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Synthesis and anti-tumor activities of methyl 2-*O*-aryl-6-*O*-aryl′-D-glucopyranosides

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ABSTRACT

A synthetic method of introducing bulky aryl groups at the 2-*O*- and 6-*O*-positions on glucopyranosides was developed. A total of 37 new compounds of this class were obtained successfully. These compounds were tested on several tumor cell lines by MTT assays, and some of them exhibited encouraging inhibitory activities. The most potent compound, CAB-SHZH-**27**, exhibited EC_{50} values of 14, 12, and 10 μmol/L on A549, MDA-MB-231 and HeLa cells, respectively. A preliminary structure–activity relationship analysis indicates that the two free hydroxyl groups on the D-glucose core are indispensable for the biological activities of this class of compounds, and the aryl group at the 6-*O*-position has a more obvious impact than the one at the 2-*O*-position. An interesting 'on–off' mechanism of this class of compounds was also observed in our MTT assays, which remains to be explored.

Carbohydrate-based compounds are associated with a range of biological activities. They thus have received special attention in drug discovery for years. A recent review published by Meutermans et al.[1] pointed out that carbohydrate-based compounds are characterized by a relatively rigid core and multiple hydroxyl groups which can be differentially modified with orthogonally protected carbohydrate scaffolds. The unique structural diversity of carbohydrate-based compounds allows them to be good mimetics of known drugs or other bioactive compounds with appropriate substituent groups installed at appropriate positions. For example, compound **1** (Fig. 1) exhibit an IC_{50} value of 53 nM against human somatostatin receptor subtype 4 (SSTR4).[1,2] The well known antibiotic clindamycin (compound **2**) has a D-galactose core bearing a lipophilic pyrrolidinyl group. Epirubicin (compound **3**) bears a tri-deoxy arabinopyranoside group, which is used primarily as an anthracycline drug against breast cancer, ovarian cancer, gastric cancer, lung cancer, and lymphomas.[3] Thus, creating new classes of carbohydrate-based compounds and exploring their biological activities is an intriguing aim in medicinal chemistry.

In this study, we have designed and synthesized a class of methyl D-glucopyranosides **4** (Fig. 1) with two aryl groups substituted at the 2-*O*- and 6-*O*-positions. Such a molecule combines hydrophilic and hydrophobic features. They basically have a linear shape, which may be preferable for achieving desired cell permeability. These compounds were tested on several selected tumor cell lines. The synthesis method and anti-tumor activities of this new class of compounds are described in this Letter.

Few known compounds have phenyl ether moieties attached directly to the carbohydrate core structure. Considering that substituent at the 6-*O*-position is relatively easy, we aimed to introduce

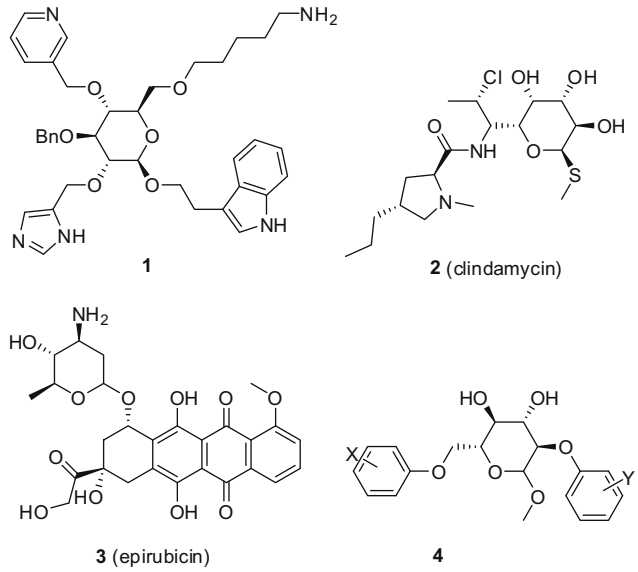

Figure 1. Chemical structures of some carbohydrate-based bioactive compounds.

