25 °C for 18 h. Few frank DNA strand breaks were evident under these DNA cleavage reaction conditions; however, when DNA cleavage incubations were followed by brief (90 s) heating at 70 °C, much more DNA cleavage was evident. The DNA cleavage products were quantified by agarose gel electrophoresis followed by ethidium bromide staining and quantification by a fluorimager. An image of the separated, stained DNA fragments from a typical DNA cleavage reaction involving compound **10b** is shown in Figure 1. DNA cleavage results are presented as EC_{25} values, the concentration of each compound required to produce 25% normalized DNA cleavage, which were determined by interpolation of concentration versus normalized DNA cleavage curves.

The results of the DNA cleavage assays for the bis(propargylic)sulfone crown ethers **10a–d** and model bis(propargylic)sulfone **9** are presented in Table 2. All of the compounds examined here are modest DNA cleavage agents at pH 7.4, with EC_{25} values in the millimolar range. Nicolaou and coworkers have demonstrated that propargylic sulfones cleave DNA optimally at a pH of 8.5. Under optimal pH conditions (pH 8.5) bis(properglyic) sulfone crown ether **10a** cleaves DNA more efficiently, with an EC_{25} of 200 μM,[5] which is comparable to the cleavage efficiency reported for other macrocyclic bis(propargylic) sulfones.[1] While most of the bis(propargylic) sulfone crown ethers did not show a strong dependence of DNA cleavage ability on alkali metal ion identity, compounds **10c** and **10d** both show a modest enhancement of cleavage of Na^+DNA and

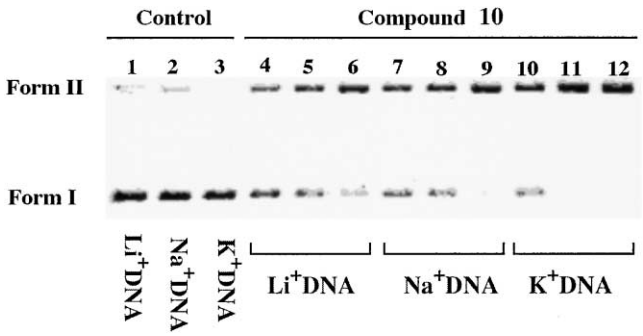


Figure 1. Cleavage of supercoiled (Form I) Li^+-, Na^+-, and K^+DNA by bis(propargylic) sulfone crown ether **10b**. Solutions of pH 7.4 M^+DNA (23 μM base pairs) containing 12.5% v/v DMSO were incubated for 18 h at 25 °C with compound **10b**. Samples were heated at 70 °C for 90 s then mixed with loading buffer, and the nicked DNA products (form II) separated from supercoiled (form I) DNA by electrophoresis (0.7% agarose, TBE buffer, 45 V, 3 h) followed by ethidium bromide fluorescence imaging. Lane 1, Li^+DNA control; lane 2, Na^+DNA control; lane 3, K^+DNA control; lane 4, **10b** (7.5 mM) + Li^+DNA; lane 5, **10b** (10 mM) + Li^+DNA; lane 6, **10b** (15 mM) + Li^+DNA; lane 7, **10b** (7.5 mM) + Na^+DNA; lane 8, **10b** (10 mM) + Na^+DNA; lane 9, **10b** (15 mM) + Na^+DNA; lane 10, **10b** (7.5 mM) + K^+DNA; lane 11, **10b** (10 mM) + K^+DNA; lane 12, **10b** (15 mM) + K^+DNA.

K^+DNA as compared to Li^+DNA. As expected, the model sulfone **9** has no alkali metal ion preference in its DNA cleavage reactions.

Cytotoxicity

Two of the bis(propargylic) sulfone crown ethers, compounds **10c** and **10d**, displayed the highest sodium and potassium metal ion binding in the series, and showed a modest enhancement in DNA cleavage in the presence of sodium and potassium. These two bis(propargylic) sulfone crown ethers were assayed against a range of cancer cell lines from nine different panels by the NCI. The results of these assays, summarized in Table 3, demonstrate that both of these crown ethers are more growth inhibitory than the non-crown ether bis(propargylic) sulfone **6**. In particular, compound **10c** is a moderately strong inhibitor ($GI_{50} < 10$ μM) against two leukemia cell lines, one non-small cell lung cancer cell line, two colon cell lines, and two melanoma cell lines.

