Organic & Biomolecular Chemistry

Synthesis and antitumor activities of aquayamycin and analogues of derhodinosylurdamycin A

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-01-2019-000121.R1
Article Type:	Paper
Date Submitted by the Author:	06-Feb-2019
Complete List of Authors:	Acharya, Padam Prasad; University of Toledo, Chemistry and Biochemistry Khatri, Hem Raj; University of Toledo, Chemistry and Biochemistry Janda, Sandip; University of Toledo, Chemistry and Biochemistry Zhu, Jianglong; University of Toledo, Chemistry and Biochemistry

Organic & Biomolecular Chemistry

PAPER

Synthesis and antitumor activities of aquayamycin and analogues of derhodinosylurdamycin A†

Padam P. Acharya, Hem Raj Khatri, Sandip Janda, and Jianglong Zhu*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Total syntheses of aquayamycin (**3**) and a number of analogues of angucycline antitumor antibiotic derhodinosylurdamycin A bearing various 2-deoxy sugar subunits (**4**-**7**) have been achieved. These molecules (**3**-**7**) were synthesized based on a convergent strategy for the synthesis of derhodinosylurdamycin A (**2**) previously reported from our group. In particular, our recently developed mild cationic gold-catalyzed glycosylation with *S*-but-3-ynyl thioglycoside donors was employed for the synthesis of analogues (**6** and **7**) bearing disaccharide subunits containing α-L-olivoside and α-L-olioside moiety, respectively. Aquayamycin (**3**), analogues (**4**-**7**), and our previously synthesized derhodinosylurdamycin A (**2**) were then submitted to the Development Therapeutics Program of the National Cancer Institute of National Institutes of Health for the NCI-60 Human Tumor Cell Lines Screening using standard protocols. It was found that derhodinosylurdamycin A (**2**), aquayamycin (**3**), and three other analogues (**5**-**7**) bearing sugar subunits did not show significant antiproliferative activity against those cancer cell lines. Interestingly, analogue (**4**) bearing no sugar subunit demonstrates good potential for growth inhibition and cytotoxic activity against a variety of human cancer cell lines.

Introduction

Angucycline antibiotics are a class of bioactive natural products containing an angularly assembled tetracyclic ring frame and diverse sugar subunits, and they exhibit a wide range of biological activities such as antimicrobial, antifungal, antitumor, enzyme inhibiting, and platelet aggregation inhibiting activities.[1-4] The urdamycins[5-9] (*cf.* **1** and **2**, **Fig. 1**) are a subclass of angucyclines first isolated in 1986 and, structurally, they are related to an early isolated molecule, aquayamycin (**3**).[10] Aquayamycin was also known as urdamycinone A and it contains a *C*-glycosylated benz[a]anthraquinone core and a *cis*-diol at the ring junction (**3**).[10] Other known aquayamycin-type angucycline antibiotics include saquayamycins,[11] vineomycin A_1,[12] and PI-080.[13] Among urdamycin family antiobiotics, urdamycin A and derhodinosylurdamycin A share the same 2-deoxy trisaccharide subunit consisting of two D-olivoses and one L-rhodinose *C*-linked to the tetracyclic core (**Fig. 1**). Structurally, urdamycin A (**1**)[5] was identical to a previously isolated molecule Kerriamycin B, while derhodinosylurdamycin A (**2**)[14-15] was the same as Kerriamycin C.[16] The urdamycins were reported to be active against Gram-positive bacteria and murine L1210 leukemia stem cells. For instance, urdamycin A and derhodinosylurdamycin A possess significant anticancer activity against L1210 Leukemia stem cells with an IC_{50} value of 0.55 and 0.75 µg/ml, respectively. In addition, aquayamycin (**3**) was found to inhibit tyrosine hydroxylase and is active against Gram-positive bacteria, Ehrlich ascites carcinoma, and Yoshida rat sarcoma cells.[17]

The complex structures and interesting biological properties of the urdamycins have drawn a great deal of attention. Since their isolation, numerous synthetic studies have been reported toward the urdamycin family antibiotics including aquayamycin (urdamycinone A) tetracyclic core structure[18] as well as urdamycinone B[19-23] and related structures.[24-25] However, due to the highly labile nature of the aquayamycin core system,[26] thus far only two groups have independently accomplished the total synthesis of aquayamycin (**3**). In 2000, Suzuki and co-workers reported the first total synthesis of aquayamycin.[27-29] Their key strategy involves a Hauser annulation[30-31] of β-*C*-glycosylated 3-(benzenesulfonyl)phthalide and a complex cyclohexanone followed by an intramolecular pinacol coupling to construct the tetracyclic angular aglycon. Recently in 2016, Toshima and co-workers[26] reported the second synthesis of aquayamycin and key steps include: 1) a diastereoselective 1,2-addition of *C*-glycosyl naphthyllithium to a cyclic ketone derived from D-(-)-quinic acid; 2) an indium-mediated site-selective allylation-rearrangement; and 3) a diastereoselective intramolecular pinacol coupling. This synthetic route was relatively shorter and may be more practical to access aquayamycin-type angucycline antibiotics.

While there has not been a total synthesis of urdamycin A reported thus far, in 2015 our group accomplished the first and only total synthesis of derhodinosylurdamycin A (**2**).[32] As shown in **Scheme 1**, the synthesis was achieved by employing a modified strategy based on Suzuki's work[27-29] and the strategy was specifically designed in order to facilitate the preparation of derhodinosylurdamycin A analogues bearing different sugar subunits for structure and activity relationship (SAR) studies. In brief, Hauser annulation of cyanophthalide **8** and known complex enone **9**[28] followed by pinacol coupling and necessary functional group manipulations afforded tetracyclic aryliodide **10** (confirmed by single-crystal *X*-ray crystallographic analysis). Next, Stille coupling of glycal stannane **11** with tetracyclic aryliodide **10** followed by stereoselective reduction[33-34] of the resulting enol ether and desilylation provided β-C-arylglycoside **12**. Stereoselective α-glycosylation between disaccharide donor **13** and acceptor **12** gave derhodinosylurdamycin A (**2**).

Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, The University of Toledo, Toledo, OH, 43606, USA. Email: Jianglong.Zhu@utoledo.edu
†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Organic & Biomolecular Chemistry

