Bioorganic & Medicinal Chemistry Letters 21 (2011) 2591–2596

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Effect of carbohydrate amino group modifications on the cytotoxicity of glycosylated 2-phenyl-benzo[*b*]thiophenes and 2-phenyl-benzo[*b*]furans

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ARTICLE INFO

Article history:
Received 9 January 2011
Revised 5 February 2011
Accepted 14 February 2011
Available online 17 February 2011

Keywords:
Structure–activity relationship
Amino group
Cytotoxicity

ABSTRACT

In previous studies, we have identified a family of benzo[*b*]furan and benzo[*b*]thiophene derivatives linked to amino sugars (**1**–**6**) that are cytotoxic to a range of cancer cell lines. We describe here an exploration of the effect of structural modification of the amino group on one of the carbohydrate residues (4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside) on in vitro cytotoxicity. It has been found that maintaining at least one basic functional group around the C-4 position in the carbohydrate moiety is crucial for cytotoxicity. Furthermore, it appears that modifications around the C-4 position are limited by suitable hydrophilic/hydrophobic and/or ionic interactions, as well as steric constraints.

Previous reports from our laboratory have detailed the design, synthesis and cytotoxicity of novel glycosylated benzo[*b*]furan and benzo[*b*]thiophene derivatives (e.g., **1**–**6**, Chart 1).[1–4] These compounds are cytotoxic to mammalian cells,[3] and also possess weak bacteriocidal activity against gram-positive bacteria.[2] The mode of action of these compounds remains unclear. However, some of the active compounds have been demonstrated to bind to DNA[1] and also inhibit topoisomerase I and topoisomerase II,[3] albeit at relatively high concentration.

Encouraged by low micromolar in vitro cytotoxicity of these compounds, and their relatively simple structure and ease of synthesis,[1–3] we endeavoured to identify key structural features in these molecules. We envisioned that knowledge of the structural motifs essential for cytotoxicity would benefit future efforts towards the preparation of more potent analogues, and in turn would facilitate the discovery of their molecular targets. Our first structure–activity relationship (SAR) study on analogs containing the 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (4-*N*-TDTH) moiety (e.g., **5** and **6**)[4] demonstrated that the orientation of the substituents at C-1 and C-4 appeared not to be important for cytotoxicity. In addition, the alkyne linker was only slightly preferred to the alkane. These studies have also confirmed the crucial role of the amino group at C-4 for cytotoxicity. Reported here is an investigation of how structural modifications of the amino group influence the cytotoxicity of these compounds.

In view of the in vitro anticancer activity of the compounds previously described,[1–3] the benzo[*b*]furan system was employed in initial studies. To avoid potential bioactivity loss due to steric hindrance, small acyl and alkyl groups were first installed for a preliminary study (Scheme 1). To explore the effect of *N*-acylation, the acetylated compound **7**, and a derivative (**9**) in which the amine was *N*-acylated with L-alanine were prepared from **5** in good yield. The purpose of preparing **9** was to keep a free amino group in the molecule while the amine on the carbohydrate ring was masked. Probing the effect of *N*-alkylation involved first the synthesis of the ethylamino and isopropylamino analogues **10** and **11**, via reductive amination with either acetaldehyde or acetone, respectively. In this one-pot process, the condensation of the amino group with the aldehyde or ketone was followed by in situ reduction of the intermediate imines with sodium cyanoborohydride ($NaCNBH_3$). Under these conditions it was possible to generate the desired products in a yield of 85% for **10** and 82% for **11**.

Next, we assayed **5**, **7**, and **9**–**11** against three cancer cell lines—MCF-7 breast cancer, HT29 colon cancer, and HepG2/C3A liver cancer (Table 1). These results demonstrated that acylation (**7**) was detrimental to cytotoxicity, presumably due to the loss of a basic nitrogen. This was supported by the observation that **9**, which contained both an amide bond and additional basic nitrogen atom, was cytotoxic at a level comparable to **5**. Although **10** and **11** showed similar cytotoxicity against HT29 cells, the former showed better cytotoxicity for the other two cancer cell lines. Based on the results in Table 1, we proceeded with reductive amination of **5** with aldehydes to enrich the diversity of our compound library for more reliable SAR interpretation.

