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Structure–activity relationships in glycosylated 2-phenyl-indoles, 2-phenyl-benzo[*b*]thiophenes and 2-phenyl-benzo[*b*]furans as DNA binding and potential antitumor agents

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ABSTRACT

In earlier investigations we have described the synthesis and biological evaluation of a panel of novel glycosylated heteroaromatics (**1**–**12**). It was found that these compounds can bind to DNA in vitro and are cytotoxic against several cancer cell lines at low micromolar concentration. We report here structure–activity studies of these molecules with respect to DNA binding and cytotoxicity. In particular the structure of the linker moiety between the carbohydrate and the intercalator, the stereochemistry at the anomeric position, and the substituents and stereochemistry at C-4 in one of the carbohydrate residues (4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranose) are investigated. All these structural features were identified to have a clear influence on DNA binding; however, only the substituents at C-4 in the carbohydrate residue exhibited an obvious impact on cytotoxicity. It was found that the amino group at C-4 was favored over all other substituents with regard to both DNA binding and cytotoxicity. The information gathered from these structure–activity investigations suggested that future work on the preparation of additional analogues should focus on molecules containing an amino sugar moiety.

1. Introduction

DNA binding is one of the common modes of action for many clinically-used anticancer agents, such as dactinomycin, mitomycin, daunorubicin, and trabectedin.[1] Using a molecular hybridization approach,[2] we recently designed and synthesized a small panel of novel glycoconjugates (**1**–**12**, Chart 1).[3,4] These molecules comprise three functional modules: a flat aromatic moiety that can potentially intercalate between DNA base pairs, a minor-groove binding carbohydrate moiety,[5] and a linker, which we selected to be a propargylic group. By using direct and indirect fluorescence measurements, it was confirmed that **1**–**12** bind to DNA in vitro with moderate affinity.[3] It was also demonstrated that some of these analogues showed low micromolar antibacterial[4] and anticancer[6] activity in vitro against one strain of gram-positive bacteria and three solid-tumor cell lines.

In this paper, we investigated the effect of structural features in the carbohydrate and the propargyl linker in these compounds on cytotoxicity and DNA binding. Among the three configurations of the carbohydrate domain, we decided to concentrate our structure–activity relationship (SAR) analysis on the 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (4-*N*-TDTH) system (**9**–**12**) for two reasons. First, and most importantly, this series of analogues exhibited representative properties in terms of DNA binding, antibacterial activity, and cytotoxicity. Second, compared to daunosamine (**1**–**4**) and acosamine systems (**5**–**8**), the 4-*N*-TDTH system has one stereogenic center fewer, which simplifies SAR analysis. It should be noted that because of the dramatically lower cytotoxicity of free indole analogues (**4**, **8**, and **12**) compared to molecules with the other three aromatic domains,[6] the SAR analysis did not include analogues possessing a free indole core.

2. Results and discussion

2.1. Effect of stereochemistry at the anomeric position in the carbohydrate residue

2.1.1. DNA binding

It has been found that the configuration at the anomeric carbon can have a significant influence on the biological activity of carbohydrate-containing compounds, such as the anthracycline antibiotics.[7,8] Whereas the α-configured anthracyclines usually have potent anticancer activity and strong DNA binding affinity, their β-configured counterparts exhibit both lower cytotoxicity and much weaker DNA binding.[7,8] With enough quantities of the β-configured analogues **18**–**25** in hand (Chart 2),[4] we first investigated the effect of the anomeric configuration on biological activity.

Using a previously described ethidium bromide (EtBr) fluorescent intercalator displacement (FID) assay,[3] the DNA binding ability of analogues **13**–**25** were determined at 50 μM. As presented in

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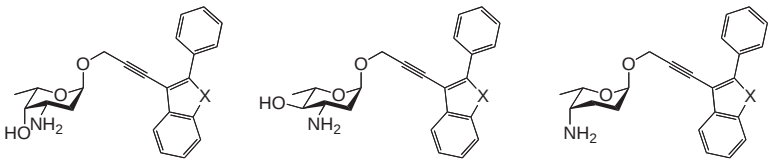
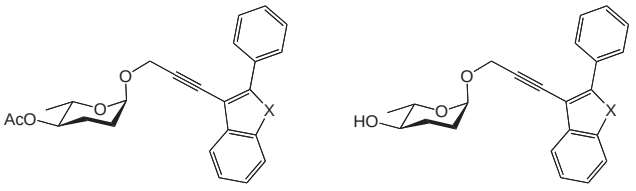


1 X = O
2 X = S
3 X = NTs
4 X = NH

5 X = O
6 X = S
7 X = NTs
8 X = NH

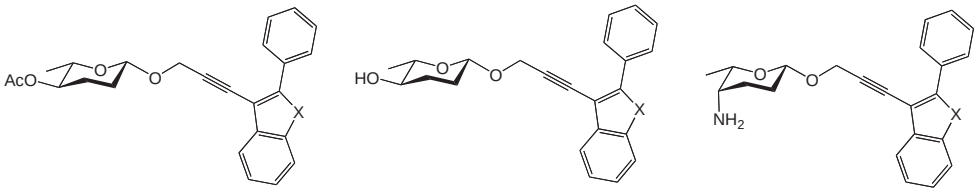
9 X = O
10 X = S
11 X = NTs
12 X = NH

Chart 1. Structures of anthracycline analogues **1**–**12**.



13 X = O
14 X = S

15 X = O
16 X = S
17 X = NTs



18 X = O
19 X = S

20 X = O
21 X = S
22 X = NTs

23 X = O
24 X = S
25 X = NTs

Chart 2. Structures of **13**–**25**.

Table 1, those compounds possessing an amino group (**23**–**25**), were shown to displace EtBr from DNA, thus indicating their ability to bind to DNA. However, no DNA binding could be detected by this assay for those compounds in which the amino group is replaced with a hydroxyl functionality (**15**–**17** and **20**–**22**). The O-acetylated analogues, **13**, **14**, **18**, and **19**, were not evaluated due to their poor solubility in the aqueous buffer required for the assay.

From these data, it is clear that the β-configured amino analogues **23**–**25** exhibited stronger DNA binding affinity (a larger amount of EtBr was displaced) than their α-configured equivalents **9**–**11**. Compared to the *N*-tosyl protected indole core (**11** vs. **25**), the enhancement is more pronounced for the benzo[*b*]furan (**9** vs. **23**) and benzo[*b*]thiophene systems (**10** vs. **24**). Subsequently, an FID titration experiment was performed to obtain the relative binding constant for **23**–**25** using the binding constant of EtBr as a reference (Fig. 1). Due to relatively weak DNA binding affinity, a linear relationship between fluorescence intensity and

Table 1
The effect of α and β configuration on DNA binding affinity[a]

Compd	% Remaining EtBr
9 (α)	68.5 ± 1.9
10 (α)	79.9 ± 1.0
11 (α)	38.6 ± 1.9
15 (α)	96.7 ± 3.2
16 (α)	102.6 ± 4.7[b]
17 (α)	111.2 ± 3.6[b]
20 (β)	103.2 ± 3.4[b]
21 (β)	99.9 ± 1.8
22 (β)	109.2 ± 5.1[b]
23 (β)	26.9 ± 1.1
24 (β)	26.6 ± 1.4
25 (β)	19.8 ± 1.0

[a] All the compounds were tested at 50 μM.
[b] The percentage is slightly over 100% presumably due to the weak fluorescence enhancement effect between the assayed compounds and free EtBr or the EtBr–DNA complex.

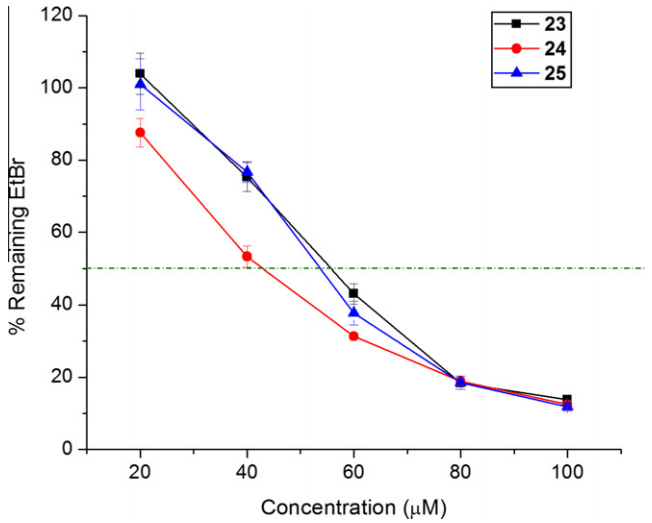


Figure 1. FID titration curves of β-configured analogues **23**–**25**.

concentration was not seen when the concentration of titrants was larger than 60 μM. Cognizant of the fluorescence interference for compounds **23** and **25** at 20 μM, which is indicated by a fluorescence intensity of over 100%, only the percentages of remaining EtBr between 40 and 60 μM were used to estimate the concentration at which there is a 50% fluorescence loss of EtBr. This value is approximately 55 μM for **23**, 45 μM for **24**, and 55 μM for **25**. Using the equation $K_{Ligand} = K_{EtBr}[EtBr]/[Ligand]_{50\%}$,[9,10] the DNA binding affinity of the β-configured analogues is 8–10-fold less than EtBr, but is at least 2–4-fold stronger than the corresponding α-configured isomers.