PAPER

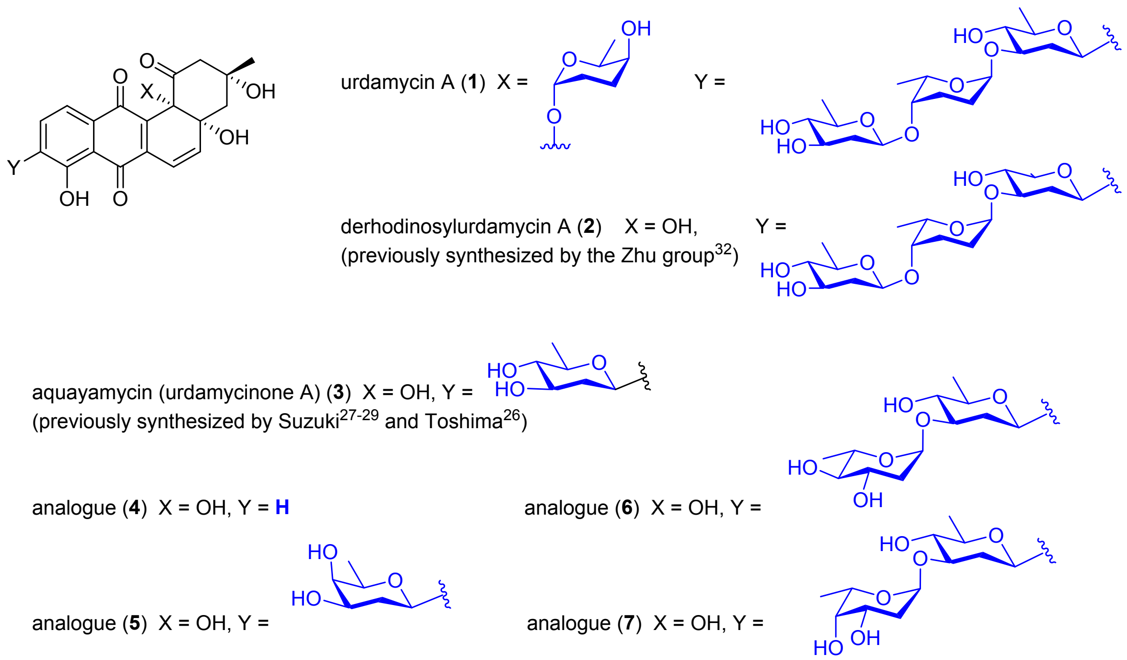
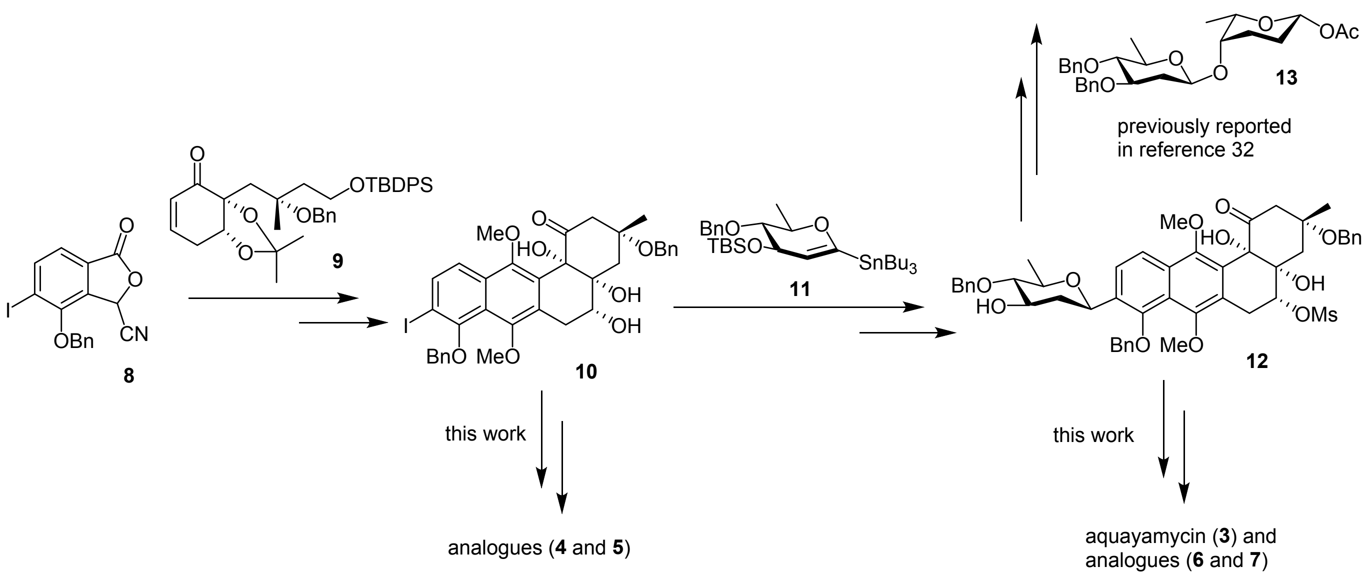


Fig. 1 Urdamycin A, derhodinosylurdamycin A, aquayamycin, and related molecules.

Previously, limited structure and activity relationship (SAR) studies of urdamycin family antitumor antibiotics were reported based on the derivatization of limited amounts of isolated naturally occurring molecules.[14] Since the actual mode of action of the urdamycins is not exactly known, it would be appealing to chemically prepare these molecules as well as their analogues for biological studies. With the successful development of a convergent strategy amenable to the preparation of analogues of derhodinosylurdamycin A, we decided to carry out the synthesis and explore the structure and activity relationship of by varying the structures of the 2-deoxy sugar subunits. Herein, we wish to report the synthesis of aquayamycin (**3**) and several analogues of derhodinosylurdamycin A (*cf.* **4**-**7**, **Fig. 1**) as well as their antitumor activities.

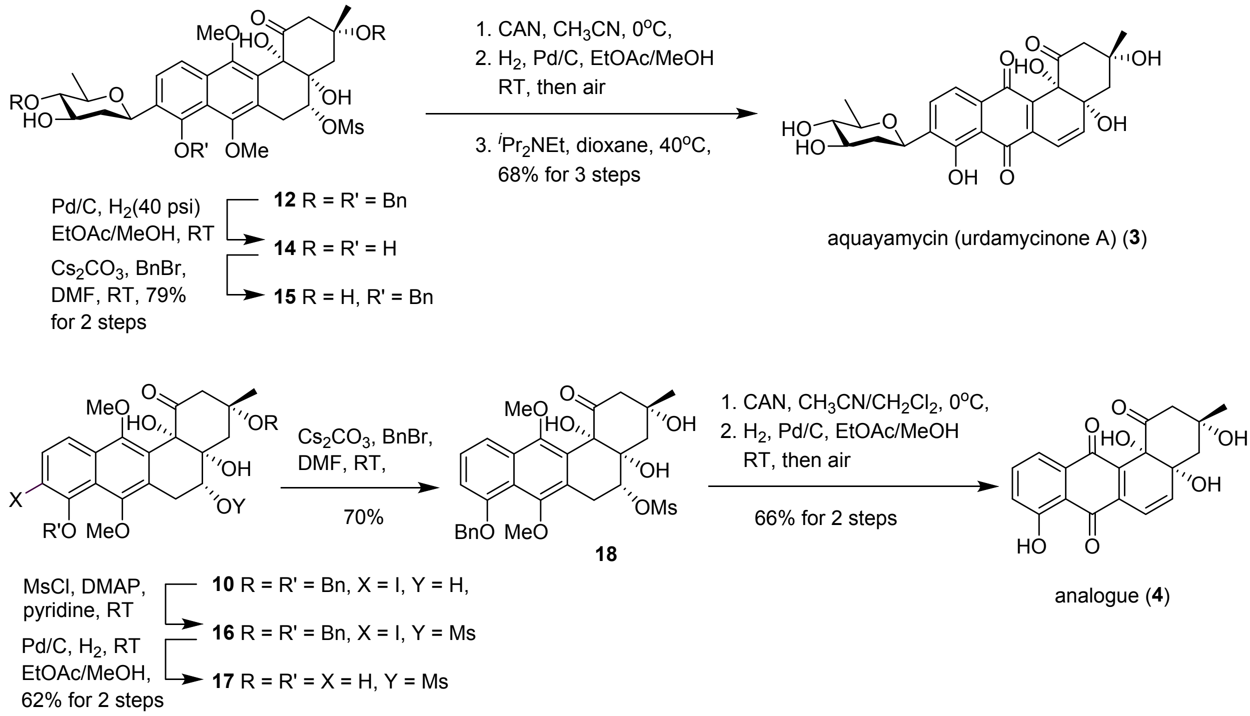


Scheme 1 Our strategies for the synthesis of derhodinosylurdamycin A (**2**), aquayamycin (**3**), and analogues (**4**-**7**).