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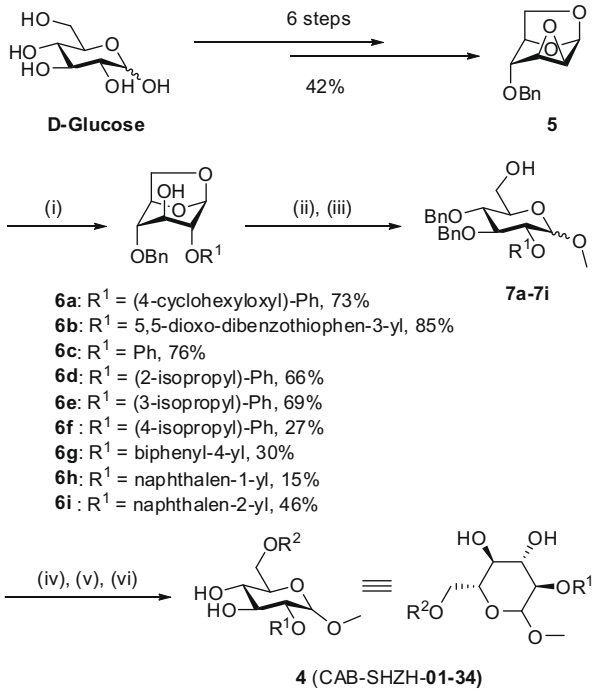


Scheme 1. Synthesis of Methyl 6-*O*-R^2-2-*O*-R^1-D-glucopyranosides CAB-SHZH-**01–34**. Reagents and conditions: (i) R^1OH, NaH, EGDME, reflux; (ii) NaH, BnBr, Bu_4NI, THF, 0 °C to rt; (iii) $TMSOCH_3$, TMSOTf, 3 Å MS, CH_3CN, −45 °C to 45 °C; (iv) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt; (v) R^2OH, NaH, DMF, 80 °C; (vi) Pd/C, H_2, EtOAc/CH_3OH = 2:1.

substituent R^1 at the 2-*O*-position first. Unfortunately, the regular method using nucleophile to replace the axial bond leaving group to form an equatorial bond did not work. Thus, we tried to make the precursor epoxide undergo a ring opening reaction with a nucleophile to install two hydroxyl groups at the 2- and 3-positions with the expected configuration.

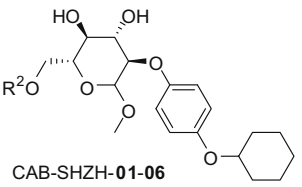
2,3-Epoxide **5** was prepared from D-glucose following the methods reported in literature[4–10] with 42% yield in six steps. Precursor **5** was treated with a panel of substituted sodium phenolates (R^1ONa) in ethylene glyco dimethyl ether (EGDME), and the 2,3-epoxide was opened to form 2-position axial hydroxyl bond. The typical by-product at this step was the dimer molecule formed by an extra step of epoxide opening reaction. Sometimes even the trimer could be obtained, which was not easy to separate using silica gel flash column chromatography (see Supplementary data).

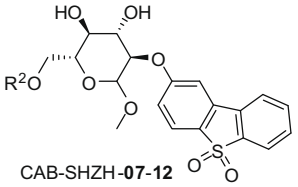
After 3-O-benzylation, $TMSOCH_3$ with TMSOTf could make the 1,6-anhydro bond crashed to form a mixture of the α,β-anomers of **7**. This mixture could not be separated at this step in most cases. Nevertheless, if exposed to different substituted sodium phenolates (R^2ONa), **7** could be transformed into 6-*O*-aryl‘ compounds after further tosylation. After the catalytic hydrogenation step, α,β-anomers could be separated by silica gel flash column chromatography. The desired target compounds (CAB-SHZH-**01** to **34**) were obtained so (Scheme 1).