2.1.2. Cytotoxicity

Next, compounds **13**–**25** were screened against three cancer cell lines. Because the observed trends are the same for all the three aromatic systems, only the data for the benzo[*b*]thiophene analogues are listed in Table 2 for the sake of brevity; data for the other ring systems can be found in Table S2. The effect of the anomeric configuration on the cytotoxicity is subtle. Compared to the α-configured 4-amino analogues, the strong enhancement of DNA binding affinity but rather constant cytotoxicity of the β-glycoside analogues further confirmed our previous conclusion using **1**–**12** that DNA binding alone is not the major mode of action of this class of molecules.[6] In addition, compared to the compounds with an α configuration, the cancer cell selectivity of β-configured analogues did not show any significant improvement (Table S1 in Supplementary data).

2.2. Effect of substituents and stereochemistry at C-4 in the carbohydrate domain

In previous studies with **1**–**12** it was found that different substituents at C-4 in the carbohydrate domain, such as *O*-acetyl, hydroxyl, azido, and amino groups, had some effect on both DNA binding and cytotoxicity.[6] These trends are also observed from the data presented above. For example, in Table 1, the replacement of an axial amino group (**11** and **23**–**25**) with an equatorial hydroxyl group (**17** and **20**–**22**) led to the total loss of DNA binding affinity. Furthermore, in Table 2, the compounds with an equatorial *O*-acetyl (**14** and **19**) or an equatorial hydroxyl group (**16** and **21**) at C-4 exhibited a pronounced decrease in cytotoxicity in comparison with the amino analogues (**10** and **24**). However, to make SAR interpretation more conclusive, it was necessary to synthesize additional analogues with these groups installed in the opposite orientation at C-4 (**27** and **29**, Scheme 1). No significant differences in biological activity were observed for the different aromatic cores when the attached sugar was 4-*N*-TDTH.[6] Therefore, we chose only the benzo[*b*]thiophene system for these investigations and, given the results discussed above comparing the α- and β-stereochemistry, we propose that similar trends will be observed for the other aromatic rings.

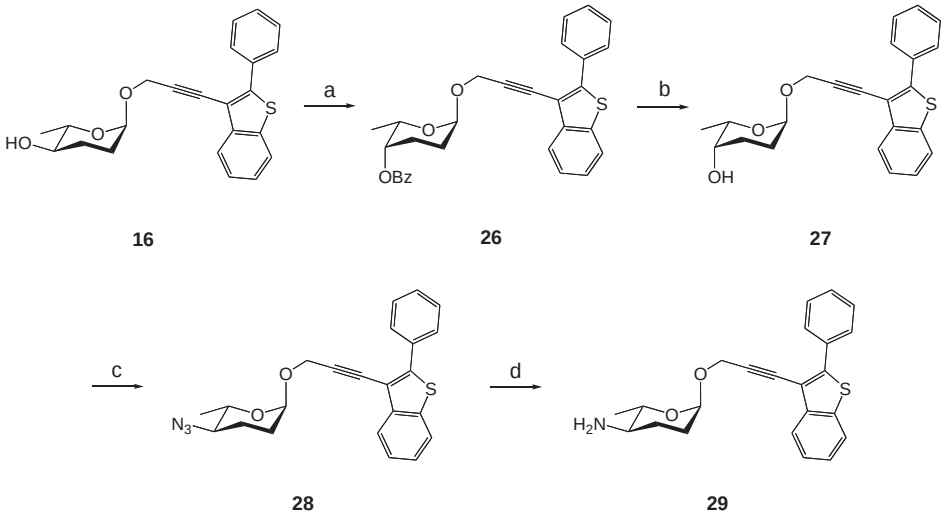
2.2.1. Chemistry

The preparation of **27** and **29** is shown in Scheme 1. Under Mitsunobu conditions with benzoic acid, alcohol **16** was converted in

Table 2
Cytotoxicity of α- and β-configured analogues of the benzo[*b*]thiophene system against three solid-tumor cell lines[a]

Compd	Cell viability (%) at 25 μM			IC_{50} (μM)		
	MCF7	HT29	HepG2[b]	MCF7	HT29	HepG2
10 (α, 4-NH_2)	<1	<1	<1	7.1 ± 1.2	5.9 ± 0.5	8.8 ± 0.9
14 (α, 4-OAc)	27 (0.9)	<1	<1	—	7.2 ± 1.0	12.3 ± 1.4
16 (α, 4-OH)	51 (3.2)	6 (0.7)	25 (0.3)	—	11.1 ± 1.1	16.4 ± 0.4
19 (β, 4-OAc)	40 (3.5)	<1	8 (2.1)	—[c]	10.0 ± 0.7	15.7 ± 1.6
21 (β, 4-OH)	23 (1.2)	2 (0.7)	7 (0.9)	—	12.8 ± 0.8	13.6 ± 1.0
24 (β, 4-NH_2)	<1	<1	<1	7.7 ± 0.4	4.6 ± 0.5	4.9 ± 0.5

[a] The numbers in parentheses represent the standard deviation.
[b] HepG2/C3A is simplified as HepG2.
[c] '—' = not determined.



Scheme 1. Synthesis of 4-*N*-TDTH compounds **29** with an equatorial amino group. Reagents and conditions: (a) PPh_3, benzoic acid, DIAD, −20 °C→rt, 79%; (b) $NaOCH_3$ methanol, 99%; (c) PPh_3, diphenylphosphoryl azide, DIAD, −20 °C→rt, 56%; (d) PPh_3, H_2O, THF, 70 °C, 75%.

benzoate ester **26** in 79% yield. The removal of the benzoyl group in **26** was achieved in nearly quantitative yield and a second Mitsunobu reaction with diphenylphoshoryl azide was used to install the azido group in **28**. The yield for this conversion (56%) was rather low due to the generation of a significant amount of the two possible elimination products as was observed earlier for a similar reaction.[4] Finally, Staudinger reduction led to the production of **29** with the desired equatorial amino group in 75% yield.

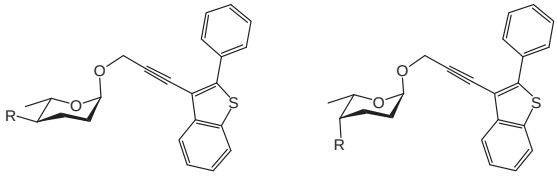
2.2.2. DNA binding

With enough quantities of **29** in hand, we first assayed its DNA binding affinity using the FID assay. Originally, we speculated that compared to the anomeric configuration (**10** vs. **24**, Table 1), the stereochemistry at C-4 would have a less pronounced effect on DNA binding because the relative spatial relationship between the carbohydrate and aromatic domains would be less influenced by such an apparently small substituent change. However, to our surprise, at a concentration of 100 μM, the percentage of remaining EtBr for **29** is comparable to that for β-configured analogue **24** (Fig. 2). This indicates much stronger binding of **29** to DNA than the α-configured analogue **10** (~80% remaining EtBr). Based on the titration curves, the concentration at which there is a 50% fluorescence loss of EtBr is approximately 65 μM for **29** and 45 μM for **24**, which indicates that compared to EtBr, the binding constant of **24** is around 1.5-fold larger than **29**. When **10** was tested in the FID assay, minimal EtBr displacement at 100 μM was observed, and consequently no titration was carried out. Therefore, the order of DNA binding affinity is **10** ≪ **29** < **24** and based on these results we propose that the relative position of the C-1 and C-4 substituents on the carbohydrate ring appears to be more important than their individual orientations. In particular, stronger DNA binding affinity is achieved when these substituents are *cis* on the ring.

2.2.3. Cytotoxicity

The stereochemistry and identity of different substituents at C-4 also showed some effect on cytotoxicity (Table 3). When the substituent at C-4 was oriented equatorially, esterification of the hydroxyl group improved the cytotoxicity (**14** and **30** vs. **16**).[6] On the other hand, esterification of the free hydroxyl group appeared to have little effect on the cytotoxicity of **31** compared to **27**,[6] in which this group was axial. For the same substituent, the equatorial orientation seems slightly favored over the axial for most of the cell lines. The significant loss of the cytotoxicity for **26** could be due to the bulkiness of the benzoyl group, but this remains to be investigated. Regardless of the orientation at C-4, the introduction of an amino group dramatically improved the in vitro anticancer activity, especially for MCF-7 cells (compounds **29** and **10**).