Results and discussion

Chemistry

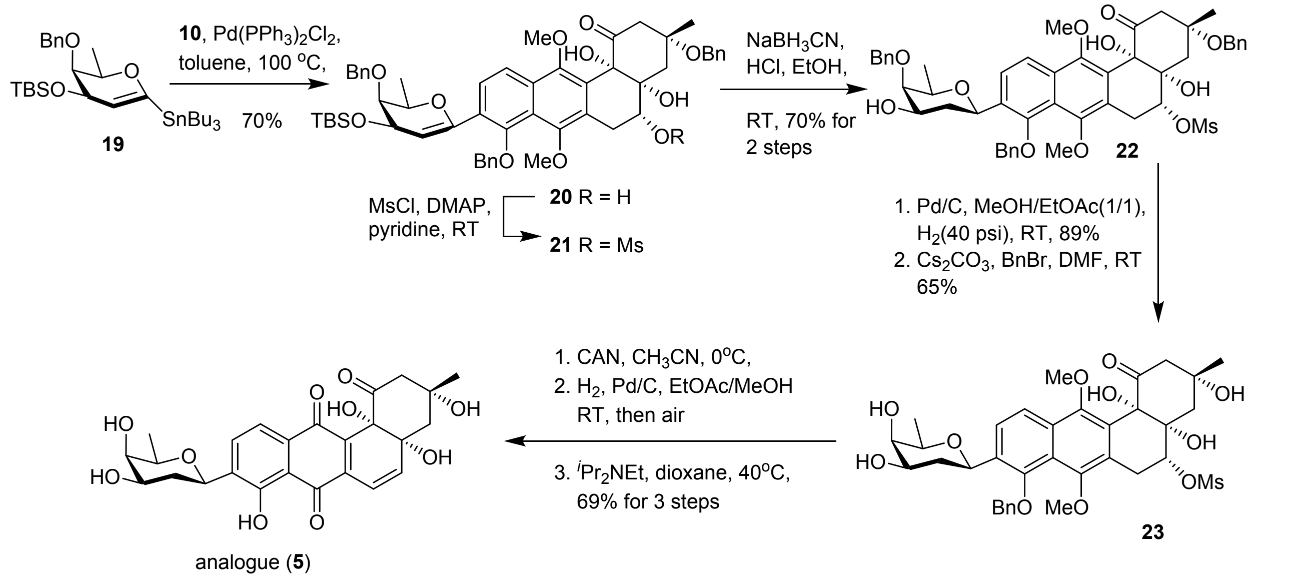
In general, our syntheses of aquayamycin (**3**) and analogues (**4**-**7**) follow our previously reported strategy for the synthesis of derhodinosylurdamycin A (**2**). The highly functionalized building blocks, including tetracyclic aryliodide (**10**) and partially protected β-*C*-arylglycoside (**12**), were prepared in good quantities from commercially available starting substrates following previously disclosed strategies and procedures.[32] As shown in **Scheme 2**, global debenzylation of known β-*C*-arylglycoside **12**[32] via palladium on carbon-catalyzed global hydrogenolysis afforded desired product **14** which upon selective protection of the phenol provided key intermediate **15** (79% yield over 2 steps). Next, compound **15** was converted to aquayamycin (**3**) in 68% yield following our previously reported three-step sequence: 1) cerium(IV) ammonium nitrate (CAN)-mediated oxidation of **15** to the corresponding quinone; 2) quick Pd/C-catalyzed hydrogenolysis to remove the phenolic benzyl ether and concomitant reduction of the quinone to hydroquinone followed by oxidation of the hydroquinone back to quinone via exposure to the air; 3) *N*,*N*-diisopropylethylamine-mediated elimination of the mesylate. Similarly, analogue (**4**) was prepared from known tetracyclic aryliodide (**10**) in five steps: 1) selective mesylation of the secondary alcohol of **10** to give **16**; 2) Pd/C-catalyzed global hydrogenolysis of **16** for removal of aryl iodide and benzyl ethers to produce **17** (62% yield in two steps); 3) selective protection of the phenol as its benzyl ether **18** (70% yield); 4) CAN oxidation of **18** to the corresponding quinone; 5) Pd/C-catalyzed hydrogenolysis to remove the phenolic benzyl ether and concomitant reduction of the quinone to hydroquinone followed by air-mediated oxidation of the hydroquinone back to quinone and subsequent spontaneous elimination (66% over 2 steps).



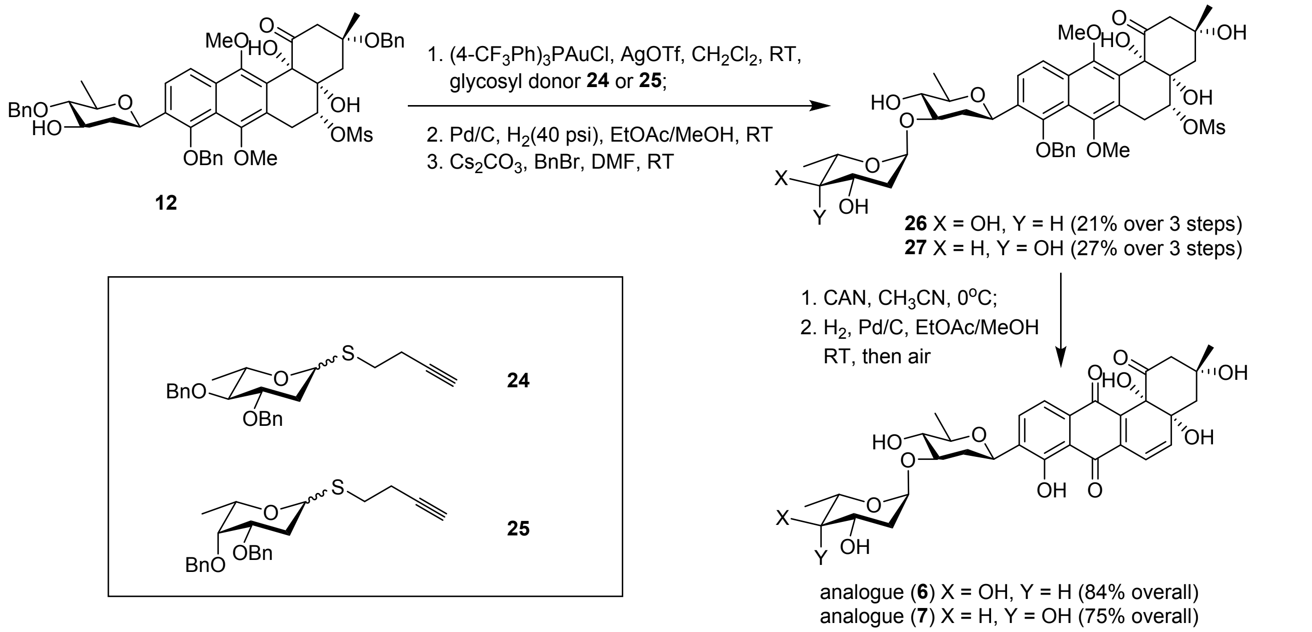
Scheme 2 Synthesis of aquayamycin (**3**) and analogue (**4**).

The synthesis of analogue (**5**) commenced with the preparation of D-fucal stannane **19** which was obtained from 3,4-di-*O*-*tert*-butyldimethylsilyl-D-fucal[35] in four steps:[36] 1) lithiation of the C1 of glycal followed by stannylation; 2) global silyl ether deprotection to the diol; 3) regioselective silylation of the hydroxyl group at C3; and 4) benzylation of the remaining C4-hydroxyl group. Next, Stille coupling of D-fucal stannane **19** with tetracyclic aryliodide **10** which provided *C*-arylated D-fucal **20** in 70% yield (**Scheme 3**). Mesylation of the secondary alcohol of **20** followed by stereoselective reduction of the enol ether moiety and concomitant acid-mediated desilylation afforded β-*C*-arylglycoside **22** (70% yield over two steps). Likewise, analogue (**5**) was prepared from **22** in five steps: 1) removal of all three benzyl ethers of **22** by Pd/C-catalyzed global hydrogenolysis and subsequent selective protection of the phenol as its benzyl ether **23** (65% yield over two steps); 2) CAN oxidation of **23** to the corresponding quinone; 3) Pd/C-cataylzed hydrogenation to remove the phenolic benzyl ether and concomitant reduction of the quinone to hydroquinone followed by air-mediated oxidation of the hydroquinone back to quinone; 4) *N*,*N*-diisopropylethylamine-mediated elimination of the mesylate (69% over 3 steps).