In our study, the 4-cyclohexyloxylphenol bearing a highly lipophilic substitute on the phenyl ring as R^1OH was chosen at first to react with **5** to obtain **6a**. Considering the similarity in molecular shape, another substituted phenol 5,5-dioxo-5*H*-5λ^6-dibenzothiophen-3-ol was also chosen to react with **5** to obtain **6b**. In the following reactions, different aryl groups were appended to the 6-*O*-position on the carbohydrate core to produce compounds CAB-SHZH-**01** to CAB-SHZH-**12** (Table 1). Here, 5,6,7,8-tetrahydronaphthalen-1-yl and 5,6,7,8-tetrahydro-naphthalen-2-yl were prepared from naphthalen-1-yl and naphthalen-2-yl through in situ reduction at a catalytic hydrogenation reaction condition.

Since this is a new class of compounds, it is difficult to predict their potential pharmaceutical applications. Anyway, we started our testing with tumor cell lines. The first batch of obtained compounds (CAB-SHZH-**01** to CAB-SHZH-**12**) were tested on three human breast tumor cell lines, including MDA-MB-231, MDA-MB-435, and MCF7, by standard MTT assays (see Supplementary data). These tumor cell lines were chosen since they are routinely used in our laboratory. The results indicated that CAB-SHZH-**03** and CAB-SHZH-**05** exhibit modest inhibitory activities on all three cell lines with EC_{50} values ranging from 17 to 36 μM. The different potency of CAB-SHZH-**05** and CAB-SHZH-**11**, CAB-SHZH-**03** and CAB-SHZH-**09** disregarding the configuration at 1-position of the glucopyranosides indicates that 4-cyclohexyloxyl-phenyl at the 2-*O*-position is

Table 1
Chemical structures and inhibitory activities on tumor cell lines of the compounds in the first batch





Compds	R^2	OCH_3	Yield[a] (%)	Inhibition % at 20 μM			EC_{50}[b] (μM)		
				MDA-MB-231	MDA-MB-435	MCF7	MDA-MB-231	MDA-MB-435	MCF7
CAB-SHZH-**01**	Ph	α	59	−11	−8	−9	N.D.	N.D.	N.D.
CAB-SHZH-**02**	Ph	β	17	−9	−14	−5	N.D.	N.D.	N.D.
CAB-SHZH-**03**	4-Biphenyl	α	22	64	30	51	36	26	18
CAB-SHZH-**04**	5,6,7,8-Tetra-*H*-naphthalen-1-yl	α	22	−8	−1	3	N.D.	N.D.	N.D.
CAB-SHZH-**05**	5,6,7,8-Tetra-*H*-naphthalen-2-yl	α	33	24	16	45	20	26	17
CAB-SHZH-**06**	5,6,7,8-Tetra-*H*-naphthalen-2-yl	β	13	0	1	14	N.D.	N.D.	N.D.
CAB-SHZH-**07**	Ph	α	37	2	4	7	N.D.	N.D.	N.D.
CAB-SHZH-**08**	Ph	β	65	−16	0	7	N.D.	N.D.	N.D.
CAB-SHZH-**09**	4-Biphenyl	β	66	29	12	15	N.D.	N.D.	N.D.
CAB-SHZH-**10**	5,6,7,8-Tetra-*H*-naphthalen-1-yl	α	32	−8	−5	4	N.D.	N.D.	N.D.
CAB-SHZH-**11**	5,6,7,8-Tetra-*H*-naphthalen-2-yl	α	41	30	17	2	N.D.	N.D.	N.D.
CAB-SHZH-**12**	5,6,7,8-Tetra-*H*-naphthalen-2-yl	β	38	14	−4	7	N.D.	N.D.	N.D.

[a] Yield rate of the final hydrogenation reaction.
[b] N.D. = not determined due to poor potency observed in the preliminary screening conducted at 20 μM.