Discussion

We have prepared a homologous series of bis(propargylic) sulfone crown ethers in order to explore the role of alkali metal ion binding on the DNA cleavage efficiency and cancer cell cytotoxicity in these compounds. Only two of the compounds in this series, crown ethers **10c** and **10d,** showed any significant affinity for Na^+ or K^+ ions as determined by the picrate extraction method. Using a DNA cleavage assay at physiologically relevant pH conditions, all of the bis(propargylic) sulfones examined cleave double-stranded DNA to afford products of single-strand DNA scission, with significantly more cleavage observed after brief heat treatment of the DNA cleavage reactions. These results are commensurate with an electrophilic DNA cleavage mechanism (Scheme 1, path a). We noted an effect of the crown ether ring size on the DNA cleavage ability in this series of bis(propargylic) sulfone crown ethers. Figure 2 demonstrates the relationships between DNA cleavage efficiency, alkali metal ions, and ring size for bis(propargylic) sulfone crown ethers **10a–d** and the model bis(propargylic) sulfone compound **9**. Examination of Figure 2 demonstrates that the DNA cleavage ability of model compound **9** (ring size 13) and the crown ethers **10a** (ring size 19) and **10b** (ring size 22) is insensitive to the nature of the alkali metal ion present, and that these three compounds cleave DNA with approximately the same efficiency. In contrast, the higher crown ether homologues **10c** (ring size 25) and **10d** (ring size 28) are much less efficient DNA cleavage

Table 2. Cleavage of Li^+-, Na^+-, and K^+-DNA by bis(propargylic) sulfones

Compound	EC_{25} (mM)[a]		
	Li^+DNA	Na^+DNA	K^+DNA
10a	4.6±0.5	3.7±0.9	3.1±0.4
10b	3.2±0.9	3.0±0.4	1.9±0.2
10c	8.7±0.9	4.1±0.5	4.2±0.5
10d	11.8±0.8	6.6±0.9	8.3±0.6
9	1.9±0.5	1.7±0.4	2.4±0.7

[a]Concentration required to effect 25% normalized cleavage of supercoiled DNA at pH 7.4, 25 °C for 18 h. All values are expressed as the mean±SD for three separate determinations.

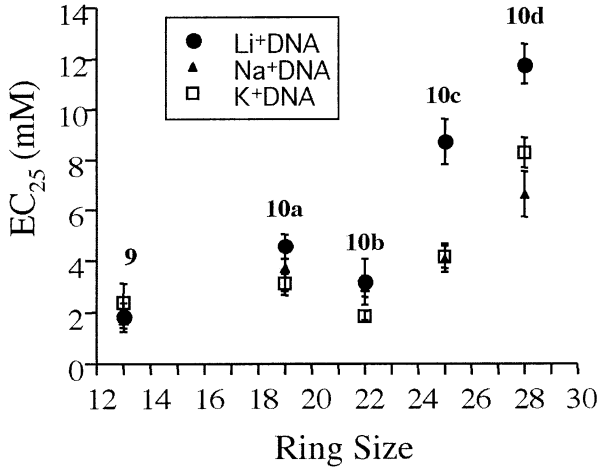


Figure 2. DNA cleavage efficiency, expressed as EC_{25}, the concentration of compound require to effect 25% normalized cleavage of supercoiled DNA, as a function of ring size and alkali metal ion for bis(propargylic) sulfones **9** and **10a–d**.

Table 3. Cancer cell growth inhibition by bis(propargylic) sulfone crown ethers

Panel/cell line	Compound **10c** GI_{50} (μM)[a]	Compound **10d** GI_{50} (μM)[a]	Compound **6** GI_{50} (μM)[a]
Leukemia			
HL-60 (TB)	13.5	20.9	50.1
K-562	11.2	23.9	63.1
MOLT-4	3.7	19.9	39.8
RPMI-8226	nd[b]	17.4	nd[b]
SR	8.2	18.9	39.8
Non-small cell lung cancer			
A-549/ATCC	20.2	61.7	>100
EKVX	15.1	20.9	>100
HOP-62	21.7	21.0	>100
HOP-92	10.9	19.6	31.6
NCI-H226	39.7	86.9	nd[b]
NCI-H322M	17.5	32.3	>100
NCI-H460	19.3	38.0	>100
NCI-H522	2.86	10.6	20.0
Colon cancer			
COLO 205	7.2	14.7	>100
HCC-2998	19.1	17.7	63.1
HCT-116	8.1	15.3	>100
HCT-15	15.5	32.7	>100
HT29	13.8	18.7	>100
KM12	16.2	20.7	>100
SW-620	10.4	14.3	39.8
CNS cancer			
SF-268	24.2	30.5	nd[b]
SF-295	32.0	81.2	>100
SF-539	11.1	26.2	>100
SNB-19	18.2	27.9	nd[b]
SNB-75	19.9	32.6	79.4
U251	16.9	18.3	>100
Melanoma			
LOX IMVI	11.1	19.9	63.1
MALME-3M	2.7	14.6	20.0
M14	nd[b]	16.9	39.8
SK-MEL-2	16.1	17.3	>100
SK-MEL-28	18.9	17.9	79.4
SK-MEL-5	16.8	18.1	>100
UACC-257	11.6	21.2	>100
UACC-62	0.28	17.1	>100
Ovarian cancer			
IGROV1	16.3	44.6	39.8
OVCAR-3	15.8	19.7	>100
OVCAR-4	19.8	27.3	>100
OVCAR-5	17.1	18.0	>100
OVCAR-8	14.2	33.3	>100
SK-OV-3	36.0	32.2	>100
Renal cancer			
786-0	11.0	18.4	>100
A498	16.9	54.7	>100
ACHN	13.5	22.4	>100
CAKI-1	12.2	21.2	nd[b]
RXF 393	15.1	27.0	>100
SN12C	13.2	18.8	>100
TK-10	13.9	23.0	>100
Prostate cancer			
PC-3	18.6	43.4	nd[b]
DU-145	32.8	>100	nd[b]
Breast cancer			
MCF7	10.1	22.0	nd[b]
MCF7/ADR-RES	25.7	>100	nd[b]
MDA-MB-231/ ATCC	20.0	23.5	nd[b]
HS 578T	40.7	52.8	nd[b]
MDA-MB-435	13.3	20.4	nd[b]
MDA-N	14.2	18.9	nd[b]
BT-549	14.1	20.0	nd[b]
T-47D	20.6	24.5	nd[b]