In order to prepare analogues (**6**) and (**7**) bearing 2-deoxy disaccharide subunit, we chose to utilize our recently developed mild cationic gold(I)-catalyzed glycosylation methodology employing *S*-but-3-ynyl thioglycoside donors.[37] In the event, cationic (4-$CF_3Ph)_3PAuOTf$-catalyzed glycosylation between readily available L-olivose-derived *S*-but-3-ynyl thioglycoside donor **24**[36] and β-*C*-arylglycoside **12** acceptor afforded corresponding disaccharide in 91% yield as a mixture of inseparable α/β anomers (α/β, 2/1).[36] The inseparable α/β anomers were then subjected to global debenzylation by Pd/C-catalyzed hydrogenolysis. Once all benzyl ethers were removed, α/β anomers can be separated and the α-disaccharide was obtained in 36% yield after purification. The α-anomer was then subjected to selective benzylation of the phenol to provide desired product **26** in 63% yield (21% yield over three steps). Next, intermediate **26** was converted to analogue (**6**) following aforementioned strategy: 1) CAN oxidation of **26** to the corresponding quinone; 2) Pd/C-cataylzed hydrogenation to remove the phenolic benzyl ether and concomitant reduction of the quinone to hydroquinone; 3) air-mediated oxidation of the hydroquinone back to quinone followed by spontaneous elimination of the mesylate (84% overall). Similarly, cationic $(4\text{-}CF_3Ph)_3PAuOTf$-catalyzed glycosylation between readily available L-oliose-derived *S*-but-3-ynyl thioglycoside donor **25**[36] and β-*C*-arylglycoside **12** acceptor afforded corresponding disaccharide in 84% yield as a mixture of inseparable α/β anomers. Upon global debenzylation of this mixture of inseparable α/β anomers by Pd/C-catalyzed hydrogenolysis, the α-disaccharide was separated from its β-anomer and obtained in 56% yield after purification. The α-anomer was then subjected to selective benzylation of the phenol to provide desired product **27** in 57% yield (27% yield over three steps). Likewise, intermediate **27** was converted to analogue (**7**) following aforementioned two-step sequence (75% overall).



Scheme 3 Synthesis of analogue (**5**) bearing β-*C*-aryl D-olioside moiety.



Scheme 4 Synthesis of analogues (**6** and**7**).

Biological studies

Quinonoid molecules, especially naphthoquinones, demonstrate excellent efficiency in cancer chemotherapeutic treatment. They are well-known as oxidizing or dehydrogenating agents which undergo reduction very easily.[38] In addition, quinones are electrophiles which can be attacked by a variety of nucleophiles. The presence of an electron-withdrawing group or electron-donating substitution on the quinonoid structures may significantly influence their oxidative/reductive properties. In general, the cytotoxicity of quinonoid molecules are attributed to two major mechanisms: 1) promoting oxidative stress; and 2) deactivation of cellular nucleophiles via alkylation/nucleophilic addition. For instance, β-lapachone, a recently naturally occurring *ortho*-naphthoquinone and a novel drug for cancer treatment, has been known to induce apoptosis in various human cancer cell lines by inhibiting DNA topoisomerase I and II.[39] Recently, it was also found that the anticancer activity of β-lapachone may be due to its inhibition of allosteric 5-lipoxygenase.[40] In addition, β-lapachone and a structurally related *ortho*-naphthoquinone, rhinacanthone,[41] were also extensively investigated in SAR studies[42-44] for their cytotoxicity against cancer cell lines.

With newly synthesized aquayamycin (**3**), analogues (**4**-**7**) and previously prepared derhodinosylurdamycin A (**2**) in hand, we decided to evaluate their antiproliferative activities against various cancer cell lines. After careful consideration, we submitted these molecules to the Development Therapeutics Program of the National Cancer Institute of National Institutes of Health for the NCI-60 Human Tumor Cell Lines Screening using standard protocols. These known 60-cell panel contains a variety of human cell lines of leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer, would be ideal for *in vitro* assay of our synthesized molecules.

Initially, six compounds **2**-**7** were evaluated for their antiproliferative activities against 60 human cancer cell lines at 10 μM concentration. The growth percent for selected cancer cells in the presence of each of these six molecules at 10 μM concentration are shown in **Table 1** and also illustrated using by dynamic heat map presentation. The growth percent for all 60 cancer cells in the presence of each of these six molecules at 10 μM concentration are provided in the Supporting Information. Despite that it was known that derhodinosylurdamycin A (**2**) is active against L1210 Leukemia stem cells[14] and aquayamycin (**3**) is active against Ehrlich ascites carcinoma and Yoshida rat sarcoma cells,[17] to our surprise, in this NCI-60 Human Tumor Cell Lines Screening none of derhodinosylurdamycin A (**2**), aquayamycin (**3**), and analogues **5**-**7** bearing various 2-deoxy sugar subunits showed significant antiproliferative activities against these 60 human cancer cell lines. Gratifyingly, analogue **4** demonstrated potent growth inhibition against a broad range of human cancer cell lines, especially for some colon, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Despite numerous previous reports indicating that sugar play critical roles in the bioactivity of their carrier molecules,[45-46] the biological studies herein indicated that sugars do not help improve the anticancer activity of these aquayamycin-type of angucycline antibiotics.

Table 1 Selected Results showing antiproliferative activity of compounds **2**-**7** in the NCI-60 human tumor cell lines screening at 10 μM.

CELL NAME		2	3	4	5	6	7
Leukemia	CCRF-CEM	90.5	89	0.4	88.9	86.4	93.8
	HL-60(TB)	99.4	100.6	-43.8	99.2	99.2	99.5
	K-562	94.4	86.9	5.4	86.8	95.3	91.3
	MOLT-4	86.6	93.5	-12.8	99.5	97.3	91.5
	RPMI-8226	95.3	90.8	-0.2	89.1	92.7	92
	SR	89.6	77.2	10.5	85.4	71.7	76.7
Non-Small Cell Lung Cancer	HOP-62	112.9	109.3	49	98.2	106.4	107.1
	HOP-92	149.1	119.1	-12.2	125.4	116.9	119.9
	NCI-H23	100.1	93.1	-6.8	95.3	101.6	93.2
	NCI-H522	41.5	78.7	-59.5	85.3	86.7	82.8
Colon Cancer	COLO 205	-19.1	62.4	-81.3	103.2	113.1	96.9
	HCT-116	91.1	97.2	-32.1	97.8	98.7	98.9
	HCT-15	94.8	100	-45.5	97.6	96.9	98.9
	HT29	107.8	106.4	3.9	106.8	109.5	105.8
	SW-620	94.4	101.3	-55.5	107.1	104.8	95
CNS Cancer	SNB-75	90.7	91.5	20	98.9	89	86.8
Melanoma	LOX IMVI	94.6	96.6	-51.5	93.7	92.6	91
	MALME-3M	65.4	80.4	-96.7	87.4	99.2	90.7
	M14	109.8	104.2	-24.8	113.2	104.6	98.4
	MDA-MB-435	105.6	105.2	-79.8	105	108.1	103.2
	SK-MEL-28	116.5	109.5	-37.4	117.8	117.3	112.6
	SK-MEL-5	100.6	99.9	-53.7	99.3	99.6	100.2
	UACC-62	97.8	90.7	16.5	98.8	91.4	94.2
Ovarian Cancer	IGROV1	96.4	91.9	27.1	98.2	83.4	91.2
	OVCAR-3	98.1	105	-32.8	111	104.3	105.4
	OVCAR-4	87.1	84.8	-97.9	87.9	86	92
	OVCAR-5	110	106.6	38.8	105.4	99.9	102
	OVCAR-8	98.2	92	-12.6	100.5	93.4	96.7
	NCI/ADR-RES	97	99.2	47.2	99.9	97.9	91.8
Renal Cancer	786-0	102.8	98.1	9.8	101.5	102.6	100.8
	ACHN	90.8	99.5	-100	109.2	101.4	102.3
	CAKI-1	93.3	91.5	-59.8	91.3	86.8	88.1
	RXF 393	113	105	-85.3	107.7	96.9	106.1
	SN12C	98.4	94.9	3.2	95.4	91.7	94.2
	TK-10	97.4	98.6	-82.8	102	104.6	101.1
	UO-31	82.8	84.4	-96.6	87.4	77.6	78.9
Prostate Cancer	DU-145	110.3	106.9	-82.3	109	115.3	104.4
Breast Cancer	MCF7	86.4	88	-60.2	93.7	82.3	91.8
	MDA-MB-231/ATCC	104.8	100.1	30.2	104	107.7	102
	BT-549	100.1	98.5	45.2	101.3	97.5	100.4
	T-47D	78.7	101.4	-45.5	94.2	89.9	93.8
	MDA-MB-468	106.2	104.7	-57.5	97.6	103.4	106.5
		Range	149.1	0	-100		