Table 3
The effects of the stereochemistry at C-4 on cytotoxicity against three solid-tumor cell lines[a]



14 R = OAc
16 R = OH
29 R = NH2
30 R = HCOO

26 R = OBz
27 R = OH
10 R = NH2
31 R = HCOO

Compd	Cell viability (%) at 25 μM			IC_{50} (μM)		
	MCF7	HT29	HepG2[b]	MCF7	HT29	HepG2
10	<1	<1	<1	7.1 ± 1.2	5.9 ± 0.5	8.8 ± 0.9
14	27 (3.5)	<1	6 (1.1)	–[c]	7.2 ± 0.9	12.3 ± 1.4
16	51 (3.2)	6 (0.7)	25 (0.3)	–	11.1 ± 1.1	16.4 ± 0.4
26	100 (7.3)	81 (2.1)	99 (1.2)	–	–	–
27	56 (1.9)	10 (1.1)	10 (1.2)	–	13.4 ± 1.1	15.6 ± 1.7
29	<1	<1	<1	6.6 ± 1.0	5.3 ± 0.7	4.7 ± 0.8
30	37 (0.7)	<	<1	–	7.7 ± 0.6	17.4[d]
31	59 (2.1)	2 (0.3)	17 (1.1)	–	12.6 ± 1.3	19.8[d]

[a] The numbers in parentheses represent the standard deviation.
[b] HepG2/C3A is simplified as HepG2.
[c] '–' = not determined.
[d] Only assayed once.

2.3. Effect of the intercalator–carbohydrate linker

2.3.1. Analogues without carbohydrate moieties

After the investigation of the 4-*N*-TDTH carbohydrate moiety, we turned our attention to another structural component, the

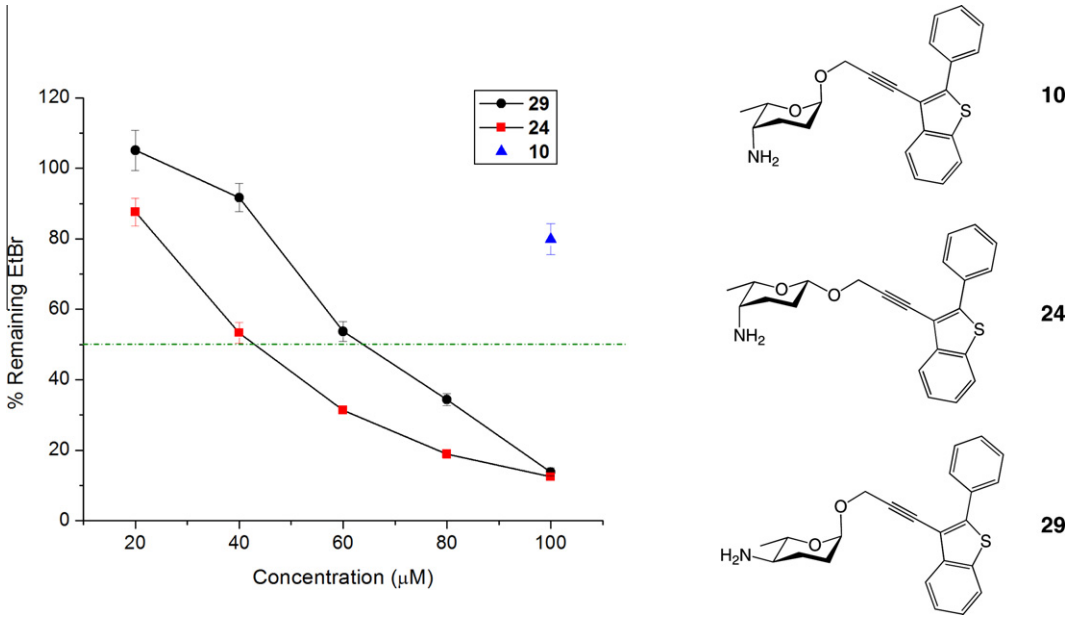


Figure 2. FID titration results of **10**, **24**, and **29**.

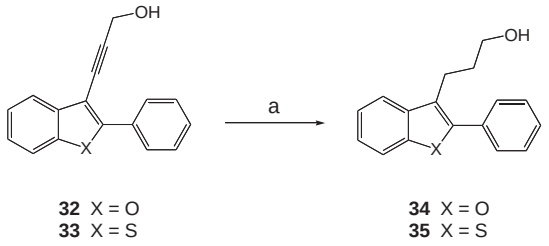
linker moiety. The easiest modification of the propargyl linker is to reduce the triple bond to a single bond by hydrogenation. Before applying this modification to the target compounds, a simpler system was used to explore both the chemistry required and the effect of alkyne reduction on cytotoxicity. In our previous studies, compounds **32** and **33** (Scheme 2), which contain only the aromatic domain and the propargyl linker, showed potent cytotoxicity (cell viability <10%) at 100 μM, especially against HT29 colon and HepG2/C3A liver cancer cells.[6] However, potent cytotoxicity was lost when the concentration was decreased to 25 μM (Table 4). Therefore, we first chose to determine if a change in cytotoxicity would be observed upon reduction of the alkyne in **32** and **33** to an alkane (**34** and **35**).

Using standard palladium-catalyzed hydrogenation conditions, the conversion of **32**–**34**, and **33**–**35** proceeded smoothly in yields of 82% and 89%, respectively. When **34** was screened against three cancer cell lines, no significant variation in cytotoxicity was observed compared to the parent alkyne (Table 4). However, a very pronounced improvement was achieved with **35**, specifically against HT29 colon cancer cells (IC_{50} ~16 μM). We next therefore investigated the effect of replacing the proparyl linker with a propyl group on compounds containing a carbohydrate residue.

2.3.2. Analogues with carbohydrate moieties

2.3.2.1. Chemistry. Unfortunately, under the hydrogenation conditions used for the synthesis of **34** and **35**, the direct conversion of 4-*N*-TDTH analogues **9** and **10** to their propyl-linked counterparts **36** and **37** (Scheme 3) was not very efficient regardless of the reaction time and amount of catalyst used. In particular, it was difficult to obtain the desired products pure, and they were always contaminated by minor impurities of unknown structure. This is presumably due to the poisoning effect of the amino group through the coordination to the palladium catalyst. We therefore synthesized **38** and **39** from **13** and **14**, respectively (Scheme 3).

Under standard hydrogenation conditions, the alkyne was reduced to the alkane in 97% yield for the synthesis of **38** from **13**, and in 99% yield for the synthesis of **39** from **14**. Using potassium carbonate in methanol, removal of the acetyl group in **38** and **40** afforded **40** and **41** in 93% and 96% yield, respectively. Mitsunobu reaction with diphenyl phosphoryl azide was then used to convert the hydroxyl group in **40** and **41** to an azido group in **42** and **43**. The yields of this transformation (96% for **42** and 95% for **43**) was significantly (~40%) higher than the previous corresponding reaction (56% for **28** in Scheme 1) presumably because the leaving group is equatorial and therefore less prone to elimination. Indeed, smaller amounts of elimination products were detected by TLC. The final conversion of the azido group to the amino group was achieved by the Staudinger reaction to give **36** and **37** in 88% and 85% yield, respectively.



Scheme 2. Synthesis of compounds **34** and **35** via reduction of the alkyne to an alkane. Reagents and conditions: (a) H_2, 10% Pd/C, ethyl acetate, 82% for **34** and 89% for **35**.

Table 4
Cytotoxicity of **32**–**35** against three solid-tumor cell lines[a]

Compd	Cell viability (%)					
	100 μM			25 μM		
	MCF7	HT29	HepG2[b]	MCF7	HT29	HepG2
32	2 (0.3)	<1	<1	86 (3.7)	86 (5.7)	88 (6.4)
33	37 (3.2)	8 (1.9)	17 (2.0)	90 (5.8)	68 (3.4)	97 (3.8)
34	16 (1.1)	<1	<1	94 (2.1)	76 (2.1)	91 (6.2)
35	<1	<1	<1	79 (2.9)	<1[c]	87 (7.1)

[a] The numbers in parentheses represent the standard deviation.
[b] HepG2/C3A is simplified as HepG2.
[c] IC_{50} = 16.3 ± 1.5 μM.

2.3.2.2. DNA binding. The relative DNA binding affinity of **36** and **37** was assayed by the EtBr FID assay (Table 5). From the assay results, there was a moderate increase of the DNA binding affinity for both **36** and **37** measured as a decrease (~25%) in the percentage of remaining EtBr. Although the effect is small, we postulate that the greater flexibility of the alkane chain helps the propyl analogues adopt conformations that allow stronger interaction with DNA.

2.3.2.3. Cytotoxicity. Next, all intermediates and final products with the propargyl or propyl linker were assayed for their in vitro anticancer activity. Because similar trends were obtained for the both benzo[*b*]furan and benzo[*b*]thiophene systems, only the cytotoxicity for the benzo[*b*]thiophene system is summarized in Table 6; data for the benzo[*b*]furan system can be found in Tables S3. In contrast to the previous observation for **33** versus **35** (Table 4) that the propyl linker benefited cytotoxicity, it was found that, to some extent, compounds containing a propargyl linker exhibited better cytotoxicity than their equivalents with the propyl linker. This trend is most pronounced for analogues lacking an amino group (**14** vs. **39**, **16** vs. **41**) against HepG2/C3A liver cancer cells, but becomes ambiguous for the amino-containing analogues. The overall conclusion is that modification of the propargyl group to the propyl group does not change the cytotoxicity in a predictable manner. Moreover, the contrast between the increase of DNA binding affinity and the fluctuation of the cytotoxicity change from alkyne **10** to alkane **37** further suggests that DNA binding is not the major biological target of this class of compounds.