Reductive amination of aldehydes and ketones is a general approach for the alkylation of amines.[5–8] There are two general procedures: one-pot (direct)[5,7,8] or stepwise (indirect).[5,6] The latter method is usually employed for synthesizing monoalkylated compounds only when the former method fails. A panel of aliphatic and

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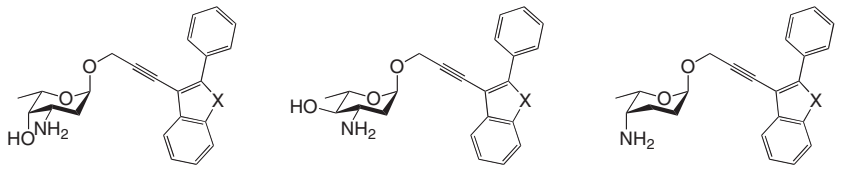
doi:10.1016/j.bmcl.2011.02.051

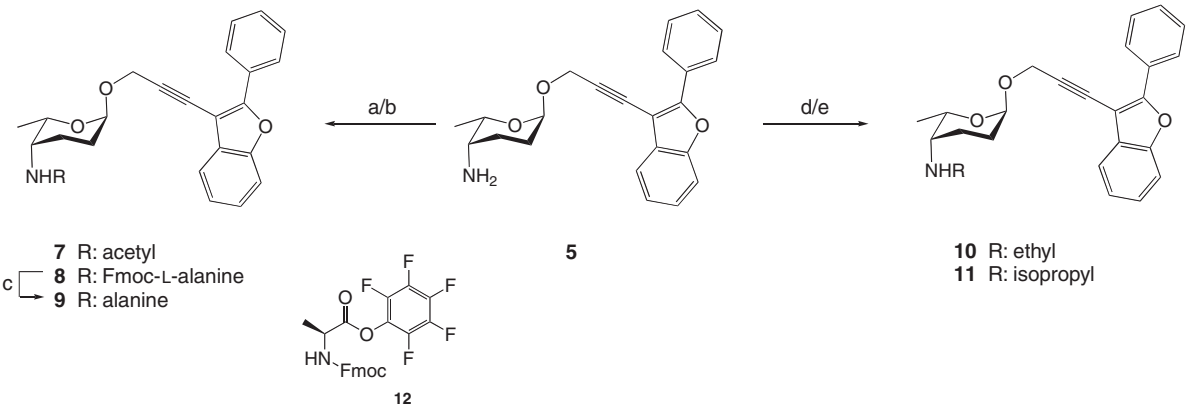


1 X = O
2 X = S

3 X = O
4 X = S

5 X = O
6 X = S

Chart 1. Structures of glycosylated bezno[*b*]furan and benzo[*b*]thiophene analogues **1**–**6**.



7 R: acetyl
8 R: Fmoc-L-alanine
9 R: alanine

5

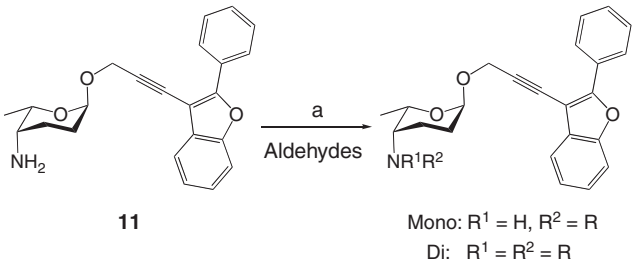
10 R: ethyl
11 R: isopropyl

12

Scheme 1. Synthesis of **7**–**11** from **5**. Reagents and conditions: (a) acetyl chloride, Et_3N, 86%; (b) **12**, CH_2Cl_2; (c) piperidine, CH_2Cl_2, 55% over two steps; (d) acetaldehyde, $NaCNBH_3$, CH_3OH, 85% for **10**; (e) acetone, $NaCNBH_3$, CH_3OH, 82% for **11**.