[a]Concentration of compound required to inhibit cell line growth by 50%, as determined by the sulforhodamine B assay.
[b]Not determined.

agents in the presence of Li^+ ions. However, these two crown ethers, which are the only two in the series that bind Na^+ and K^+ ions with appreciable affinity, display slightly enhanced DNA cleavage efficiency in the presence of Na^+ and K^+ ions. Because compounds **10a–d** do not bind Li^+ ions with appreciable affinity, the general trend of decreasing DNA cleavage in the presence of Li^+ ions with increasing ring size in this series must be due to ring size effects independent of metal ion coordination effects. It appears that metal ion coordination with Na^+ or K^+ ions can serve to partially rescue the larger crown ethers **10c** and **10d** from this deleterious ring size effect; however, even in the presence of Na^+ or Li^+ ions, **10c** and **10d** do not cleave DNA as well as the catechol-based compound **9**. Dai and coworkers have discussed the role of intercalation in the DNA cleavage efficiency of properglyic sulfones.[11] It is possible that the cleavage efficiency of compound **9** is enhanced by intercalation interactions between the catechol ring of **9** and the DNA. The alkali metal ion enhanced DNA cleavage due to **10c** and **10d**, although modest, appears to be due to specific interactions between these crown ethers and the alkali metal ions. Weak metal ion binders like **10a–b**, and **9** do not show any difference in DNA cleavage ability in the presence of different alkali metal ions, ruling out metal ion-induced changes in the DNA as the origin of the effect.

The two bis(propargylic) sulfone crown ethers, **10c–d,** that demonstrated some degree of alkali metal ion-dependent DNA cleavage were examined for their growth inhibitory effects against a variety of human cancer cell lines. Both compounds are moderately effective at inhibiting the growth of all cell lines examined, with little selectivity for any particular panel of cell lines. Crown ether **10c**, the more potent of the two compounds, is also the more efficient DNA cleavage agent; however, the concentrations required for growth inhibition are orders of magnitude less than those required in the DNA cleavage assays. A similar discrepancy has been noted in the DNA cleavage versus cytotoxicity of enediyne antitumor compounds.[16] In comparison, the previously reported bis(propargylic) sulfone **6** is much less growth inhibitory than crown ethers **10c–d**, displaying weak inhibitory activity mainly against the leukemia cell lines. Alkali metal ion concentrations within cells are regulated during the cell cycle[17] and may be significantly altered in cancer cells versus normal cells.[18] The anticancer activity of **10c** and **10d** relative to the macrocyclic bis(propargylic) sulfone **6** may reflect the ability of **10c** and **10d** to cleave DNA better under the conditions of elevated sodium or potassium levels that may exist, at least transiently, in cancer cells. Further investigation with more potent non-metal ion-binding DNA cleavage agents, such as compound **9**, is required in order to address the role of alkali metal ion binding in the anticancer activity of these crown ethers.

In conclusion, we have shown that within a series of bis(propargylic) sulfone crown ethers, compounds **10c** and **10d**, which are the best ligands for Na^+ and K^+ ions, display alkali metal ion-regulated DNA cleavage, although the effect of the nature of the alkali metal ion on DNA cleavage is modest. These two crown ethers also inhibit the growth of a series of human cancer cell lines, and are more potent than the non-crown ether cyclic bis(propargylic) sulfone **6**. Studies to elucidate the molecular nature of the metallo-regulated DNA cleavage ability and the role of metal ion binding in the cytotoxicity of these bis(propargylic) sulfone crown ethers are underway, and will be reported in due course.