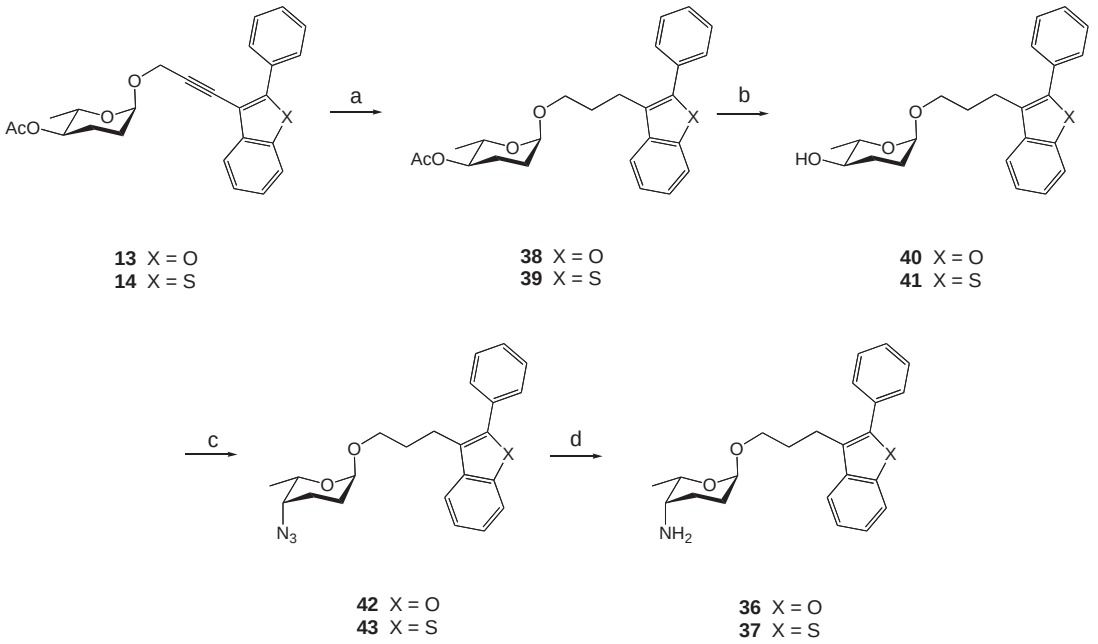
3. Conclusions

In summary (Fig. 3), the linker moiety, the stereochemistry at the anomeric position, and the substituents and stereochemistry at C-4 in the 4-*N*-TDTH system all influence DNA binding affinity of this family of compounds. On the other hand, except for the substituent at C-4, the other structural features examined did not have a significant impact on cytotoxicity. The information gathered from these SAR studies further confirmed our previous conclusions[6] that the cytotoxicity of this class of compounds does not arise as a result of DNA binding. In view of the importance of the amino group at C-4 for both DNA binding and cytotoxicity, further SAR studies on the modification of the amino group is warranted.

4. Experimental section

4.1. Chemistry

All reagents were purchased from commercial sources and were used without further purification unless noted. Before use, reaction solvents were purified by successive passage through columns of alumina and copper in a PURESOLV-400 System from Innovative Technology Inc. under argon atmosphere. Unless stated otherwise, all reactions were carried out under a positive pressure of argon



Scheme 3. Synthesis of 4-*N*-TDTH compounds **36** and **37** with the propyl linker. Reagents and conditions: (a) H_2, 10% Pd/C, ethyl acetate, 97% for **38** and 99% for **39**; (b) K_2CO_3, methanol, 93% for **40**, 96% for **41**; (c) PPh_3, DPPA, DIAD, −20 °C→rt, 96% for **42**, 95% for **43**; (d) PPh_3, H_2O, THF, 70 °C, 88% for **36**, 85% for **37**.

Table 5
The percentage of remaining EtBr of **9** versus **36** and **10** versus **37** at 100μM

Compd	Linker	% Remaining EtBr
9	Propargyl	68.5 ± 1.9
10		79.9 ± 1.0
36	Propyl	43.5 ± 1.0
37		51.9 ± 1.0

and were monitored by TLC on silica gel G-25 UV_{254} (0.25 mm, Macherey–Nagel). Spots were detected under UV light and/or by charring with 10% H_2SO_4 in ethanol, or in acidified ethanolic anisaldehyde or vanillin. Optical rotations were measured at 22 ± 2 °C. Melting points are uncorrected. ^{1}H NMR spectra were recorded on VARIAN INOVA-NMR spectrometers at 400, 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$), or the solvent residual $CHCl_3$ (7.26, $CDCl_3$), or CD_2HOD (4.78, CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz, and ^{13}C chemical shifts are referenced to $CDCl_3$ (77.23 ppm, CD_3Cl_3) or CD_3OD (49.00 ppm, CD_3OD). ^{1}H data are reported as though they were first order. The errors between the coupling constants for two coupled protons were less than 0.5 Hz, and the average number was reported. Assignment of NMR resonances was done based on ^{1}H–^{1}H COSY, HMQC, and in some cases HMBC experiments. In the interpretation of the NMR data for methylene protons on the carbohydrate ring, 'a' and 'e' refer to axial and equatorial orientation, respectively. In the cases where no clear assignment of these hydrogens could be made based on all NMR data, assignments were made taking into consideration the anisotropy effect of a ring σ bond, which results in equatorial hydrogens resonating more downfield than axial hydrogens.[11] Electrospray mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. Optical rotations were measured on a Perkin–Elmer 241 Polarimeter at the sodium D line (589 nm). Optical rotations are in units of deg mL(dm g)$^{-1}$. IR spectra were recorded on the Nicolet Magna 750 FTIR spectrometer. The reported purity values were obtained with a Varian HPLC system, using an evaporative light scattering detector (ELSD) 2000ES from Alltech, and a Varian Microsorb-MV 100-5 C18 column. The eluant consisted of acetonitrile and water, the ratio of which depends on the compound. For all basic amino compounds, 0.1% by volume of trifluoroacetic acid was added to facilitate elution and avoid aggregation. When the purity derived from HPLC analysis is greater than 99.5%, it is reported as >99%.

4.1.1. General procedures

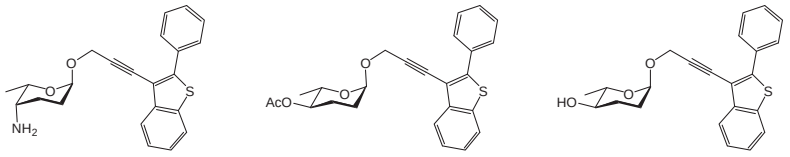
4.1.1.1. Deacetylation. The acetyl protected compound (1 equiv) was dissolved in CH_3OH (30–40 mL per mmol). K_2CO_3 (30 mol %) was added, and then the reaction mixture was stirred at rt and followed by TLC (∼12 h). After the evaporation of the solvent, the residue was diluted with water and extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography.

4.1.1.2. Mistunobu reaction. To a solution of the hydroxyl compound (1 equiv) in THF (15–20 mL per mmol) was added PPh_3 (3 equiv) at −20 °C. To this mixture was added a solution of DIAD (2.5 equiv) and DPPA (2.5 equiv) in THF (5–7 mL per mmol) at −20 °C. The reaction mixture was then allowed to warm to room temperature and followed by TLC (∼12 h). The resulted solution was then diluted with Et_2O, washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography.

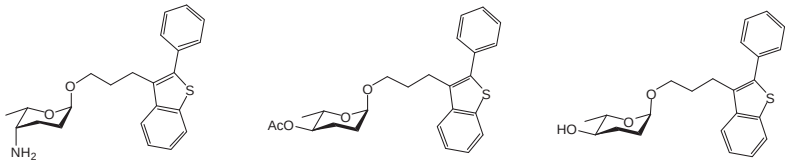
4.1.1.3. Staudinger reaction. To a solution of azide compound (1 equiv) in THF (30–40 mL per mmol) and H_2O (>50 equiv) was added PPh_3 (2.5 equiv), and the reaction was stirred under reflux and followed by TLC (∼10 h). After evaporation, the residue was purified by column chromatography to yield the pure amino compound.

4.1.1.4. Palladium-catalyzed hydrogenation. A solution of the alkyne in EtOAc (10 mL) was hydrogenated in the presence of 10% Pd/C at room temperature and normal pressure. After stirring for 3–8 h, the reaction mixture was filtered through a Celite pad and concentrated. The crude product was purified by column chromatography.