aromatic aldehydes was chosen to make the *N*-alkyl amino analogues from **5** (Scheme 2). We found that the one-pot approach, when carried out in two different solvent systems, can be used to prepare either monoalkylated or dialkylated analogues, depending on the aldehyde substrates. In most cases, treatment of **5** with the aldehyde and sodium cyanoborohydride in methanol led to monoalkylation. Under these reaction conditions, dialkylation occurred mostly with linear or branched aliphatic aldehydes. On the other hand, by switching the solvent to DMF containing 1% acetic acid, dialkylated analogues could be prepared from some aromatic or cyclic aliphatic aldehydes. The alkylated derivatives **12**–**28** made under either of the above two conditions are listed in Tables 2 and 3.

However, not all monoalkylated or dialkylated analogues could be efficiently made under these conditions. For example, it was difficult to control monomethylation using formaldehyde. Once the monomethylated product was formed, it reacted with another molecule of formaldehyde at a rate faster than the starting material. Attempts to quench the reaction before completion failed and the major component was either **5**, or the dimethylated product **12**. The very similar R_f values, as well as the high polarity, of the free, monomethylated, and dimethylated amino compounds made the purification impossible. Use of the stepwise process was also unsuccessful.



11

Mono: $R^1 = H$, $R^2 = R$
Di: $R^1 = R^2 = R$

Scheme 2. Monoalkylation and dialkylation of compound **5** via reductive amination. Reagents and conditions: (a) $NaCNBH_3$, CH_3OH or DMF containing 1% acetic acid. See Supplementary data for the experimental details.

We also found that for most of the aromatic aldehydes, it was difficult to obtain the dialkylated products, even in DMF containing 1% acetic acid, conditions that favored dialkylation. For some bulky aromatic aldehydes, monoalkylation of **5** was also difficult. This is presumably due to the axial orientation of the amino group on the carbohydrate ring, which would be expected to react slowly with bulky aldehydes. In cases where dialkylation was successful, the reaction required substantially longer reaction times. These difficulties, coupled with the fact that the majority of the dialkylated compounds had very poor water solubility and thus were difficult to assay led us not to pursue the synthesis of dialkylated compounds from aromatic aldehydes.

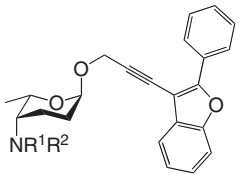
After the assembly of this small library consisting of 18 alkylated analogues of **5**, we assayed their cytotoxicity against three cancer cell lines. The data were summarized in Table 2 for aliphatic substituents and Table 3 for aromatic substituents, respectively. Two obvious trends emerge from these data. First, except for small aliphatic substituents such as methyl (**12**) and ethyl (**13**) groups,

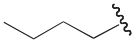
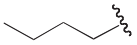
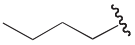
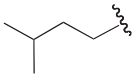
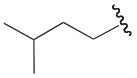
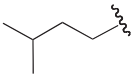
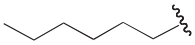
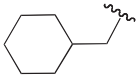
Table 1
Cytotoxicity of compounds **5**, **7**, and **9**–**11**

Compound	IC_{50} (μM)		
	MCF-7	HT29	HepG2[a]
5	10.3 ± 0.2	6.5 ± 0.5	7.9 ± 0.7
7	23.4 ± 2.3	13.3 ± 1.2	18.2 ± 1.9
9	8.0 ± 1.0	6.8 ± 0.9	4.1 ± 0.7
10	9.2 ± 1.1	5.8 ± 0.8	6.0 ± 1.0
11	12.6 ± 1.0	5.8 ± 0.6	13.0 ± 1.2

[a] HepG2/C3A is simplified as HepG2.