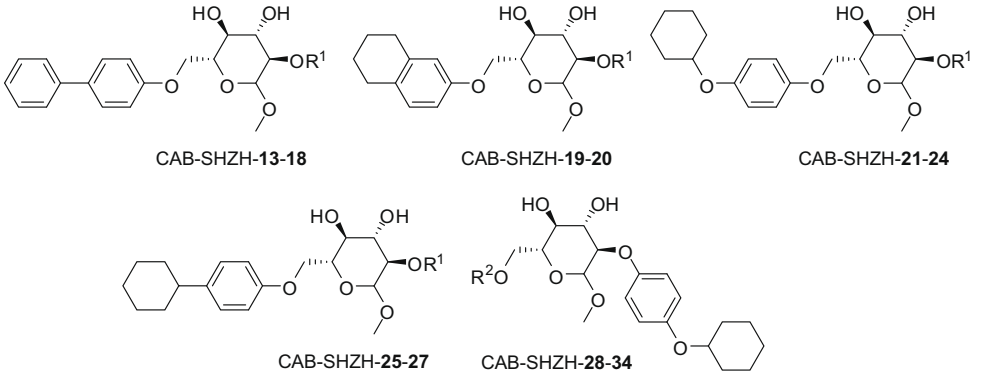
more effective than 5,5-dioxo-5*H*-5λ^6-dibenzothiophen-3-yl for achieving the potency of these compounds. Also, comparing the activities of CAB-SHZH-**01** to CAB-SHZH-**06**, one can see that 4-biphenyl or 5,6,7,8-tetrahydro-naphthalen-2-yl at the 6-*O*-position is more effective than phenyl and 5,6,7,8-tetrahydronaphthalen-yl.

Encouraged by the anti-tumor activities exhibited by CAB-SHZH-**03** and CAB-SHZH-**05**, we launched another round synthesis by transplanting the three important groups identified in the first batch of compounds onto the 6-*O*- and the 2-*O*-positions on the glucopyranosides. Some other substituted phenols were used to react with **5**. Additional epoxide ring opening compounds **6c**–**6i** were obtained so, expanding the diversity of R^1. Here, R^2 used on these new compounds included 4-biphenyl, 5,6,7,8-tetrahydronaphthalen-2-yl, and 4-cyclohexyloxyl-phenyl group, which had been proved effective for the biological activities of this class of compounds. Compounds CAB-SHZH-**13** to CAB-SHZH-**27** (Table 2) were obtained through the same synthetic route. Here, the 4-biphenyl group on some compounds, to our surprise, was found to be hydrogenated to 4-cyclohexylphenyl group besides the reduction of naphthalenyl to 5,6,7,8-tetrahydronaphthalenyl group.

The second batch of compounds were tested on A549 (human alveolar epithelial cell), MDA-MB-231 (human breast tumor cell), and HeLa (human cervical tumor cell) cells by MTT assays. As summarized in Table 2, CAB-SHZH-**03** and CAB-SHZH-**13** to **18** have different aryl groups as R^1 but all share the same biphenyl group as R^2. Their potency on MDA-MB-231 cells are essentially the same ($EC_{50} \geqslant 32$ μM), indicating that modification of R^1 is not that important when R^2 is an appropriate aryl group. In contrast, CAB-SHZH-**03**, CAB-SHZH-**05**, CAB-SHZH-**23**, CAB-SHZH-**24**, and CAB-SHZH-**27** have the same 4-cyclohexyloxylphenyl group as R^1 and different aryl groups as R^2. They do exhibit notably different potency on MDA-MB-231 with EC_{50} = 12–36 μM. This observation further supports that modification on R^2 has priority over R^1 in terms of exploring the biological activities of this class of compounds.

The most potent compound among all of the compounds obtained by us is CAB-SHZH-**27**, which has EC_{50} values of 14, 12, and 10 μmol/L on A549, MDA-MB-231 and HeLa cells, respectively (Table 2). Three derivatives of this compound, that is, CAB-SHZH-**35** to **37**, were prepared by protecting the two free hydroxyl groups on the D-glucose core with different substituent groups (Table 3). They were also tested on the three tumor cell lines. Interestingly, these compounds lost the inhibitory activities demonstrated by CAB-SHZH-**27** completely. One may suspect that the structure–activity relationship observed for this class of compounds arises from different levels of general lipophilicity or cell permeability.