Table 6
Cytotoxicity of benzo[*b*]thiophene analogues with the propyl linker and their counterparts with the propargyl linker against three solid-tumor cell lines[a,b]



10 **14** **16**



37 **39** **41**

Compd	Cell viability (%) at 25 μM			IC_{50} (μM)		
	MCF7	HT29	HepG2[c]	MCF7	HT29	HepG2
10	<1	<1	<1	7.1 ± 1.2	5.9 ± 0.5	8.8 ± 0.9
14	27 (3.5)	<1	6 (1.1)	–[d]	7.2 ± 0.9	12.3 ± 1.4
16	51 (3.2)	6 (0.7)	25 (0.3)	–	11.1 ± 1.1	16.4 ± 0.4
37[e]	<1	<1	<1	11.8 ± 1.7	8.2 ± 1.1	7.5 ± 1.1
39	47 (2.4)	9 (1.7)	72 (1.2)	–	10.2 ± 1.5	–
41	68 (4.0)	19 (2.2)	84 (6.1)	–	15.3[f]	–

[a] The numbers in parentheses represent the standard deviation.
[b] The compounds are grouped based on the heteroatom and the linker.
[c] HepG2/C3A is simplified as HepG2.
[d] '–' = not determined.
[e] The underlined number indicates an analogue with a propyl linker.
[f] Only assayed once.

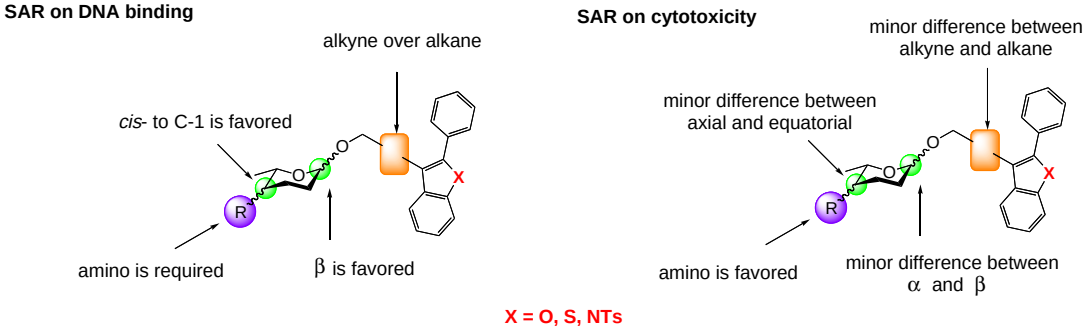


Figure 3. Schematic representation of structure–activity relationships on DNA binding and cytotoxicity.

4.1.2. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-prop-2-ynyl 4-*O*-benzyl-2,3,6-trideoxy-α-L-*threo*-hexopyranoside (26)

To a solution of compound **16** (87 mg, 0.23 mmol) in THF (5 mL) was added PPh_3 (181 mg, 0.692 mmol) at −20 °C. To this mixture was added a solution of DIAD (117 mg, 0.582 mmol) and benzoic acid (70 mg, 0.58 mmol) in THF (3 mL) at −20 °C. The reaction was stirred for 12 h while warming to room temperature, and then diluted with Et_2O. The organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (12:1, hexanes–EtOAc) to yield pure **26** (88 mg, 79%) as a colorless foamy solid: R_f 0.27 (12:1 hexanes–EtOAc); IR: ν 2216 (C≡C), 1716 (C=O) cm^{-1}; $[\alpha]_D$ −79.9 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.13–8.16 (m, 2H, Ar), 7.99–8.03 (m, 2H, Ar), 7.96–7.99 (m, 1H, Ar), 7.80–7.83 (m, 1H, Ar), 7.57–7.61 (m, 1H, Ar), 7.37–7.51 (m, 7H, Ar), 5.26 (br d, 1H, $J_{1,2a}$ = 4.0 Hz, H-1), 5.09 (br s, 1H, H-4), 4.64 (s, 2H, $OCH_2C{\equiv}C$), 4.20 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 2.20–2.28 (m, 1H, H-3e), 2.06–2.14 (m, 1H, H-2e), 1.95–2.01 (m, 1H, H-3a), 1.68–1.74 (m, 1H, H-2a), 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 146.9 (Ar), 141.1 (Ar), 137.5 (Ar), 133.7 (Ar), 133.0 (Ar), 130.4 (Ar), 129.7 (2, Ar), 128.8 (Ar), 128.7 (2, Ar), 128.4 (4, Ar), 125.2 (Ar), 125.0 (Ar), 123.3 (Ar), 122.1 (Ar), 113.0 (Ar), 95.9 (C-1), 90.4 (≡C), 80.4 (≡C), 70.0 (C-4), 65.8 (C-5), 55.1 (OCH_2), 24.1 (C-2), 23.0 (C-3), 17.2 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{30}H_{26}O_4SNa$: 505.1444. Found: 505.1445. Purity: >99%.

4.1.3. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-prop-2-ynyl 2,3,6-trideoxy-α-L-*threo*-hexopyranoside (27)

Compound **26** (67 mg, 0.14 mmol) was dissolved in CH_3OH (10 mL). A catalytic amount of 1 M sodium methoxide in CH_3OH was added, and then the solution was stirred for 12 h. The solvent

was evaporated and the residue was purified by column chromatography (2:1, hexanes–EtOAc) to acquire pure **27** (53 mg, 99%) as a yellowish syrup: R_f 0.21 (2:1 hexanes–EtOAc); IR: ν 3445 (O–H), 2216 (C≡C) cm^{-1}; $[\alpha]_D$ −97.9 (*c* 1.1, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.96–8.00 (m, 2H, Ar), 7.92–7.96 (m, 1H, Ar), 7.79–7.82 (m, 1H, Ar), 7.36–7.49 (m, 5H, Ar), 5.13 (br s, 1H, H-1), 4.58 (s, 2H, OCH_2C≡C), 4.04 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.60 (br s, 1H, H-4), 1.99–2.10 (m, 2H, H-2e, H-3e), 1.74–1.84 (m, 2H, OH, H-3a), 1.59–1.66 (m, 1H, H-2a), 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 146.9 (Ar), 141.1 (Ar), 137.5 (Ar), 133.7 (Ar), 128.8 (Ar), 128.7 (2, Ar), 128.4 (2, Ar), 125.2 (Ar), 124.9 (Ar), 123.3 (Ar), 122.0 (Ar), 113.0 (Ar), 95.9 (C-1), 90.4 (≡C), 80.3 (≡C), 67.3 (C-4), 66.6 (C-5), 54.9 (OCH$_2$), 25.7 (C-2), 23.2 (C-3), 17.1 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{23}H_{22}O_3SNa$: 401.1182. Found: 401.1183. Purity: >99%.

4.1.4. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-prop-2-ynyl 4-azido-2,3,4,6-tetradeoxy-α-L-*erythro*-hexopyranoside (28)

This compound was synthesized as a colorless oil from **27** (60 mg, 0.16 mmol), PPh_3 (126 mg, 0.481 mmol), DIAD (81 mg, 0.40 mmol), and DPPA (110 mg, 0.402 mmol) in 56% yield by following procedure B: R_f 0.24 (20:1 hexanes–EtOAc); IR: ν 2217 cm^{-1} (C≡C), 2099 cm^{-1} (N=N=N); $[\alpha]_D$ −100.1 (*c* 0.7, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.96–8.00 (m, 2H, Ar), 7.92–7.96 (m, 1H, Ar), 7.79–7.82 (m, 1H, Ar), 7.36–7.49 (m, 5H, Ar), 5.14 (br d, 1H, $J_{1,2a}$ = 3.3 Hz, H-1), 4.57 (s, 2H, OCH_2C≡C), 3.72 (dq, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.00–3.08 (m, 1H, H-4), 1.92–2.00 (m, 3H, H-3a, H-3e, H-2e), 1.79–1.87 (m, 1H, H-2a), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 147.0 (Ar), 141.1 (Ar), 137.5 (Ar), 133.7 (Ar), 128.8 (Ar), 128.7 (2, Ar), 128.4 (2, Ar), 125.2 (Ar), 125.0 (Ar), 123.2 (Ar), 122.0 (Ar), 112.9 (Ar), 94.6 (C-1), 90.1 (≡C), 80.5 (≡C), 67.9 (C-5), 62.5 (C-4), 54.8 (OCH$_2$), 29.1 (C-2), 23.7 (C-3), 18.6 (C-6). HRMS (EI) Calcd for $C_{23}H_{21}N_3O_2S$: 403.1354. Found: 403.1351.

4.1.5. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-prop-2-ynyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (29)

This compound was synthesized as a colorless oil from **28** (20 mg, 0.051 mmol), H_2O (0.02 mL), and PPh_3 (26 mg, 0.099 mmol) in 75% yield by following procedure C: R_f 0.19 (15:1 CH_2Cl_2–CH_3OH); IR: ν 3378 (N–H), 2215 (C≡C) cm^{-1}; $[\alpha]_D$ −116.0 (*c* 2.4, CH_2Cl_2); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.94–7.98 (m, 2H, Ar), 7.86–7.89 (m, 1H, Ar), 7.82–7.84 (m, 1H, Ar), 7.36–7.48 (m, 5H, Ar), 5.03 (br s, 1H, H-1), 4.56 (s, 2H, OCH_2C≡C), 4.06 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 2.64 (ddd, 1H, $J_{3a,4}$ = 11.4 Hz, $J_{4,5}$ = 9.4 Hz, $J_{3e,4}$ = 4.3 Hz, H-4), 1.70–1.82 (m, 3H, H-2a, H-2e, H-3a), 1.58–1.67 (m, 1H, H-3e), 1.18 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 147.8 (Ar), 142.3 (Ar), 138.8 (Ar), 134.9 (Ar), 130.1 (Ar), 129.8 (2, Ar), 129.4 (2, Ar), 126.6 (Ar), 126.2 (Ar), 124.1 (Ar), 123.2 (Ar), 114.1 (Ar), 96.5 (C-1), 92.1 (≡C), 80.9 (≡C), 72.2 (C-5), 55.4 (OCH$_2$), 54.3 (C-4), 30.7 (C-2), 27.9 (C-3), 18.5 (C-6). HRMS (ESI) Calcd for (M+H) $C_{23}H_{24}NO_2S$: 378.1522. Found: 378.1524. Purity: 99.3%.