Experimental[19]

General procedure for bis(alkylation) using propargyl bromide. 1,8-Bis(2-propyn-1-oxy)-3,6-dioxaoctane (11a).[5] To a cooled (0 °C) suspension of *t*-BuOK (3.77 g, 95% w/w, 32 mmol) in 28 mL of THF under argon was added a solution of triethylene glycol (2.13 g, 14.2 mmol) in 3 mL of THF. The resulting glycol dialkoxide was allowed to warm to room temperature and was then added under argon via cannula to an ice-water bath cooled solution of propargyl bromide (6.3 mL, 80% w/w, 56.5 mmol) in 114 mL of THF. The reaction mixture was stirred with a mechanical stirrer for an additional 18 h as the ice-water bath melted and the reaction was allowed to warm to room temperature. The reaction mixture was diluted with 75 mL of 3:1 brine/water and the aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were washed with 40 mL of 1:1 brine/water and 65 mL brine. The residue upon drying and concentration was purified by flash chromatography on silica gel (50% hexanes in EtOAc) to afford bis(propargyl ether) **11a** (2.37 g, 74%) as a light golden oil: R_f 0.46 (50% hexanes in EtOAc); ^{1}H NMR δ 2.40 (t, J = 2.5 Hz, 2H), 3.63–3.72 (m, 12H), 4.18 (d, J = 2.5 Hz, 4H); ^{13}C NMR δ 58.05, 68.77, 70.08, 70.26, 74.35, 79.39; IR 2879, 2117, 1099 cm^{-1}; MS 227 (MH^+), 171, 127; HR-MS *m*/*e* calcd for $C_{12}H_{19}O_4$: 227.1283, found 227.1288.

1,11-Bis(2-propyn-1-oxy)-3,6,9-trioxaundecane (11b).[13] Following the general procedure (see compound **11a**), tetraethylene glycol (2.51 g, 12.9 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (35% hexanes in EtOAc) to afford bis(propargyl ether) **11b** (2.67 g, 77%) as a light yellow oil: R_f 0.45 (35% hexanes in EtOAc); ^{1}H NMR δ 2.37 (t, J = 2.4 Hz, 2H), 3.53–3.63 (m, 16H), 4.10 (d, J = 2.4 Hz, 4H); ^{13}C NMR δ 58.11, 68.83, 70.13, 70.31, 70.33, 74.38, 79.43; IR 2872, 2117, 1101 cm^{-1}; MS 271 (MH^+), 215, 171, 127; HR-MS *m*/*e* calcd for $C_{14}H_{23}O_5$: 271.1545, found 271.1542.

1,17-Bis(2-propyn-1-oxy)-3,6,9,12,15-pentaoxaheptadecane (11d). Following the general procedure (see compound **11a**), hexaethylene glycol (2.89 g, 10.2 mmol) gave a residue after workup that was purified by flash chromatography (5% MeOH on EtOAc) to afford bis(propargyl ether) **11d** (3.11 g, 85%) as a yellow oil: R_f 0.53 (5% MeOH in EtOAc); ^{1}H NMR δ 2.38 (t, J = 2.4 Hz, 2H), 3.55–3.66 (m, 24H), 4.14 (d, J = 2.4 Hz, 4H); ^{13}C NMR δ 58.22, 68.95, 70.24, 70.42 (4C), 74.42,

79.52; IR 2872, 2116, 1107 cm^{-1}; MS 359 (MH^+), 259, 215, 171, 127; HR-MS *m/e* calcd for $C_{18}H_{31}O_7$: 359.2070, found 359.2060.

General procedure for hydroxymethylation. 1,8-Bis(4-hydroxy-2-butyn-1-oxy)-3,6-dioxaoctane (12a).[5] An argon-flushed 100 mL three-neck round bottom flask equipped with a mechanical stirrer was charged with a solution of bis(propargyl) ether **11a** (593 mg, 2.62 mmol) in 22 mL of THF and TMEDA (4.0 mL, 26.5 mmol). The mixture was cooled with stirring to −78 °C, and *n*-BuLi (2.7 mL, 2.37 M, 6.39 mmol) was added dropwise with stirring. After an additional 5 min, a stirring suspension of paraformaldehyde (1.7 g, 56.6 mmol formaldehyde equivalents) in 7 mL of THF under argon was added quickly via cannula. After an additional 5 min the cooling bath was removed and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred an additional 1 h and diluted with 90 mL of EtOAc and 60 mL of saturated aqueous NaH_2PO_4. The aqueous layer was extracted with EtOAc (3×45 mL), and the combined organic layers were washed with 60 mL of saturated $NaHCO_3$ and 65 mL of brine. The residue upon drying and concentration was purified by flash chromatography (2% MeOH in EtOAc) to afford diol **12a** (293 mg, 39%) as a pale yellow solid: mp 33–34 °C; R_f 0.34 (2% MeOH in EtOAc); ^1H NMR δ 2.95 (s(br), 2H), 3.60–3.73 (m, 12H), 4.20 (t, $J=1.8$ Hz, 4H), 4.27 (t, $J=1.8$ Hz, 4H); ^{13}C NMR δ 50.76, 58.62, 68.99, 70.37, 70.50, 81.27, 85.05; IR 3400 cm^{-1}; MS 287 (MH^+), 269, 201, 157, 113; HR-MS *m/e* calcd for $C_{14}H_{23}O_6$: 287.1495, found 287.1485.