Table 2
Cytotoxicity of the alkylated analogues from compound **5** and aliphatic aldehydes[a]



Compound	R^1	R^2	IC_{50}[a] (μM)		
			MCF-7	HT29	HepG2[b]
5	H	H	10.3 ± 0.2	6.5 ± 1.8	7.9 ± 0.7
12			10.3 ± 0.8	6.1 ± 0.6	6.8 ± 0.8
10		H	9.2 ± 0.9	5.8 ± 1.0	6.0 ± 0.4
13			9.7 ± 1.1	6.8 ± 0.4	10.7 ± 1.2
14		H	20.8 ± 2.0	9.8 ± 1.0	19.0 ± 1.6
15			>25	>25	>25
16		H	11.3 ± 1.5	6.6 ± 0.5	13.6 ± 1.0
17			>25	>25	>25
18		H	14.8 ± 1.2	6.0 ± 0.3	14.4 ± 0.8
19		H	>25	10.2 ± 0.5	>25

[a] All analogues with measured IC_{50} values showed cell viability of less than 1% at 25 μM.
[b] HepG2/C3A is simplified as HepG2.

the dialkylated analogues had lower cytotoxicity compared to the monoalkylated compounds (**14** vs **15**, and **16** vs **17**), especially for HT29 cells. Second, for the monoalkylated analogues, the linear aliphatic groups (Table 2) usually exhibited a much stronger cytotoxic effect than cyclic aliphatic groups (compound **19**, Table 2) and most of the aromatic groups (Table 3). This effect is more pronounced for MCF-7 and HepG2/C3A cells. Both trends could be explained by two reasons: low aqueous solubility and/or steric hinderance that may interfere with the interaction with their biological target(s).

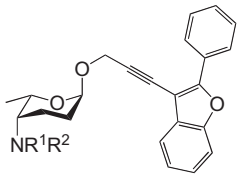
Figure 1 shows the IC_{50} values for the analogues with linear/branched aliphatic substituent(s) (excluding inactive compounds **15**, **17**, and **19**). It is of note that there is a spike in the IC_{50} trends for monobutylated analogue **14**. The increased potency of analogues **16** and **18** might imply that lipophilicity can rescue those compounds with poor aqueous solubility to some extent, presumably due to increased cell permeability.[9]

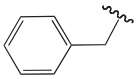
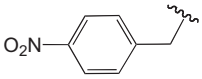
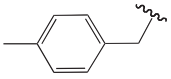
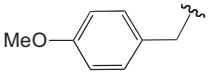
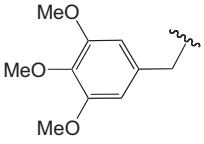
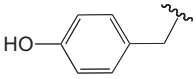
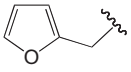
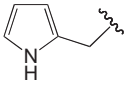
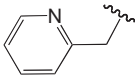
The aromatic substituents were divided into two subgroups: carbocyclic (left, Fig. 2) and heterocyclic (right, Fig. 2). Because IC_{50}s could not be obtained for some analogues, such as **20**–**22**, due to poor aqueous solubility, the cell viability data at 25 μM (Table S2 in Supplementary data) were employed here for the SAR analysis. Using the phenyl analog (compound **20**) as a reference, the incorporation of electron-withdrawing substituents into the carbocyclic aromatics, such as *p*-nitro (compound **21**), lessened the cytotoxicity, while the inclusion of electron-donating substituents, such as *p*-methyl (**22**), *p*-methoxy (**23**), 3,4,5-trimethoxy (**24**) and *p*-hydroxyl (**25**), raised the cytotoxicity, especially **25**. In addition, by comparing the cytotoxicity of analogues **26**–**28**, which contain a nitrogen-containing heterocyclic substituent, with that of compound **20**, it is clear that the presence of nitrogen-containing heterocycle (**27** and **28**) greatly enhanced the cytotoxicity, while the (2-furanyl)-methyl moiety (**35**) has little effect. The recovery of the cytotoxicity for compounds **25**, **27** and **28** implies that the interaction with their biological target(s) depends on the finely defined details of binding, but not the overall size of the substituent on nitrogen.