4.1.6. 3-(2-Phenylbenzofuran-3-yl)-propanol (34)

This compound was synthesized as a colorless oil from **32** (25 mg, 0.11 mmol) and 10% Pd/C (21 mg, 20 mol % in palladium) in 82% yield by following procedure D: R_f 0.27 (3:1 hexanes–EtOAc); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 7.82–7.85 (m, 2H, Ar), 7.59–7.62 (m, 1H, Ar), 7.47–7.52 (m, 3H, Ar), 7.36–7.43 (m, 1H, Ar), 7.29–7.33 (m, 1H, Ar), 7.24–7.28 (m, 1H, Ar), 4.70 (t, 2H, *J* = 6.3 Hz, CH_2OH), 4.70 (t, 2H, *J* = 7.7 Hz, CH_2CH$_2$CH$_2$OH), 2.01–2.07 (m, 2H, CH_2CH$_2$OH), 1.46 (br s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 153.9 (Ar), 150.9 (Ar), 131.3 (Ar), 130.4 (Ar), 128.7 (2, Ar), 128.2 (Ar), 126.9 (2, Ar), 124.4 (Ar), 122.4 (Ar), 119.5 (Ar), 115.5 (Ar), 111.1 (Ar), 62.3 (CH$_2$OH), 32.4 (*C*H$_2$CH$_2$OH), 20.4 (*C*H$_2$CH$_2$CH$_2$OH). HRMS (EI) Calcd for $C_{17}H_{16}O_2$: 252.1150. Found: 252.1152. Anal. Calcd for $C_{17}H_{16}O_2$: C, 80.93; H, 6.39. Found: C, 81.01; H, 6.36.

4.1.7. 3-(2-Phenylbenzothiophen-3-yl)-propanol (35)

This compound was synthesized as a white foamy solid from **33** (26 mg, 0.10 mmol) and 10% Pd/C (21 mg, 20 mol % in palladium) in 89% yield by following procedure D: R_f 0.47 (2:1 hexanes–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.84–7.87 (m, 1H, Ar), 7.78–7.81 (m, 1H, Ar), 7.53–7.56 (m, 2H, Ar), 7.45–7.49 (m, 2H, Ar), 7.38–7.43 (m, 2H, Ar), 7.35–7.38 (m, 1H, Ar), 4.70 (t, 2H, *J* = 5.7 Hz, CH_2OH), 4.70 (dd, 2H, *J* = 7.7 Hz, *J* = 6.1 Hz, CH_2CH$_2$CH$_2$OH), 1.90–1.96 (m, 2H, CH_2CH$_2$OH), 1.39 (br s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 140.2 (Ar), 139.3 (Ar), 138.7 (Ar), 134.6 (Ar), 131.6 (Ar), 129.7 (2, Ar), 128.7 (2, Ar), 128.1 (Ar), 124.3 (Ar), 124.2 (Ar), 122.3 (Ar), 122.2 (Ar), 62.3 (CH$_2$OH), 32.9 (*C*H$_2$CH$_2$OH), 20.4 (*C*H$_2$CH$_2$CH$_2$OH). HRMS (EI) Calcd for $C_{17}H_{16}OS$: 268.0922. Found: 268.0920. Purity: 99.3%.

4.1.8. 3-(2-Phenyl-benzo[*b*]furan-3-yl)-propyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (36)

This compound was synthesized as a colorless syrup from **42** (82 mg, 0.21 mmol), H_2O (0.08 mL), and PPh_3 (94 mg, 0.36 mmol) in 88% yield by following procedure C: R_f 0.32 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3368, 3298 (N–H) cm^{-1}; $[\alpha]_D$ −45.1 (*c* 0.8, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.77–7.80 (m, 2H, Ar), 7.53–7.58 (m, 1H, Ar), 7.41–7.46 (m, 3H, Ar), 7.32–7.36 (m, 1H, Ar), 7.24–7.28 (m, 1H, Ar), 7.18–7.22 (m, 1H, Ar), 4.61 (br d, 1H, $J_{1,2a}$ = 3.4 Hz, H-1), 3.84 (br q, 1H, $J_{5,6}$ = 6.5 Hz, H-5), 3.64 (dt, 1H, *J* = 9.8 Hz, *J* = 5.9 Hz, OCH$_2$), 3.35 (dt, 1H, *J* = 9.8 Hz, *J* = 5.8 Hz, OCH$_2$), 2.97–3.08 (m, 2H, CH_2CH$_2$CH$_2$O, 2.58 (br s, 1H, H-4), 1.94–2.04 (m, 3H, CH_2CH$_2$O, H-3e), 1.76–1.85 (m, 1H, H-2e), 1.52–1.58 (m, 1H, H-3a), 1.39–1.45 (m, 1H, H-2a), 0.98 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 155.4 (Ar), 152.0 (Ar), 132.6 (Ar), 131.6 (Ar), 129.8 (2, Ar), 129.3 (Ar), 127.9 (2, Ar), 125.6 (Ar), 123.6 (Ar), 120.7 (Ar), 116.9 (Ar), 111.9 (Ar), 98.5 (C-1), 67.1(9) (C-5), 67.1(7) (OCH$_2$), 49.4 (C-4), 30.5 (*C*H$_2$CH$_2$O), 26.9 (C-3), 24.5 (C-2), 21.7 (*C*H$_2$CH$_2$CH$_2$O), 17.8 (C-6). HRMS (ESI) Calcd for (M+H) $C_{23}H_{28}NO_3$: 366.2064. Found: 366.2066. Purity: >99%.

4.1.9. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-propyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (37)

This compound was synthesized as a yellowish syrup from **43** (76 mg, 0.17 mmol), H_2O (0.06 mL), and PPh_3 (94 mg, 0.29 mmol) in 85% yield by following procedure C: R_f 0.31 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3364 (N–H) cm^{-1}; $[\alpha]_D$ −26.7 (*c* 2.5, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.76–7.82 (m, 2H, Ar), 7.49–7.52 (m, 2H, Ar), 7.41–7.46 (m, 2H, Ar), 7.35–7.40 (m, 2H, Ar), 7.29–7.34 (m, 1H, Ar), 4.62 (br s, 1H, H-1), 3.88 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.57 (dt, 1H, *J* = 9.8 Hz, *J* = 5.8 Hz, OCH$_2$), 3.27–3.33 (m, 1H, OCH$_2$), 2.95–3.06 (m, 3H, H-4, CH_2CH$_2$CH$_2$O), 2.00–2.09 (m, 1H, H-3e), 1.86–1.92 (m, 2H, CH_2CH$_2$O), 1.69–1.78 (m, 2H, H-2e, H-3a), 1.40–1.47 (m, 1H, H-2a), 1.04 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 141.7 (Ar), 140.6 (Ar), 139.7 (Ar), 136.1 (Ar), 133.0 (Ar), 130.7 (2, Ar), 129.8 (2, Ar), 129.2 (Ar), 125.5 (Ar), 125.3 (Ar), 123.4 (Ar), 123.2 (Ar), 98.1 (C-1), 67.6 (OCH$_2$), 64.9 (C-5), 50.1 (C-4), 31.0 (*C*H$_2$CH$_2$O), 24.3 (C-3), 24.1 (C-2), 24.0 (*C*H$_2$CH$_2$CH$_2$O), 17.6 (C-6). HRMS (ESI) Calcd for (M+H) $C_{23}H_{28}NO_2S$: 382.1835. Found: 378.1834. Purity: 98.3%.