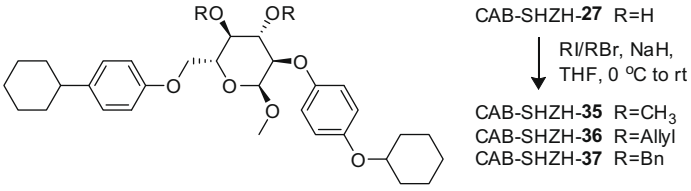
Table 2
Chemical structures and inhibitory activities on tumor cell lines of the compounds in the second batch



Compds	R^1	R^2	$-OCH_3$	Yield[a] (%)	EC_{50}[b] (μM)		
					A549	MDA-MB-231	HeLa
CAB-SHZH-**13**	Ph	4-Biphenyl	β	5	N.D.	N.D.	N.D.
CAB-SHZH-**14**	Ph	4-Biphenyl	α	17	N.A.	36*	33*
CAB-SHZH-**15**	(2-Isopropyl)-Ph	4-Biphenyl	α	68	34	33	29*
CAB-SHZH-**16**	(3-Isopropyl)-Ph	4-Biphenyl	β	23	35*	35*	32*
CAB-SHZH-**17**	(3-Isopropyl)-Ph	4-Biphenyl	α	44	30*	32*	35*
CAB-SHZH-**18**	(4-Isopropyl)-Ph	4-Biphenyl	α	36	31	32	19
CAB-SHZH-**19**	(3-Isopropyl)-Ph	5,6,7,8-Tetra-*H*-naphthalen-2-yl	β	8	N.A.	N.A.	N.A.
CAB-SHZH-**20**	(3-Isopropyl)-Ph	5,6,7,8-Tetra-*H*-naphthalen-2-yl	α	21	N.A.	40*	33*
CAB-SHZH-**21**	(4-Isopropyl)-Ph	(4-Cyclohexyloxyl)-Ph	β	14	35	32	26
CAB-SHZH-**22**	(4-Isopropyl)-Ph	(4-Cyclohexyloxyl)-Ph	α	50	25	27	18
CAB-SHZH-**23**	(4-Cyclohexyloxyl)-Ph	(4-Cyclohexyloxyl)-Ph	β	10	26	25	14
CAB-SHZH-**24**	(4-Cyclohexyloxyl)-Ph	(4-Cyclohexyloxyl)-Ph	α	45	20	18	14
CAB-SHZH-**25**	5,6,7,8-Tetra-*H*-naphthalen-2-yl	(4-Cyclohexyl)-Ph	α	17	25	17	29
CAB-SHZH-**26**	5,6,7,8-Tetra-*H*-naphthalen-1-yl	(4-Cyclohexyl)-Ph	α	15	37*	28	25
CAB-SHZH-**27**	(4-Cyclohexyloxyl)-Ph	(4-Cyclohexyl)-Ph	α	33	14	12	10
CAB-SHZH-**28**	(4-Cyclohexyloxyl)-Ph	(2-Isopropyl)-Ph	β	23	44*	41*	42*
CAB-SHZH-**29**	(4-Cyclohexyloxyl)-Ph	(2-Isopropyl)-Ph	α	48	36*	33*	47*
CAB-SHZH-**30**	(4-Cyclohexyloxyl)-Ph	(3-Isopropyl)-Ph	β	15	40*	41*	39*
CAB-SHZH-**31**	(4-Cyclohexyloxyl)-Ph	(3-Isopropyl)-Ph	α	49	38*	30*	28*
CAB-SHZH-**32**	(4-Cyclohexyloxyl)-Ph	(3,4-Dimethoxy)-Ph	β	6	N.A.	N.A.	N.A.
CAB-SHZH-**33**	(4-Cyclohexyloxyl)-Ph	(3,4-Dimethoxy)-Ph	α	44	N.A.	N.A.	N.A.
CAB-SHZH-**34**	(4-Cyclohexyloxyl)-Ph	(3,5-Dimethoxy)-Ph	α	16	N.A.	N.A.	N.A.