Although SAR analysis of the alkylated derivatives containing the benzo[*b*]furan system elucidated some of the structural features key to bioactivity, we wondered about the generality of these conclusions. Therefore, some relevant alkylated analogues containing the benzo[*b*]thiophene core were synthesized to further validate the trends deduced from the above SAR analysis.

The synthesis of the alkylated benzo[*b*]thiophene-containing analogues employed the previously established reductive amination protocol for the benzo[*b*]furan system. The structures of the synthesized analogues (**30**–**40**) are listed in Table 4. It is of note that, unlike the benzo[*b*]furan system, during the preparation of the dimethylated analogue **30**, it was found that the separation of the monoalkylated product was possible. Therefore, we explored conditions to produce a small amount of the pure monomethylated analogue for SAR analysis. We first performed the reductive amination in methanol with a large excess of free amino analogue **6**, but under these conditions the major product was still the dialkylated compound **30**, which was formed together with a 2-cyanomethylamino derivative (**31**) (Scheme 3). Byproduct **31** is presumably formed as outlined in Scheme 3; a similar method has been reported for the synthesis of 2-cyano amines from sodium cyanide and aldehydes.[10] When the reaction was carried out in DMF containing 1% acetic acid, the dialkylated analogue **32** was obtained instead of **30**. Apparently, the electron-withdrawing cyano group prevents the second methylation in methanol, but not in acidified

Table 3
Cytotoxicity of the alkylated analogues from compound **5** and aromatic aldehydes



Compound	R^1	R^2	IC_{50} (μM)		
			MCF-7	HT29	HepG2[a]
5	H	H	10.3 ± 0.2	6.5 ± 1.8	7.9 ± 0.7
20		H	>25	17.9[b]	>25
21		H	>25	>25	>25
22		H	>25	11.9 ± 1.0	>25
23		H	31 (2.2)[c]	7.9 ± 0.5	13.1 ± 0.8
24		H	30 (1.1)[c]	8.0 ± 0.6	11.3 ± 0.9
25		H	10.9 ± 0.9	5.8 ± 0.9	13.4 ± 0.7
26		H	>25	15.2 ± 0.7	>25
27		H	18.1[b]	11.1 ± 0.9	20.8[b]
28		H	14.3 ± 1.3	10.8 ± 0.6	16.3[b]

[a] HepG2/C3A is simplified as HepG2.
[b] Assayed only once.
[c] Cell viability at 25 μM. The numbers in parentheses represent the standard deviation.

DMF. Finally, by using solid paraformaldehyde in methanol rather than aqueous formaldehyde solution, monomethylated analogue **29** (>85% pure based on ^{1}H NMR spectroscopy) was obtained; the major contaminant was the free amino compound **6**.

With alkylated analogues **29**–**40** in hand, they were subsequently subjected to the in vitro cytotoxicity assay (Table 4). The same cytotoxicity profile was observed for monomethylated compound **29** as for dimethylated (**30**) and monoethylated (**33**) analogues. All the previously identified trends with the benzo[*b*]furan derivatives were confirmed with this series of analogues. For example, except for small groups, monoalkylation is favored over dialkylation (**35** vs **36**); aliphatic substituents generally provide better bioactivity than aromatic ones; and the lipophilicity has some influence on the cytotoxicity (**37** vs **35**). Finally, based on the assay data for cyano-containing analogues **31** and **32**, which have low cytotoxicity, we can further support the statement made above that increasing electron density on nitrogen enhances cytotoxicity.