4.1.10. 3-(2-Phenyl-benzo[*b*]furan-3-yl)-propyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hexopyranoside (38)

This compound was synthesized as a colorless oil from **13** (126 mg, 0.311 mmol) and 10% Pd/C (33 mg, 10 mol % in palladium) in 97% yield by following procedure D: R_f 0.50 (10:1 hexanes–EtOAc); IR: 1735 (C=O) cm^{-1}; $[\alpha]_D$ −51.3 (*c* 0.8, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.85–7.88 (m, 2H, Ar), 7.60–7.63

(m, 1H, Ar), 7.47–7.54 (m, 3H, Ar), 7.37–7.41 (m, 1H, Ar), 7.30–7.34 (m, 1H, Ar), 7.25–7.29 (m, 1H, Ar), 4.72 (br s, 1H, H-1), 4.54 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, $J_{3a,4}$ = 9.7 Hz, $J_{3e,4}$ = 4.4 Hz, H-4), 3.76–3.86 (m, 2H, H-5, OCH_2), 3.48 (dt, 1H, J = 9.8 Hz, J = 6.0 Hz, OCH_2), 3.02–3.16 (m, 2H, CH_2CH$_2$CH$_2$O), 2.05–2.12 (m, 5H, CH_2CH$_2$O, O=CCH$_3$), 1.94–2.01 (m, 1H, H-3e), 1.76–1.93 (m, 3H, H-2a, H-2e, H-3a), 1.16 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.3 (C=O), 154.0 (Ar), 150.8 (Ar), 131.3 (Ar), 130.5 (Ar), 128.7 (2, Ar), 128.1 (Ar), 126.9 (2, Ar), 124.4 (Ar), 122.4 (Ar), 119.5 (Ar), 115.7 (Ar), 111.1 (Ar), 96.3 (C-1), 73.6 (C-4), 66.6 (C-5), 66.3 (OCH$_2$), 29.6 (CH$_2$CH$_2$O), 29.3 (C-2), 24.2 (C-3), 21.2 (O=CCH$_3$), 21.0 (CH$_2$CH$_2$CH$_2$O), 17.9 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{25}H_{28}O_5Na$: 431.1834. Found: 431.1836. Anal. Calcd for $C_{25}H_{28}O_5$: C, 73.51; H, 6.91. Found: C, 73.29; H, 7.07.

4.1.11. 3-(2-Phenyl-benzo[*b*]thiophene-3-yl)-propyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hexopyranoside (39)

This compound was synthesized as a colorless oil from **14** (133 mg, 0.321 mmol) and 10% Pd/C (34 mg, 10 mol % in palladium) in 99% yield by following procedure D: R_f 0.29 (10:1 hexanes–EtOAc); IR: 1737 (C=O) cm^{-1}; $[\alpha]_D$ −61.8 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.80–7.87 (m, 2H, Ar), 7.56–7.59 (m, 2H, Ar), 7.34–7.49 (m, 5H, Ar), 4.65 (br s, 1H, H-1), 4.47–4.53 (m, 1H, H-4), 3.78 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.70 (dt, 1H, J = 9.8 Hz, J = 6.0 Hz, OCH$_2$), 3.40 (dt, 1H, J = 9.8 Hz, J = 6.2 Hz, OCH$_2$), 2.97–3.12 (m, 2H, CH_2CH$_2$CH$_2$O), 2.07 (s, 3H, O=CCH$_3$), 1.91–2.06 (m, 3H, CH_2CH$_2$O, H-3e), 1.74–1.86 (m, 3H, H-2a, H-2e, H-3a), 1.14 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.3 (C=O), 140.4 (Ar), 139.3 (Ar), 138.7 (Ar), 134.8 (Ar), 131.8 (Ar), 129.7 (2, Ar), 128.6 (2, Ar), 128.0 (Ar), 124.2 (Ar), 124.1 (Ar), 122.3 (Ar), 122.2 (Ar), 96.2 (C-1), 73.6 (C-4), 66.5 (C-5), 66.4 (OCH$_2$), 30.2 (CH$_2$CH$_2$O), 29.2 (C-2), 24.2 (C-3), 23.6 (CH$_2$CH$_2$CH$_2$O), 21.2 (O=CCH$_3$), 17.9 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{25}H_{28}O_4SNa$: 447.1606. Found: 447.1609. Anal. Calcd for $C_{25}H_{28}O_4S$: C, 70.73; H, 6.65; S, 7.55. Found: C, 70.34; H, 6.89; S, 7.67.

4.1.12. 3-(2-Phenyl-benzo[*b*]furan-3-yl)-propyl 2,3,6-trideoxy-α-L-*erythro*-hexopyranoside (40)

This compound was synthesized as a colorless oil from **38** (123 mg, 0.301 mmol) and K_2CO_3 (12 mg, 0.088 mmol) in 93% yield by following procedure A: R_f 0.26 (3:1 hexanes–EtOAc); IR: *v* 3422 (O–H) cm^{-1}; $[\alpha]_D$ −39.4 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.83–7.86 (m, 2H, Ar), 7.58–7.61 (m, 1H, Ar), 7.46–7.53 (m, 3H, Ar), 7.35–7.40 (m, 1H, Ar), 7.26–7.33 (m, 2H, Ar), 4.68 (br s, 1H, H-1), 3.76 (dt, 1H, J = 9.8 Hz, J = 6.0 Hz, OCH$_2$), 3.55 (dq, 1H, $J_{4,5}$ = 9.1 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.45 (dt, 1H, J = 9.8 Hz, J = 6.1 Hz, OCH$_2$), 3.22–3.28 (m, 1H, H-4), 3.01–3.14 (m, 2H, CH_2CH$_2$CH$_2$O), 2.03–2.10 (m, 2H, CH_2CH$_2$O), 1.70–1.90 (m, 4H, H-2a, H-2e, H-3a, H-3e), 1.47 (br s, 1H, OH), 1.23 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.0 (Ar), 150.8 (Ar), 131.3 (Ar), 130.5 (Ar), 128.7 (2, Ar), 128.1 (Ar), 126.9 (2, Ar), 124.3 (Ar), 122.4 (Ar), 119.6 (Ar), 115.6 (Ar), 111.1 (Ar), 96.2 (C-1), 72.1 (C-4), 69.5 (C-5), 66.1 (OCH$_2$), 29.7 (CH$_2$CH$_2$O), 29.5 (C-2), 27.7 (C-3), 20.9 (CH$_2$CH$_2$CH$_2$O), 17.9 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{23}H_{26}O_4Na$: 389.1723. Found: 389.1721. Anal. Calcd for $C_{23}H_{26}O_4$: C, 75.38; H, 7.15. Found: C, 75.73; H, 7.05.

4.1.13. 3-(2-Phenyl-benzo[*b*]thiophene-3-yl)-propyl 2,3,6-trideoxy-α-L-*erythro*-hexopyranoside (41)

This compound was synthesized as a colorless oil from **39** (127 mg, 0.303 mmol) and K_2CO_3 (12 mg, 0.088 mmol) in 96% yield by following procedure A: R_f 0.44 (2:1 hexanes–EtOAc); IR: 3403 (O–H) cm^{-1}; $[\alpha]_D$ −45.0 (*c* 1.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.83–7.86 (m, 1H, Ar), 7.78–7.82 (m, 1H, Ar), 7.54–7.58 (m, 2H, Ar), 7.33–7.48 (m, 5H, Ar), 4.62 (br s, 1H, H-1), 3.68 (dt, 1H, J = 9.8 Hz, J = 6.0 Hz, OCH$_2$), 3.51 (dq, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.38 (dt, 1H, J = 9.8 Hz, J = 6.2 Hz, OCH$_2$), 3.20–3.27 (m, 1H, H-4), 2.96–3.10 (m, 2H, CH_2CH$_2$CH$_2$O), 1.94–2.01 (m, 2H, CH_2CH$_2$O), 1.68–1.88 (m, 4H, H-2a, H-2e, H-3a, H-3e), 1.51 (br s, 1H, OH), 1.22 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 140.4 (Ar), 139.3 (Ar), 138.6 (Ar), 134.8 (Ar), 131.8 (Ar), 129.7 (2, Ar), 128.6 (2, Ar), 127.9 (Ar), 124.2 (Ar), 124.1 (Ar), 122.2(7) (Ar), 122.2(6) (Ar), 96.1 (C-1), 72.1 (C-4), 69.4 (C-5), 66.3 (OCH$_2$), 30.1 (CH$_2$CH$_2$O), 29.7 (C-2), 27.7 (C-3), 23.6 (CH$_2$CH$_2$CH$_2$O), 17.9 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{23}H_{26}O_3SNa$: 405.1500. Found: 405.1501. Purity: >99%.

4.1.14. 3-(2-Phenyl-benzo[*b*]furan-3-yl)-propyl 4-azido-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (42)

This compound was synthesized as a colorless oil from **40** (99 mg, 0.27 mmol), PPh_3 (212 mg, 0.808 mmol), DIAD (137 mg, 0.678 mmol), and DPPA (187 mg, 0.678 mmol) in 96% yield by following procedure C: R_f 0.26 (20:1 hexanes–EtOAc); IR: *v* 2100 cm^{-1} (N=N=N); $[\alpha]_D$ −17.9 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.82–7.85 (m, 2H, Ar), 7.58–7.61 (m, 1H, Ar), 7.45–7.53 (m, 3H, Ar), 7.35–7.40 (m, 1H, Ar), 7.29–7.34 (m, 1H, Ar), 7.23–7.28 (m, 1H, Ar), 4.76 (br s, 1H, H-1), 3.91 (dq, 1H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.7 Hz, H-5), 3.72 (dt, 1H, J = 9.8 Hz, J = 6.0 Hz, OCH$_2$), 3.45 (dt, 1H, J = 9.8 Hz, J = 6.1 Hz, OCH$_2$), 3.39 (br s, 1H, H-4), 3.01–3.13 (m, 2H, CH_2CH$_2$CH$_2$O), 2.02–2.18 (m, 3H, CH_2CH$_2$O, H-3e), 1.88–1.97 (m, 2H, H-2e, H-3a), 1.53–1.60 (m, 1H, H-2a), 1.16 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.0 (Ar), 150.9 (Ar), 131.3 (Ar), 130.4 (Ar), 128.7 (2, Ar), 128.1 (Ar), 126.9 (2, Ar), 124.4 (Ar), 122.4 (Ar), 119.5 (Ar), 115.5 (Ar), 111.1 (Ar), 96.9 (C-1), 66.3 (OCH$_2$), 65.2 (C-5), 60.0 (C-4), 29.4 (CH$_2$CH$_2$O), 24.2 (C-2), 23.0 (C-3), 20.9 (CH$_2$CH$_2$CH$_2$O), 17.9 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{23}H_{25}N_3O_3Na$: 414.1794. Found: 414.1792.