Next, due to its promise analogue (**4**) was advanced to next level and subjected to five dose testing at 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM. The GI_{50} values are provided in **Table 2** and also illustrated using by dynamic heat map presentation. The comprehensive list of GI_{50}, TGI, and LC_{50} values of analogue (**4**) as well as dose response curves and mean graphs for all 60 cell lines are provided in the Supporting Information. As shown in **Table 2**, the GI_{50} values of analogue (**4**) range from approximately 0.23 μM to 18 μM against all the 60 human cancer cell lines. It was found that analogue (**4**) was most active against colon cancer cell COLO 205 (GI_{50} 0.229 μM), Leukemia cell CCRF-CEM (GI_{50} 0.342 μM), Melanoma MALME-3M (GI_{50} 0.492 μM), Renal cancer cell ACHN (GI_{50} 0.5 μM), and breast cancer cell MCF7 (GI_{50} 0.257 μM). In general, analogue (**4**) was found not very active against CNS cancer cell lines, some of the non-small cell lung cancer cell lines and ovarian cancer cell lines. Overall, analogue (**4**) showed good potential for growth inhibition and cytotoxic activity against a variety of human cancer cell lines.

Table 2 GI_{50} values of MTS assay on survival of NCI-60 human tumor cell lines treated with analogue A (**4**) at 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM.

CELL NAME		GI_{50} (μM)	CELL NAME		GI_{50} (μM)
Leukemia	CCRF-CEM	0.342	Melanoma	M14	1.7
	HL-60(TB)	2.05		MDA-MB-435	1.85
	K-562	0.706		SK-MEL-2	2.47
	MOLT-4	1.12		SK-MEL-28	2.11
	RPMI-8226	1.64		SK-MEL-5	1.75
	SR	0.635		UACC-257	1.68
Non-Small Cell Lung Cancer	A549/ATCC	16.3		UACC-62	1.86
	EKVX	11.6	Ovarian Cancer	IGROV1	1.62
	HOP-62	15.6		OVCAR-3	1.23
	HOP-92	1.51		OVCAR-4	1.44
	NCI-H226	1.69		OVCAR-5	6.51
	NCI-H23	1.94		OVCAR-8	2.67
	NCI-H322M	14		NCI/ADR-RES	5.84
	NCI-H460	5.15		SK-OV-3	17.5
	NCI-H522	0.697	Renal Cancer	786-0	2.88
Colon Cancer	COLO 205	0.229		A498	13
	HCC-2998	7.59		ACHN	0.5
	HCT-116	0.649		CAKI-1	1.5
	HCT-15	1.19		RXF 393	1.46
	HT29	3.26		SN12C	1.72
	KM12	14		TK-10	2.27
	SW-620	1.2		UO-31	1.23
CNS Cancer	SF-268	5.16	Prostate Cancer	PC-3	2.42
	SF-295	18.3		DU-145	1.75
	SF-539	4.67	Breast Cancer	MCF7	0.257
	SNB-19	13.6		MDA-MB-231/ATCC	2.25
	SNB-75	11.4		HS 578T	2.74
	U251	6.02		BT-549	2.62
Melanoma	LOX IMVI	2.25		T-47D	1.85
	MALME-3M	0.492		MDA-MB-468	1.7
	Range	0.229	18.3		

Despite that the cytotoxic mechanism for these aquayamycin-type molecules (**2**-**7**) is not clear, it may be due to the electrophilic property of the 2-vinylnaphthoquinone moiety. In principle, protein or cellular nucleophiles may add to the 2-vinylnaphthoquinone moiety via Michael-type addition and, therefore, get deactivated. This relatively reactive 2-vinylnaphthoquinone moiety may also be responsible to the labile nature of these molecules. In addition, analogue (**4**) lacking the sugar subunit contains less polar hydroxyl groups and may be more lipophilic than derhodinosylurdamycin A (**2**), aquayamycin (**3**), and other anlogues (**5**-**7**). The more potent cytotoxicity of analogue (**4**) discovered according to our experiments is also in agreement with previous report that increase of the lipophilicity of organic molecules improved their cytotoxicity.[41]

Conclusions

In conclusion, we have described the total syntheses of aquayamycin (**3**) and a number of analogues of angucycline antitumor antibiotic derhodinosylurdamycin A bearing various 2-deoxy sugar subunits (**4**-**7**). These molecules were prepared from commercially available starting substrates in approximately 30 linear steps following a convergent strategy previously reported for the synthesis of derhodinosylurdamycin A from our group. It is worth noting that the α-L-olivoside and α-L-olioside moiety in analogues C and D were installed by using our recently developed mild cationic gold-catalyzed glycosylation using *S*-but-3-ynyl thioglycoside donors. Upon NCI-60 Human Tumor Cell Lines Screening by the Development Therapeutics Program of the National Cancer Institute of National Institutes of Health, it was found that none of aquayamycin, analogues A-D, and our previously synthesized derhodinosylurdamycin A bearing diverse 2-deoxy sugar subunits demonstrated significant antiproliferative activity against those cancer cell lines. Interestingly, analogues (**4**) bearing no sugar subunit demonstrates good potential for growth inhibition and cytotoxic activity against most of the 60 human cancer cell lines, except CNS cancer cells, some of the non-small cell lung cancer cells and ovarian cancer cell lines.

Experimental

Chemistry

Methods and materials

Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on either Bruker 600 (^{1}H NMR-600 MHz; ^{13}C NMR 150 MHz) at ambient temperature with $CDCl_3$ or CD_3OD as the solvent unless otherwise stated. Chemical shifts are reported in parts per million relative to residual protic solvent internal standard $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.36), CD_3OD (^{1}H, δ 3.31; ^{13}C δ 49.15). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (app = apparent, par obsc = partially obscure, ovrlp = overlapping, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet) and coupling constants in Hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. High resolution mass spectrometry (HRMS) was performed on a TOF mass spectrometer. Optical rotations were measured with Autopol-IV digital polarimeter; concentrations are expressed as g/100 mL. All reagents and chemicals were purchased from Acros Organics, Sigma Aldrich, Fisher Scientific, Alfa Aesar, and Strem Chemicals and used without further purification. THF, methylene chloride, toluene, and diethyl ether were purified by passing through two packed columns of neutral alumina (Innovative Technology). Anhydrous DMF and benzene were purchased from Acros Organics and Sigma-Aldrich and used without further drying. All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted. Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash column chromatography was performed using 200-400 mesh silica gel (Scientific Absorbents, Inc.). Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated.