In conclusion, although amino group modification of the 4-*N*-TDTH analogues did not further enhance the cytotoxicity compared to parent compounds **5** and **6**, several general trends have

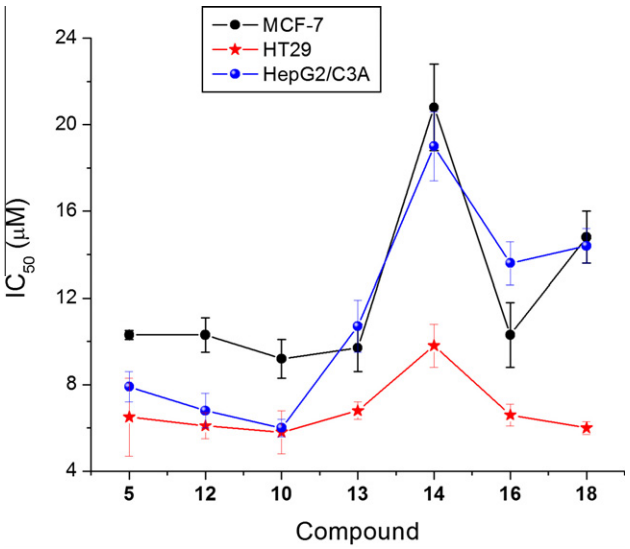


Figure 1. Cytotoxicity (IC_{50} value) trends for the aliphatic alkylated analogues.

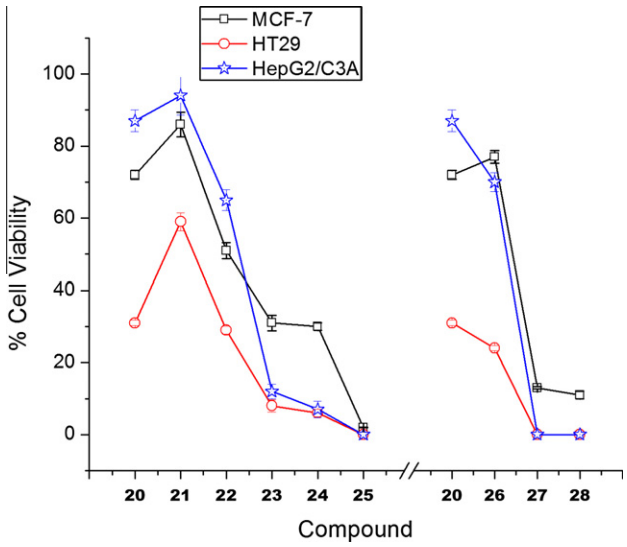
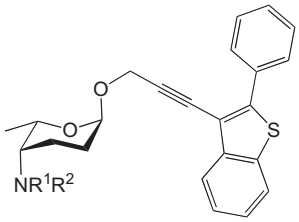


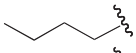
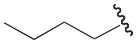
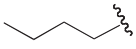
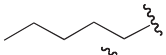
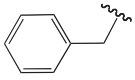
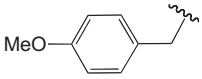
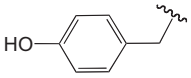
Figure 2. Cytotoxicity (cell viability at 25 μM) trends for the aromatic monoalkylated analogues.

been identified from the above SAR analysis. First, the presence of at least one basic moiety appears to be essential. This group can be either directly attached, or tethered through another group, to the carbohydrate ring. Second, modification of the amine with linear or branched aliphatic chains is favored over cyclic aliphatic or aromatic substituents; dialkylation is disfavored compared to monoalkylation. Third, given the potent cytotoxicity in the case of the *N*-(*p*-hydroxylbenzyl) group, the incorporation of extra functional groups into a linear or branched aliphatic chain may be necessary for stronger interactions between the analogue and its molecular target(s). Fourth, the proper lipophilicity of an analogue may facilitate cell membrane permeability, and thus have an influence on cytotoxicity.