[a] Yield rate of the final hydrogenation reaction.
[b] The data without an asterisk are derived from fitting the dose-dependent inhibition curves; the data with an asterisk are estimated since the dose-dependent inhibition curves are abnormal; N.D. = not determined due to poor potency observed in the preliminary screening conducted at 20 μM; N.A. = no obvious activity was observed up to 50 μM.

Table 3
Chemical structures and inhibitory activities on tumor cell lines of several derivatives of CAB-SHZH-27



Compds	R	Yield (%)	EC_{50}[a] (μM)		
			A549	MDA-MB-231	HeLa
CAB-SHZH-**35**	CH_3	94	N.A.	N.A.	N.A.
CAB-SHZH-**36**	Allyl	86	N.A.	N.A.	N.A.
CAB-SHZH-**37**	Bn	78	N.A.	N.A.	N.A.

[a] N.A. = No obvious activity was observed up to 50 μM.

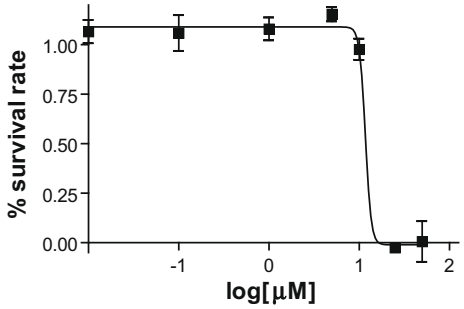


Figure 2. Dose-dependent cytotoxicity of CAB-SHZ H27 on MDA-MB-231 cells.

Nevertheless, this set of results provides convincing evidence that this class of compounds achieve their inhibitory activities on tumor cells through a specific mechanism, in which the two free hydroxyl groups at the 3- and 4-positions on the D-glucose core play an indispensable role.

Moreover, the second batch of compounds reveal a subtle role of the methoxyl group at the 1-position: the inhibitory activities of the α-anomer on three tumor cell lines are generally more obvious than the β-anomer of the same chemical structure, although the difference is only marginal. This trend can be observed virtually on all pairs of α- and β-anomers, including CAB-SHZH-**13/14,** CAB-SHZH-**16/17,** CAB-SHZH-**19/20,** CAB-SHZH-**21/22**, CAB-SHZH-**23/24**, CAB-SHZH-**28/29**, and CAB-SHZH-**30/31** (Table 2). This result also prompts that there is a specific mechanism behind the biological activities of this class of compounds. Otherwise, one probably should not observe such a consistent trend.

Most interestingly, an unexpected phenomenon was observed in our MTT assay: The dose-dependent inhibition curves of most active compounds are steep rather than a normal smooth S-shape curve (Fig. 2). It seems that these compounds are not up-taken by tumor cells up to a concentration around 10 μM, and then for some reasons they come into effect at higher concentrations. In fact, almost all active compounds could completely kill the tumor cells in our test at a concentration lower than 50 μM. Consequently, the EC_{50} values for most compounds determined by us are in a relatively narrow range between 10 and 40 μM (Tables 1 and 2). In other words, these compounds behaved basically in an 'on–off' manner in our MTT assay on tumor cells. We suspect that there is a special trans-membrane mechanism for this class of compounds, which is activated only upon a certain condition. Once these compounds enter the tumor cells, they are quite potent to regulate some biological processes. Our hypothesis, however, remains to be explored in the future.

In summary, we have developed a new synthetic method to introduce bulky aryl groups at both the 2-*O*- and 6-*O*-positions on D-glucopyranoside. A class of 37 new compounds were successfully obtained in our study. They have been tested on several selected tumor cell lines, some of which exhibited encouraging inhibitory activities against tumor cell growth. Moreover, an interesting 'on–off' mechanism was observed for some active compounds in our MTT assay. A preliminary structure–activity relationship of this class of compounds has been obtained, suggesting that their inhibitory activities on tumor cell lines are the consequence of a specific mechanism. We hope that our study adds new knowledge to the organic chemistry of carbohydrate derivatives as well as their potential pharmaceutical applications.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.045.

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