Aquayamycin (**3**). To a solution of partially protected β-*C*-arylglycoside **12**[32] (30 mg, 0.034 mmol) in 1.4 mL of mixed solvents (EtOAc/MeOH, 1/1, v/v), 10% palladium on carbon (36.2 mg, 0.034 mmol) was added. After being evacuated and filled with hydrogen five times, the reaction mixture was stirred at room temperature under positive hydrogen pressure (40 psi) for 32 h. The reaction mixture was then diluted with CH_2Cl_2/MeOH (10/1, v/v), filtered through celite, and concentrated to afford crude compound **14** (20.8 mg, quantitative) which was used directly in the next step without purification. A solution of compound **14** (20.8 mg, 0.034 mmol) in 0.74 mL DMF was cooled at 0 °C. To this solution was added Cs_2CO_3 (13.3 mg, 0.041 mmol) followed by addition of 37 μL stock solution of benzyl bromide in DMF (0.051 mmol, 1.5 eq.) (Note: the stock solution was prepared by adding 40 μL of benzyl bromide in 200 μL DMF). The reaction mixture was stirred at room temperature for 5 h before being quenched with a pinch of solid ammonium chloride. DMF was removed by air flow and the residue was purified by using preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to afford 18.9 mg (79% yield) of compound **15**. Next, A solution of **15** (14.6 mg, 0.021 mmol) in 1.3 mL of acetonitrile was cooled at 0 °C and 83 μL of stock solution of cerium ammonium nitrate in water (0.0624 mmol, 3 eq.) was added (Note: the stock solution was prepared by adding 174 mg of cerium ammonium nitrate in 400 μL water). The reaction mixture was stirred at 0 °C for 35 min before being diluted with 4 mL ethyl acetate. 0.5 mL of ice cooled saturated $NaHCO_3$ was added and the resulting mixture was stirred for 2 minutes. The organic layer was separated and passed through a small pad of Na_2SO_4, concentrated under reduce pressure, and kept in vacuum for 10 minutes. This crude material was dissolved in 0.26 mL of mixed solvents (EtOAc/MeOH, 1:1, v/v) and 10% palladium on carbon (4.4 mg, 0.0042 mmol) was added. The mixture was evacuated and filled with hydrogen for three times. After being stirring at room temperature under positive hydrogen pressure for 30 min, the reaction mixture was diluted with methanol, filtered through celite, and concentrated under reduced pressure. The resulting crude compound was dissolved in 0.8 mL of 1,4-dioxane and *N*,*N*-diisopropylethylamine (7.3 μL, 0.042 mmol) was added. After being stirred at 40 °C for 1 h, the reaction mixture was cooled down. Dioxane was removed by air flow and the residue was purified by preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to furnish 6.9 mg of aquayamycin

(**3**) as dark red solid (68% yield for 3 steps). $[\alpha]_D^{23}$ = 119.8° (c = 0.1, CH_3OH); **FT-IR (thin film):** 3367, 2963, 2921, 2852, 1723, 1637, 1563, 1260, 1055, 650 cm^{-1}; **^{1}H NMR** (600 MHz,CD_3OD) δ 7.86 (d, J=7.9 Hz, 1 H), 7.59 (d, J=7.7 Hz, 1 H), 6.87 (d, J=9.7 Hz, 1 H), 6.40 (d, J=9.7 Hz, 1 H), 4.90 (br. s., 1 H), 3.69 (ddd, J=11.2, 8.8, 5.0 Hz, 1 H), 3.42 - 3.46 (m, 1 H), 3.03 (t, J=9.0 Hz, 1 H), 2.82 (d, J=12.8 Hz, 1 H), 2.66 (dd, J=12.9, 2.1 Hz, 1 H), 2.37 - 2.46 (m, 1 H), 2.01 - 2.06 (m, 2 H), 1.37 (d, J=6.2 Hz, 4 H), 1.24 (s, 3 H) ppm; **^{13}C NMR** (150 MHz, CD_3OD) δ 206.94, 190.43, 183.60, 158.92, 145.91, 140.48, 139.89, 139.31, 134.29, 132.24, 120.03, 118.12, 115.44, 82.09, 78.80, 78.64, 77.76, 77.67, 73.57, 72.49, 53.25, 44.73, 41.11, 30.17, 18.61 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{25}H_{26}NaO_{10}$ 509.1424, found 509.1438.

Mesylate (18). To a solution of tetracyclic aryliodide **10**[32](115 mg, 0.162 mmol) in 0.8 mL anhydrous pyridine, methanesulfonyl chloride (19 µL, 0.243 mmol) and 4-dimethylaminopyridine (2 mg, 0.0162 mmol) were added. The resulting mixture was stirred at room temperature for 24 h before pyridine was removed by air flow. The reaction mixture was diluted with CH_2Cl_2, washed sequentially with saturated $CuSO_4$ solution, water, and brine. The organic layer was separated, dried over sodium sulfate, filtered, and concentrated under reduce pressure to produce crude compound **16** which was directly used in the next step. To a solution of **16** in 2 mL of EtOAc/MeOH (4/1, v/v), 10% palladium on carbon (172 mg, 0.162 mmol) was added. After the reaction mixture was evacuated and filled with hydrogen for three times, it was stirred at room temperature under positive hydrogen pressure for 13 days. The reaction mixture was diluted with CH_2Cl_2/MeOH (10:1, v/v), filtered through celite, and concentrated under reduce pressure. The residue was purified by using preparative TLC in CH_2Cl_2/MeOH (15/1, v/v) to afford 48.8 mg (62% yield for 2 steps) of desired compound **17**. To a solution of compound **17** (48.8 mg, 0.101 mmol) in 2.2 mL DMF cooled at 0 °C was added Cs_2CO_3 (40 mg, 0.122 mmol). After the addition of benzyl bromide (18 µL, 0.152 mmol), the resulting mixture was stirred at room temperature for 6 h. The reaction mixture was quenched with a pinch of solid $NaHCO_3$ and DMF was removed by air flow. The residue was purified via preparative TLC (hexanes/ethyl acetate, 1/1, v/v) to give 40.5 mg (70% yield) of the desired mesylate **18**. $[\alpha]_D^{23}$ = -75.0° (c = 0.1, $CHCl_3$); **FT-IR (thin film):** 3445, 2972, 2861, 1720, 1572, 1326, 1167, 1053, 926, 697 cm^{-1}; **^{1}H NMR (600 MHz, CD_3OD)** δ 7.63 - 7.65 (m, 1 H), 7.57 - 7.60 (m, 2 H), 7.39 - 7.44 (m, 3 H), 7.32 - 7.36 (m, 1 H), 7.11 (d, J=7.3 Hz, 1 H), 5.21 - 5.26 (m, 2 H), 5.03 (dd, J=5.8, 1.4 Hz, 1 H), 3.77 (s, 3 H), 3.69 - 3.74 (m, 1 H), 3.68 (s, 3 H), 3.51 - 3.58 (m, 1 H), 3.18 (s, 3 H), 2.76 (d, J=12.7 Hz, 1 H), 2.56 (dd, J=12.7, 2.9 Hz, 1 H), 1.98 (dd, J=14.9, 2.9 Hz, 1 H), 1.88 (d, J=14.7 Hz, 1 H), 1.16 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD_3OD)** δ 207.23, 156.29, 152.24, 151.19, 138.41, 131.73, 129.52, 128.99, 128.90, 127.72, 123.00, 122.56, 116.46, 110.27, 82.08, 79.14, 79.05, 76.07, 72.46, 63.32, 62.05, 51.46, 42.98, 38.31, 31.04, 30.47 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{29}H_{32}NaO_{10}S$ 595.1614, found 595.1637.