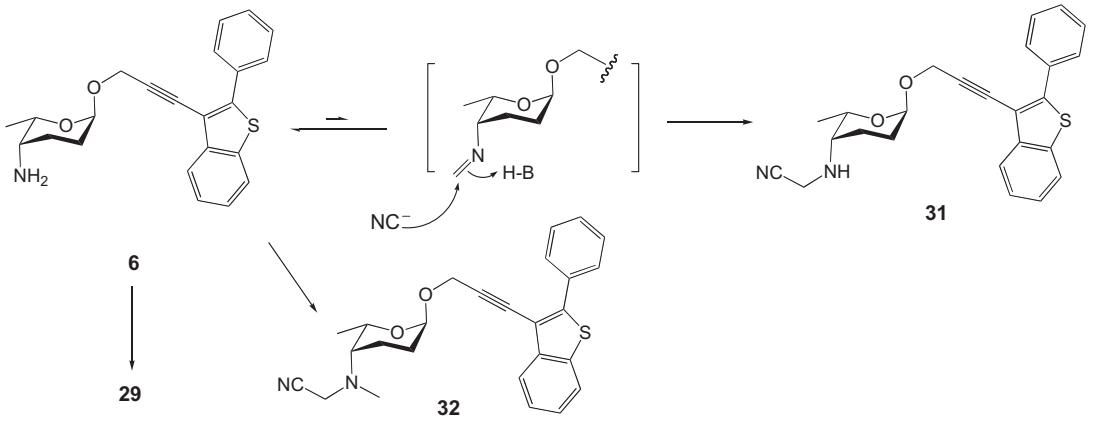
Further studies with the daunosamine or acosamine carbohydrate moiety in **1**–**4** are planned to consolidate and enrich the SAR data. In addition, functionalization of the aromatic domain is expected to be crucial for pronounced improvement in cytotoxicity. The identification of more potent compounds should facilitate the elucidation of the molecular target(s) responsible for the cytotoxicity for the class of compounds. In this regard we should note that all of the compounds reported here possess a basic nitrogen atom and bind to DNA (see Supplementary data) to varying

Table 4
Cytotoxicity of alkylated analogues from **6** and aldehydes



Compound	R^1	R^2	IC_{50} (μM)		
			MCF-7	HT29	HepG2[a]
6	H	H	7.1 ± 1.2	5.9 ± 0.5	8.8 ± 0.9
29[b]		H	7.1 ± 1.0	5.4 ± 0.2	8.8 ± 1.0
30			8.2 ± 0.9	3.3 ± 0.4	10.0 ± 0.9
31		H	10.5 ± 1.3	10.8 ± 1.0	18.0 ± 1.6
32			7.7 ± 0.8	6.2 ± 0.7	12.3 ± 1.4
33		H	5.9 ± 0.5	3.4 ± 0.4	6.6 ± 0.7
34			12.0 ± 0.7	3.9 ± 0.3	11.7 ± 0.9
35		H	10.2 ± 0.9	7.0 ± 0.5	18.6 ± 1.7
36			>25	>25	>25
37		H	6.8 ± 0.6	5.1 ± 0.4	19.1 ± 1.6
38		H	>25	19.0 ± 1.2	>25
39		H	>25	6.2 ± 0.5	>25
40		H	12.0 ± 1.0	5.1 ± 0.6	20.3 ± 0.6

[a] HepG2/C3A is simplified as HepG2.
[b] Purity ~85%.



Scheme 3.

degrees. However, the affinity is rather weak and thus it is likely that other biological targets are also involved in the observed cytotoxicity.

Acknowledgements

This work was supported by the University of Alberta, the Natural Sciences and Engineering Research Council of Canada and the Alberta Ingenuity Centre for Carbohydrate Science. WS was the recipient of a Studentship from the Alberta Ingenuity Fund.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.051.

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