1,11-Bis(4-hydroxy-2-butyn-1-oxy)-3,6,9-trioxaundecane (12b). Following the general procedure (see compound **12a**), compound **11b** (769 mg, 2.85 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (5% MeOH in EtOAc) to afford diol **12b** (450 mg, 48%) as a pale yellow oil: R_f 0.3 (5% MeOH in EtOAc); ^1H NMR δ 2.86 (s(br), 2H), 3.60–3.70 (m, 16H), 4.20 (t, $J=1.9$ Hz, 4H), 4.26 (t, $J=1.9$ Hz, 4H); ^{13}C NMR δ 50.75, 58.60, 68.96, 70.37, 70.49 (2C), 81.28, 85.07; IR 3400 cm^{-1}; MS 331 (MH^+), 313, 201, 157, 113; HR-MS *m/e* calcd for $C_{16}H_{27}O_7$: 331.1757, found 331.1756.

1,17-Bis(4-hydroxy-2-butyn-1-oxy)-3,6,9,12,15-pentaoxaheptadecane (12d). Following the general procedure (see compound **12a**), compound **11d** (755 mg, 2.11 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (10% MeOH in EtOAc) to afford compound **12d** (357 mg, 40%) as a pale yellow oil: R_f 0.22 (10% MeOH in EtOAc); ^1H NMR δ 2.70 (s(br), 2H), 3.58–3.70 (m, 24H), 4.20 (t, $J=1.9$ Hz, 4H), 4.26 (t, $J=1.9$ Hz, 4H); ^{13}C NMR δ 50.78, 58.61, 69.01, 70.38 (4C), 70.51, 81.32, 85.03; IR 3442 cm^{-1}; MS 419 (MH^+), 401, 289, 245, 201; HR-MS *m/e* calcd for $C_{20}H_{35}O_9$: 419.2281, found 419.2284.

General procedure for bromination. 1,8-Bis(4-bromo-2-butyn-1-oxy)-3,6-dioxaoctane (13a). To an ice-water bath cooled solution of PPh_3 (457 mg, 1.74 mmol) in 4 mL of CH_2Cl_2 under argon was added Br_2 (87 μL, 1.7 mmol). After an additional 5 min, a solution of diol **12a** (202 mg, 0.707 mmol) in 1.9 mL of CH_2Cl_2 was added dropwise via cannula over 3 min, and the resulting reaction mixture was stirred for an additional 5 h as the reaction mixture was allowed to warm to room temperature. The reaction mixture was diluted with 40 mL of EtOAc and washed with 15 mL of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc (3×10 mL), and the combined organic layers were washed with 20 mL of brine, dried, and concentrated to a residue that was purified by flash chromatography on silica gel (3% hexanes in EtOAc) to afford dibromide **13a** (239 mg, 82%) as a pale yellow oil: R_f 0.81 (3% hexanes in EtOAc); ^1H NMR δ 3.59–3.65 (m, 12H), 3.90 (t, $J=2.0$ Hz, 4H), 4.20 (t, $J=2.0$ Hz, 4H); ^{13}C NMR δ 14.18, 58.56, 69.14, 70.29, 70.48, 81.30, 82.79; IR 617 cm^{-1}; MS 411 (MH^+), 263, 219, 175; HR-MS *m/e* calcd for $C_{14}H_{21}Br_2O_4$: 410.9807, found 410.9802.

1,11-Bis(4-bromo-2-butyn-1-oxy)-3,6,9-trioxaundecane (13b). Following the general procedure (see compound **13a**), diol **12b** (220 mg, 0.668 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (EtOAc) to afford dibromide **13b** (275 mg, 90%) as a pale yellow oil: R_f 0.73 (EtOAc); ^1H NMR δ 3.55–3.65 (m, 16H), 3.90 (t, $J=2.0$ Hz, 4H), 4.20 (t, $J=2.0$ Hz, 4H); ^{13}C NMR δ 14.15, 58.54, 69.13, 70.27, 70.45, 70.48, 81.28, 82.78; IR 614 cm^{-1}; MS 456 (MH^+), 377, 309, 265, 221, 177; HR-MS *m/e* calcd for $C_{16}H_{25}Br_2O_5$: 455.0069, found 455.007.