Analogue (4). A solution of mesylate **18** (15 mg, 0.026 mmol) in 2.0 mL of CH_3CN/CH_2Cl_2 (5/1, v/v) was cooled to 0 °C. 104 µL of stock solution of cerium ammonium nitrate in water (0.079 mmol, 3 eq.) was added (Note: the stock solution was prepared by adding 174 mg of cerium ammonium nitrate in 400 µL water). The reaction mixture was stirred at 0 °C for 1 h before being diluted with 2 mL ethyl acetate. 0.5 mL of ice cooled saturated $NaHCO_3$ was added and the resulting mixture was stirred for 5 minutes. The organic layer was separated and passed through a small pad of Na_2SO_4, concentrated under reduce pressure, and kept in vacuum for 10 minutes. This crude material was dissolved in 0.34 mL of mixed solvents (EtOAc/MeOH, 1:1, v/v) and 10% palladium on carbon (28 mg, 0.0262 mmol) was added. The mixture was evacuated and filled with hydrogen for three times. After being stirring at room temperature under positive hydrogen pressure for 1.5 h, the reaction mixture was diluted with methanol, filtered through celite, and concentrated under reduced pressure. The residue was purified by preparative TLC in CH_2Cl_2/MeOH (20/1, v/v) to furnish 6.2 mg of analogue A (**4**) as dark red solid (66 % yield for 2 steps). $[\alpha]_D^{23}$ = -48.0° (c = 0.1, CH_3OH); **FT-IR (thin film):** 3369, 2976, 2930, 1725, 1635, 1456, 1086, 1045, 696 cm^{-1}; **^{1}H NMR (600 MHz, CD_3OD)** δ 7.71 (dd, J=8.3, 7.5 Hz, 1 H), 7.58 (dd, J=7.5, 1.1 Hz, 1 H), 7.31 (dd, J=8.4, 0.9 Hz, 1 H), 6.87 (d, J=9.9 Hz, 1 H), 6.41 (d, J=9.7 Hz, 1 H), 2.84 (d, J=12.8 Hz, 1 H), 2.67 (dd, J=12.9, 2.3 Hz, 1 H), 2.03 - 2.05 (m, 2 H), 1.24 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD_3OD)** δ 206.96, 189.47, 183.73, 162.69, 146.35, 140.37, 139.90, 138.00, 133.59, 125.29, 120.07, 118.22, 115.95, 82.10, 78.67, 77.72, 53.24, 44.72, 30.16 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{19}H_{16}NaO_7$ 379.0794, found 379.0796.

Glycal stannane (19). To a flame-dried round-bottomed flask containing potassium *tert*-butoxide (2.4 g, 21.2 mmol) in 15 mL dry THF (dried with *n*-BuLi using 1,10-phenanthroline as an indicator) cooled at -78 °C, was added 22 mL of 1.6 M *n*-BuLi (35.4 mmol). To this mixture was added a solution of 3,4-di-*O*-*tert*-butyldimethylsilyl-D-fucal[35] (4.23 g, 11.8 mmol) in 8.5 mL dry THF and the resulting mixture was stirred at -78 °C for 1 h. 9.5 mL of tri-*n*-butyltin chloride (35.4 mmol) was then added, and the resulting mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic fractions were washed with water and brine, dried over sodium sulfate, and concentrated under reduce pressure. The residue was then passed through a small pad of silica using hexanes as the eluent, and the organic fractions were concentrated to afford crude glycal stannane which was used directly in the next step. To a solution of this crude glycal stannane in 39 mL THF cooled at 0 °C was added 35.4 mL of 1.0 M tetra-*n*-butyl ammonium fluoride (35.4 mmol) and the resulting mixture was stirred at room temperature for 10 h. Saturated aqueous $NaHCO_3$ solution was added and THF was removed under reduce pressure. The aqueous layer was extracted with ethyl acetate and combined organic extracts were washed sequentially with water and brine, dried over sodium sulfate, and concentrated. The crude residue was purified by silica gel flash column chromatography (hexanes/ethyl acetate, 10/1 to 4/1, with 1% Et_3N) to provide 2.13 g (43% yield for 2 steps) of diol. To a solution of this diol (1.13 g, 2.69 mmol) in 2.7 mL DMF were added Et_3N (1.87 mL, 13.5 mmol) and *tert*-butyldimethylsilyl chloride (0.44 g, 2.96 mmol). The resulting mixture was stirred at room temperature for 10 h before being quenched with water. The mixture was extracted with ethyl acetate, and combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated under reduce pressure. The crude residue was purified by silica gel flash chromatography (hexanes/ethyl acetate, 10/1, with 1% Et_3N) to afford 1.33 g (93% yield) of glycal stannane 3-*O*-TBS ether. To a solution of this glycal stannane 3-*O*-TBS ether (1.33 g, 2.49 mmol) in 8.3 mL DMF cooled at 0 °C was added sodium hydride (0.2 g, 4.98 mmol) and the mixture was stirred at 0 °C for 45 minutes. Benzyl bromide (0.36 mL, 2.99 mmol) was then added and the resulting mixture was warmed up to room temperature and stirred for 30 h before being quenched with water. The aqueous mixture was extracted with ethyl acetate and combined organic extracts were washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. Purification on silica gel flash column chromatography (hexanes/dichloromethane = 40/1, with 1% Et_3N) provided 1.1 g (71% yield) of corresponding glycal stannane (**19**). $[\alpha]_D^{23}$ = -60.7° (c = 0.1, $CHCl_3$); **FT-IR (thin film):** 3071, 2953, 2925, 2856, 2674, 2559, 1677, 1288, 1072, 702 cm^{-1}; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.36 - 7.40 (m, 2 H), 7.29 - 7.33 (m, 2 H), 7.23 - 7.26 (m, 1 H), 4.97 (d, J=12.1 Hz, 1 H), 4.66 (dd, J=2.8, 1.3 Hz, 1 H), 4.61 (d, J=11.9 Hz, 1 H), 4.46 - 4.51 (m, 1 H), 3.99 - 4.05 (m, 1 H), 3.51 (dt, J=3.7, 2.0 Hz, 1 H), 1.48 - 1.55 (m, 6 H), 1.31 (dq, J=14.8, 7.4 Hz, 6 H), 1.23 (d, J=6.8 Hz, 3 H), 0.86 - 0.94 (m, 24 H), 0.11 (d, J=4.2 Hz, 6 H) ppm; **^{13}C NMR (150 MHz, $CDCl_3$)**

δ 163.09, 139.66, 128.39, 128.02, 127.50, 113.71, 76.05, 73.34, 73.00, 29.27, 27.55, 26.27, 18.60, 16.79, 14.07, 10.02, -4.02, -4.32 ppm; **ESI-HRMS [M+H]$^+$** Calculated for $C_{31}H_{57}O_3SiSn$ 625.3099, found 625.3113.