4.1.15. 3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-propyl 4-azido-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (43)

This compound was synthesized as a colorless oil from **42** (96 mg, 0.25 mmol), PPh_3 (197 mg, 0.752 mmol), DIAD (127 mg, 0.629 mmol), and DPPA (173 mg, 0.629 mmol) in 95% yield by following procedure C: R_f 0.29 (20:1 hexanes–EtOAc); IR: *v* 2097 cm^{-1} (N=N=N); $[\alpha]_D$ −29.2 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.84–7.87 (m, 1H, Ar), 7.78–7.81 (m, 1H, Ar), 7.54–7.58 (m, 2H, Ar), 7.33–7.48 (m, 5H, Ar), 4.70 (br s, 1H, H-1), 3.88 (dq, 1H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.7 Hz, H-5), 3.65 (dt, 1H, J = 9.9 Hz, J = 6.0 Hz, OCH$_2$), 3.37–3.43 (m, 2H, H-4, OCH$_2$), 2.97–3.10 (m, 2H, CH_2CH$_2$CH$_2$O), 2.04–2.14 (m, 1H, H-3e), 1.85–2.01 (m, 4H, H-2e, H-3a, CH_2CH$_2$O,), 1.47–1.52 (m, 1H, H-2a), 1.16 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 140.4 (Ar), 139.3 (Ar), 138.7 (Ar), 134.8 (Ar), 131.7 (Ar), 129.7 (2, Ar), 128.6 (2, Ar), 128.0 (Ar), 124.2 (Ar), 124.1 (Ar), 122.3 (Ar), 122.2 (Ar), 96.8 (C-1), 66.5 (OCH$_2$), 65.1 (C-5), 60.0 (C-4), 30.1 (CH$_2$CH$_2$O), 24.1 (C-2), 23.5 (C-3), 23.0 (CH$_2$CH$_2$CH$_2$O), 18.0 (C-6). HRMS (ESI) Calcd for (M+Na) $C_{23}H_{25}N_3O_2SNa$: 430.1565. Found: 430.1568.

4.2. DNA binding fluorescence assay

The fluorescence was measured according to the literature reported procedure.[3,4] Sheared herring sperm DNA (10 mg/mL in 10 mM Tris–HCl, 10 mM NaCl, 1 mM EDTA, pH 7.5) was purchased from Promega and diluted with BPE (bis-phosphate EDTA) buffer to 2 mg/mL as the stock solution. BPE buffer consists of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.0 in Milli-Q water. Molecular-biological grade DMSO and ethidium bromide (EtBr) were purchased from Sigma–Aldrich. A stock solution of EtBr (1 mM) was prepared in BPE buffer. The test analogues were dissolved in DMSO to make 10 mM stock solutions and further diluted with DMSO, as needed, to the desired concentration prior

to the assay. Fluorescence was measured on a PTI (Photon Technology International) MP1 fluorescence system.

4.2.1. Single-point fluorescent intercalator displacement (FID) assay

Three individual fluorescence measurements were required for this assay. First, to a fluorescence microcell was added 98.5 μL of an ethidium bromide (EtBr) solution in PBE buffer (5 μM), and its emission curve was measured from 540 nm to 700 nm at an excitation wavelength of 520 nm. Second, to the above EtBr solution was added 1 μL of a 2 mg/mL stock solution of hsDNA in PBE buffer. After mixing and a short-time equilibration (~10 s), the emission scan was performed under the same set of parameters until the error between the maxima of two continuous emission curves was <10%. The intensity difference at the emission maximum from the two previous measurements was set as 100% for normalization. Finally, 0.5 μL of a 10 mM stock solution of a test compound in DMSO was added to the mixture in the cell, which gave a final concentration of 50 μM. After mixing and equilibration (~3 min), the emission scan was performed until steady measurements were obtained. The normalized intensity difference at the emission maximum between the first measurement and this measurement was used to represent the percentage of remaining EtBr. Alternatively, the normalized intensity difference between the second and third measurements was used to represent the percentage of displaced EtBr. Under the above assay conditions, no fluorescence was observed for all the reagents and their combinations except for EtBr. Each compound was assayed in triplicate, and the values shown in the table reflect an average of this data. In cases where the percentage is slightly over 100%, this is presumably due to the weak fluorescence enhancement between the assayed compounds and free EtBr or the EtBr–DNA complex.

4.2.2. Multiple-point fluorescent intercalator displacement (FID) assay

The assay is nearly identical to the above single-point assay. The major difference is that in step 3, 0.2 μL aliquots of a 10 mM stock solution of a test compound in DMSO was added to the fluorescing solution in the cell, and the fluorescence was measured after each addition until a 50% reduction of fluorescence occured, corresponding to a 50% displacement of EtBr by the test compound. The apparent binding constant of the test ligand was then calculated from the equation:

$$K_{\text{Ligand}} = K_{\text{EtBr}} \times \frac{[\text{EtBr}]}{[\text{Ligand}]_{50\%}}$$

where $[\text{Ligand}]_{50\%}$ is the concentration of ligand that gives a 50% reduction of fluorescence, [EtBr] is the concentration of EtBr, and K_{EtBr} is the binding constant for ethidium bromide.

4.3. Cytotoxicity assay

CellTiter 96® aqueous one solution MTS dye, a mixture of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS), inner salt and an electron coupling reagent (phenazine ethosulfate, PES), was obtained from Promega. DMEM high glucose culture media with sodium pyruvate, DMEM-F12 culture media, trypsin-EDTA, L-glutamine, fetal bovine serum (FBS), human transferin, bovine insulin, and phosphate-buffered saline (PBS, calcium and magnesium free), a solution of penicillin and streptomycin were purchased from Invitrogen Corp. Human albumin was purchased from Sigma. Daunomycin and Doxorubicin were obtained from IFFECT ChemPhar (Hong Kong) Company Limited.

4.3.1. Cell culture

Three cancer cell lines (MCF-7, HT-29, and HepG2/C3A) and three non-tumor cell lines (CRL-7761, Vero, and NIH/3T3) were purchased from the American Type Culture Collection (ATCC). NIH/3T3 mouse lung cells were maintained in DMEM/HIGH culture media supplemented with 10% calf serum, 2 mM L-glutamine, 100 unit penicillin, and 100 μg streptomycin. Vero monkey kidney cells were maintained in DMEM/HIGH culture media supplemented with 5% FBS, 2 mM L-glutamine, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid), 100 unit penicillin, and 100 μg streptomycin. All the rest four cell lines were maintained in DMEM/HIGH culture media supplemented with 10% FBS and 2 mM L-glutamine. Cell cultures were grown in monolayers in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture media were changed every 2–3 days. Cell cultures were passaged once a week using trypsin-EDTA (0.25%) to detach the cells from their culture flasks.

4.3.2. MTS non-radioactive cell proliferation assay

Rapidly growing cells were counted and seeded at a concentration of 1×10^4 cells/well in 100 μL total volume per well into a 96-well microtiter plate. After incubation at 37 °C for 24 h, the culture medium was removed and the cell assay media (DMEM-F12 media with 1.0 mg/mL human albumin, 5.0 mg/L human transferin, and 5.0 mg/L bovine insulin) with or without tested compounds in 100 μL total volume were added to each well containing the exponentially growing cancer cells. Culture wells (triplicate to quintuplicate per sample) were incubated for 3 days at 37 °C (5% CO_2, 95% air). Then 20 μL of MTS dye solution was added to each sample well. After 2–2.5 h of incubation at 37 °C, the absorbance of formazan was recorded at 490 nm with a SPECTRAmax absorbance microtitre plate reader. Cell viability was calculated as a percentage of control wells, which contained the cell assay media only. Statistical and graphical analysis information was determined using Origin Pro 7.5 software (OriginLab Corp.) and Microsoft Excel (Microsoft Corp.). The determination of IC_{50} values was performed using nonlinear regression analysis based on the Boltzmann function as follows:

$$y = A_2 \times \frac{A_1 - A_2}{1 + e^{(x - x_0)/dx}}$$

where y is the dependent variable (percentage of growth inhibition), x is the independent variable (concentration), and A_1, A_2, x_0, and dx are the parameter values (A_1 is initial value, A_2 is final value, x_0 is center, and dx is time constant).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.014.

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