1,17-Bis(4-bromo-2-butyn-1-oxy)-3,6,9,12,15-pentaoxaheptadecane (13d). Following the general procedure (see compound **13a**), diol **12d** (427 mg, 1.02 mmol) gave a light tan solid after workup that contained dibromide **13d** (1.17 g, 43% w/w **13d**, 90%) and Ph_3PO as an inseparable mixture. Analytical data for dibromide **13d**: R_f 0.56 (5% MeOH in EtOAc); ^1H NMR δ 3.55–3.70 (m, 24H), 3.93 (t, $J=2.2$ Hz, 4H), 4.24 (t, $J=2.2$ Hz, 4H); ^{13}C NMR δ 14.20, 58.66, 69.26, 70.39, 70.60 (4C), 81.39, 82.91; MS 544 (MH^+), 307; HR-MS *m/e* calcd for $C_{20}H_{33}Br_2O_7$: 543.0593, found 543.0583.

$Na_2S{\cdot}Al_2O_3$ reagent. To $Na_2S{\cdot}9H_2O$ (7.1 g, 0.03 mmol) that had been rinsed with a small amount of distilled, deionized water and placed in a flask under argon was added 18 mL of warm, distilled, deionized water that had been boiled to remove CO_2. The resultant solution was poured into a flask containing Al_2O_3 (neutral, Brockmann Activity I, 80–200 mesh, 8.7 g, 0.085 mmol), and the water was removed in vacuo via rotary evaporator with gentle heating in a warm water bath. The material was then activated by heating in vacuo (95 °C, 0.1 torr) for 1.5 h until the material (21.2% w/w Na_2S) was a free-flowing pink powder. The reagent was stored under argon and used shortly after it was prepared.

General procedure for macrocyclization. 6,9,12,15-Tetraoxa-1-thiacyclononadeca-3,17-diyne (14a).[5] To a solution of dibromide **13a** (150 mg, 0.364 mmol) in 24 mL of 5:1 CH_2Cl_2/EtOH was added in one portion

$Na_2S{\cdot}Al_2O_3$ (0.311 g, 22% w/w Na_2S, 0.877 mmol). The reaction mixture was allowed to stir under argon at room temperature for 3 days. The heterogeneous reaction mixture was filtered through Celite and the solids were washed with EtOAc. The combined eluant was concentrated, and the residue was purified by flash chromatography on silica gel (3% hexanes in EtOAc) to afford sulfide **14a** (85 mg, 82%) as a colorless solid: mp 42–43 °C; R_f 0.52 (3% hexanes in EtOAc); ^{1}H NMR δ 3.49 (t, J = 1.9 Hz, 4H), 3.58–3.75 (m, 12H), 4.25 (t, J = 1.9 Hz, 4H); ^{13}C NMR δ 19.13, 58.64, 68.78, 70.35, 70.51, 79.70, 81.23; IR 1142, 1100 cm^{-1}; MS 285 (MH^+); HR-MS m/e calcd for $C_{14}H_{21}O_4S$: 285.1161, found 285.1164.

6,9,12,15,18-Pentaoxa-1-thiacyclodocosa-3,20-diyne (14b). Following the general procedure (see compound **14a**), dibromide **13b** (563 mg, 1.24 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (EtOAc) to afford sulfide **14b** (285 mg, 70%) as a pale yellow oil: R_f 0.36 (EtOAc); ^{1}H NMR δ 3.47 (t, J = 2.2 Hz, 4H), 3.62–3.73 (m, 16H), 4.23 (t, J = 2.2 Hz, 4H); ^{13}C NMR δ 19.05, 58.69, 68.80, 70.41 (2C), 70.82, 79.60, 81.34; IR 1135, 1101 cm^{-1}; MS 329 (MH^+); HR-MS m/e calcd for $C_{16}H_{25}O_5S$: 329.1423, found 329.1423.

6,9,12,15,18,21-Hexaoxa-1-thiacyclopentacosa-3,23-diyne (14c).[13] Following the general procedure (see compound **14a**), dibromide **13c** (382 mg, 0.764 mmol), prepared previously,[13] gave a residue after workup that was purified by flash chromatography on silica gel (5% MeOH in EtOAc) to afford sulfide **14c** (213 mg, 75%) as a pale yellow oil.

6,9,12,15,18,21,24-Heptaoxa-1-thiacyclooctacosa-3,26-diyne (14d). Following the general procedure (see compound **14a**), dibromide **13d** (379 mg, 43% w/w mixture with Ph_3PO, 0.3 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (10% MeOH in EtOAc) to afford 30.9 mg of pure sulfide **14d** as a colorless oil. Preparative TLC (1 mm silica gel plate, 10% MeOH in EtOAc) of **14d**-containing fractions that were contaminated with Ph_3PO yielded an additional 20.7 mg of pure **14d** (41% for combined material). Analytical data for compound **14d**: R_f 0.33 (10% MeOH in EtOAc); ^{1}H NMR δ 3.46 (t, J = 2.2 Hz, 4H), 3.62–3.70 (m, 24H), 4.23 (t, J = 2.2 Hz, 4H); ^{13}C NMR δ 19.2, 58.69, 68.91, 70.43, 70.61 (2C), 70.67 (2C), 79.37, 81.43; IR 1115, 1097 cm^{-1}; MS 417 (MH^+); HR-MS m/e calcd for $C_{20}H_{33}O_7S$: 417.1947, found 417.1944.

General procedure for sulfone formation. 6,9,12,15-Tetraoxa-1-thiacyclononadeca-3,17-diyne 1,1 Dioxide (10a).[5] To an ice-water bath cooled solution of sulfide **14a** (77.7 mg, 0.274 mmol) in 2.6 mL of MeOH was added dropwise a suspension of Oxone® (1.086 g, 0.877 mmol) in 2.6 mL of water and 1.24 mL of 2.5 M, pH 5 aqueous potassium citrate. Stirring was continued for an additional 18 h as the reaction mixture warmed to room temperature. The reaction mixture was diluted with 85 mL of water in a separatory funnel and extracted with 3×60 mL of $CHCl_3$. The chloroform extracts were washed with 55 mL of water and 65 mL of brine. Concentration of the organic layer afforded sulfone **10a** (80.2 mg, 93%) as a white solid. An analytical sample recrystallized from 40% EtOAc in hexanes gave the following: mp 132–133 °C; R_f 0.5 (5% MeOH in EtOAc); ^{1}H NMR δ 3.58–3.74 (m, 12H), 4.12 (t, J = 1.8 Hz, 4H), 4.27 (t, J = 1.8 Hz, 4H); ^{13}C NMR δ 43.84, 58.55, 69.29, 70.32, 70.51, 73.35, 84.76; IR 1328 cm^{-1}; MS 317 (MH^+); HR-MS m/e calcd for $C_{14}H_{21}O_6S$: 317.1059, found 317.1054.

6,9,12,15,18-Pentaoxa-1-thiacyclodocosa-3,20-diyne 1,1-dioxide (10b). Following the general procedure (see compound **10a**), sulfide **14b** (29.3 mg, 89.3 μmol) gave, after workup, sulfone **10b** (29.9 mg, 93%) as a colorless solid. An analytical sample recrystallized from 40% EtOAc in hexanes gave the following: mp 72.5–73.5 °C; R_f 0.54 (10% MeOH in EtOAc); ^{1}H NMR δ 3.57–3.72 (m, 16H), 4.10 (t, J = 1.8 Hz, 4H), 4.26 (t, J = 1.8 Hz, 4H); ^{13}C NMR δ 43.64, 58.51, 69.22, 70.34, 70.43, 70.72, 73.45, 84.57; IR 1338 cm^{-1}; MS 361 (MH^+), 317; HR-MS m/e calcd for $C_{16}H_{25}O_7S$: 361.1321, found 361.1324.

6,9,12,15,18,21-Hexaoxa-1-thiacyclopentacosa-3,23-diyne 1,1-Dioxide (10c). Following the general procedure (see compound **10a**), sulfide **14c** (57.4 mg, 0.154 mmol) gave a residue after workup that was purified by flash chromatography on silica gel (10% MeOH in EtOAc) to afford sulfone **10c** (60.4 mg, 97%) as colorless solid: mp 63–64 °C; R_f 0.44 (10% MeOH in EtOAc); ^{1}H NMR δ 3.56–3.73 (m, 20H), 4.12 (t, J = 2.0 Hz, 4H), 4.26 (t, J = 2.0 Hz, 4H); ^{13}C NMR δ 43.63, 58.55, 69.66, 70.45, 70.71, 70.76, 70.82, 73.57, 84.66; IR 1337 cm^{-1}; MS 405 (MH^+), 361, 341; HR-MS m/e calcd for $C_{18}H_{29}O_8S$: 405.1583, found 405.1593.

6,9,12,15,18,21,24-Heptaoxa-1-thiacyclooctacosa-3,26-diyne 1,1-dioxide (10d). Following the general procedure (see compound **10a**), sulfide **14d** (41.5 mg, 99.8 μmol) gave sulfone **10d** (40.8 mg, 91%) after workup. An analytical sample recrystallized from 40% EtOAc in hexanes gave the following: mp 87–88 °C; R_f 0.32 (10% MeOH in EtOAc); ^{1}H NMR δ 3.52–3.71 (m, 24H), 4.11 (s(br), 4H), 4.24 (s(br), 4H); ^{13}C NMR δ 43.56, 58.45, 69.17, 70.42, 70.60 (4C), 73.57, 84.53; IR 1355 cm^{-1}; MS 449 (MH^+), 404, 384, 361; HR-MS m/e calcd for $C_{20}H_{33}O_9S$: 449.1845, found 449.1849.

Alkali metal picrate extraction

The general procedure employed was as previously described.[12] Briefly, in a typical extraction, 350 μL of 3.0 mM aqueous metal picrate and 350 μL of 15.0 mM host in $CHCl_3$ were placed in a 0.5 gram vial. The vials were then vortexed for 1 min and centrifuged for 15 min to effect complete phase separation. A 50 μL aliquot of the $CHCl_3$ layer was removed and diluted to 1.0 mL with MeCN. Absorbance of this solution at 380 nm was used to calculated the association constants (K_a's) by the method of Cram.[15] The values presented in Table 1 represent the mean plus or minus one standard deviation from two separate extractions.

Preparation of alkali metal ion DNA

Supercoiled DNA was prepared as previously reported.[12] Briefly, DH5α *Escherichia coli* cells transfected with that pGAD424 were grown, collected, and lysed. The plasmid DNA was isolated with a QIAGEN miniprep spin kit according to the manufacturer's instructions, eluting the DNA from the column with distilled water. Aliquots (10 μL) of this solution were gently mixed with 212.2 μL of sterile, pH 7.4 aqueous alkali metal phosphate solution. The plasmid DNA so obtained was determined spectrophotometrically to be 17 μM base pair and typically contained 75–85% supercoiled (Form I) DNA. For EC_{25} experiments, the concentration of alkali metal ion before addition of the aqueous DNA solution was 20 mM.

Quantification of DNA cleavage by propargylic sulfones

In a typical cleavage experiment, 2 μL of a freshly prepared solution of bis(propargylic) sulfone compound in DMSO-d_6 was added to a sterile Eppendorf tube followed by 14 μL of an aqueous solution of alkali metal ion-containing DNA prepared as above. The contents were mixed by brief (5 s) centrifugation at 6000 rpm and allowed to stand at room temperature for 18 h. Tubes were then heated for 90 s at 70 °C. Upon cooling, 2 μL of 8× loading dye (0.13 g/mL each of Bromophenol Blue and Xylene Cyanol in 30% aqueous glycerol) was added to each tube, the contents were gently homogenized, and 5 μL of the resulting solution was loaded onto a 0.7% w/v agarose gel. The DNA cleavage products were separated by electrophoresis in 1× TBE running buffer at 45 V for 2.25 h. The agarose gel was stained for 15 min in TBE buffer that contained 0.25 μg/mL ethidium bromide and then destained in distilled water for 15–30 min. The gel was scanned with a Molecular Dynamics Fluorimager and the quantities of Forms I, II and III DNA were assessed with the ImageQuaNT software program. The degree of cleavage of Form I DNA was determined using eq 1.

$$\text{Percent cleavage} = \frac{(2 \times [\text{Form III}] + [\text{Form II}]}{(2 \times [\text{Form III}] + [\text{Form II}] + [\text{Form I}]} \times 100 \quad (1)$$

The reported, normalized percent cleavage accounts for cleavage in control samples under the reaction conditions employed and this was calculated according to eq (2).

$$\text{Normalized percent cleavage} = \frac{\%\ \text{cleavage (drug)} - \%\ \text{cleavage (control)}}{100 - \%\ \text{cleavage control}} \quad (2)$$

The reported EC_{25} values were obtained by interpolation from plots of normalized percent cleavage versus concentration. The EC_{25} values reported represent the average ± standard deviation for three or more separate determinations.

Cytotoxicity assay

The cytotoxicity assays were conducted at the NCI and were performed as described.[20] Briefly, cell suspensions that were diluted according to the particular cell type and the expected target cell density (5000–40,000 cells per well based on cell growth characteristics) were added by pipet (100 μL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37 °C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100-μL aliquots to the microtiter plate wells. Test compounds were evaluated at five 10-fold dilutions with the highest well concentration being 100 μM. Incubations lasted for 48 h in 5% CO_2 atmosphere and 100% humidity. The cells were assayed by using the sulforhodamine B assay.[21,22] A plate reader was used to read the optical densities, and a microcomputer program was used to process the optical densities into the GI_{50} concentration, the concentration of test drug where $100\times(T-T_0)/(C-T_0)=50$, where the optical density of the test well after a 48-h period of exposure to test drug is T, the optical density at time zero is T_0, and the control optical density is C.

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