2-Deoxy β-C-glycoside (22). Glycal stannane **19** (1.32 g, 2.11 mmol), aryl iodide **10**[32] (0.50 g, 0.70 mmol) and $Pd(PPh_3)_2Cl_2$ (0.1 g, 0.141 mmol) were taken into a flame dried flask, evacuated, and filled with argon. 7 mL of degassed dry toluene was added to the flask and the resulting mixtures was stirred at 100 ˚C for 8 h. The reaction mixture was cooled to room temperature and directly subjected to purification via silica gel flash column chromatography (hexanes/ethyl acetate = 10/1, 2% Et_3N) to affording 452 mg (70% yield) of the C1-arylated glycal **20**. To a solution of C1-arylated glycal **20** (424 mg, 0.463 mmol) in 2.3 mL pyridine were added methanesulfonyl chloride (54 µL, 0.70 mmol) and 4-dimethylaminopyridine (5.6 mg, 0.046 mmol). After the resulting mixture was stirred at room temperature for 36 h, pyridine was removed by air flow. The residue was diluted with CH_2Cl_2, washed sequentially with aqueous saturated $CuSO_4$ solution, water, and brine. The organic solution was dried over sodium sulfate, filtered, and concentrated under reduce pressure to produce the crude mesylate **21** which was used directly in the next step. The mesylate **21** was dissolved in 8.8 mL ethanol and a pinch of bromocresol green was added as an indicator. To the mixture was added sodium cyanoborohydride (0.056 g, 0.89 mmol) followed by addition of 0.5 M HCl in methanol (3.6 mL, 1.8 mmol). The resulting reaction mixture was stirred at room temperature for 15 minutes. A second batch of sodium cyanoborohydride (0.056 g, 0.886 mmol) and 0.5 M HCl in methanol (3.6 mL, 1.772 mmol) were added, and the reaction mixture was stirred at room temperature for 30 h before being quenched with saturated $NaHCO_3$ solution. The aqueous mixture was extracted with ethyl acetate and combined organic extracts were washed with water, dried over sodium sulfate, filtered, and concentrated under reduce pressure. Purification on silica gel flash column chromatography (toluene/ethyl acetate = 20:1 to 5:1) furnished 310 mg (70% yield for 2 steps) of 2-deoxy β-*C*-glycoside (**22**). $[\alpha]_D^{23}$ = -26.0˚ (*c* = 0.1, $CHCl_3$); **FT-IR (thin film):** 3407, 2934, 2883, 1682, 1453, 1326, 1027, 698 cm^{-1}; **^{1}H NMR (600 MHz, MHz, $CDCl_3$)** δ 7.88 (d, *J*=8.8 Hz, 1 H), 7.74 (d, *J*=8.8 Hz, 1 H), 7.31 - 7.50 (m, 15 H), 5.04 - 5.09 (m, 2 H), 5.02 (s, 1 H), 4.81 - 4.92 (m, 3 H), 4.68 - 4.72 (m, 2 H), 4.41 (d, *J*=9.5 Hz, 1 H), 4.34 (s, 1 H), 3.96 (d, *J*=19.6 Hz, 1 H), 3.84 (s, 3 H), 3.80 (d, *J*=3.5 Hz, 1 H), 3.72 (s, 3 H), 3.57 (d, *J*=6.6 Hz, 1 H), 3.54 (d, *J*=3.1 Hz, 1 H), 3.38 (dd, *J*=19.8, 5.9 Hz, 1 H), 3.18 (dd, *J*=13.4, 2.9 Hz, 1 H), 3.13 (s, 3 H), 2.75 (d, *J*=13.4 Hz, 1 H), 2.15 (dd, *J*=14.9, 2.9 Hz, 1 H), 1.90 - 2.00 (m, 2 H), 1.81 - 1.88 (m, 2 H), 1.38 (d, *J*=6.4 Hz, 3 H), 1.32 (s, 3 H) ppm; **^{13}C NMR (150 MHz, $CDCl_3$)** δ 205.48, 150.72, 150.59, 150.52, 138.77, 137.86, 137.06, 134.04, 130.29, 129.03, 128.96, 128.88, 128.76, 128.73, 128.57, 128.41, 128.33, 128.18, 127.27, 125.56, 123.86, 121.72, 119.87, 81.52, 80.98, 79.55, 78.72, 78.48, 76.30, 75.40, 72.57, 70.78, 65.02, 63.55, 61.76, 44.69, 43.38, 38.87, 37.29, 30.39, 25.74, 18.34 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{49}H_{54}NaO_{13}S$ 905.3183, found 905.3214.

Mesylate (23). To a solution of 2-deoxy β-*C*-glycoside **22** (50 mg, 0.057 mmol) in 1.6 mL of mixed solvents (EtOAc/MeOH, 1/1, v/v) was added 10% palladium on carbon (60 mg, 0.057 mmol). The reaction mixture was evacuated and filled with hydrogen for five times and then stirred at room temperature under positive hydrogen pressure (40 psi) for 50 h. The reaction mixture was then diluted with CH_2Cl_2/MeOH (10/1, v/v), filtered through celite, and concentrated. The residue was purified via preparative TLC (CH_2Cl_2/MeOH, 10/1, v/v) to afford 31 mg (89% yield) of desired product. To a solution of this product (29.1 mg, 0.0476 mmol) in 1 mL DMF cooled at 0˚C were added Cs_2CO_3 (18.6 mg, 0.0571 mmol) and benzyl bromide (8.5 µL, 0.071 mmol). The reaction mixture was stirred at room temperature for 5 h and then quenched with a pinch of solid ammonium chloride. DMF was removed by air flow and the residue was purified by using preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to furnish 21.7 mg (65% yield) of the desired mesylate (**23**). $[\alpha]_D^{23}$ = -100.0˚ (*c* = 0.1, $CHCl_3$); **FT-IR (thin film):** 3407, 2934, 2978, 1716, 1331, 1166, 1041, 905, 530 cm^{-1}; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.85 (d, *J*=8.8 Hz, 1 H), 7.67 (d, *J*=8.8 Hz, 1 H), 7.38 - 7.48 (m, 5 H), 5.23 (s, 1 H), 5.08 - 5.14 (m, 2 H), 4.78 - 4.84 (m, 2 H), 4.13 - 4.18 (m, 2 H), 3.97 (d, *J*=19.4 Hz, 1 H), 3.77 - 3.83 (m, 1 H), 3.74 - 3.76 (m, 6 H), 3.63 - 3.67 (m, 1 H), 3.56 (d, *J*=6.8 Hz, 1 H), 3.39 (dd, *J*=19.8, 5.9 Hz, 1 H), 3.16 (s, 3 H), 2.77 - 2.83 (m, 2 H), 2.29 (d, *J*=7.9 Hz, 1 H), 2.09 - 2.14 (m, 1 H), 2.00 - 2.06 (m, 2 H), 1.83 (dd, *J*=14.7, 2.0 Hz, 1 H), 1.71 (q, *J*=12.7 Hz, 1 H), 1.34 (d, *J*=6.4 Hz, 3 H), 1.23 (s, 3 H) ppm; **^{13}C NMR (150 MHz, $CDCl_3$)** δ 206.11, 150.70, 150.57, 150.52, 137.75, 133.89, 130.31, 128.97, 128.62, 128.53, 126.66, 125.23, 123.93, 121.42, 119.98, 80.01, 78.53, 77.72, 77.08, 75.06, 74.83, 72.91, 71.20, 70.37, 63.32, 61.84, 50.44, 41.85, 39.07, 36.09, 30.70, 29.85, 17.66 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{35}H_{42}NaO_{13}S$ 725.2244, found 725.2236.

Analogue (5). A solution of mesylate **23** (17 mg, 0.024 mmol) in 1.5 mL of acetonitrile was cooled at 0 ˚C. 96 µL of stock solution of cerium ammonium nitrate in water (0.073 mmol, 3 eq.) was added (Note: the stock solution was prepared by adding 174 mg of cerium ammonium nitrate in 400 µL water). The reaction mixture was stirred at 0 ˚C for 35 min before being diluted with 2 mL ethyl acetate. 0.5 mL of ice cooled saturated $NaHCO_3$ was added and the resulting mixture was stirred for 5 minutes. The organic layer was separated and passed through a small pad of Na_2SO_4, concentrated under reduce pressure, and kept in vacuum for 10 minutes. This crude material was dissolved in 0.3 mL of mixed solvents (EtOAc/MeOH, 1:1, v/v) and 10% palladium on carbon (5.1 mg, 0.0048 mmol) was added. The mixture was evacuated and filled with hydrogen for three times. After being stirring at room temperature under positive hydrogen pressure for 1 h, the reaction mixture was diluted with methanol, filtered through celite, and concentrated under reduced pressure. This residue was dissolved in 0.93 mL of dioxane and *N,N*-diisopropylethylamine (8.5 µL, 0.048 mmol) was added. After being stirred at 40 ˚C for 1 h, the reaction mixture was cooled down. Dioxane was removed by air flow and the crude material was purified by preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to furnish 8.1 mg of analogue B (**5**) as dark red solid (69% yield for 3 steps). $[\alpha]_D^{23}$ = 42.3˚ (*c* = 0.1, CH_3OH); **FT-IR (thin film):** 3384, 2961, 2923, 2853, 1725, 1637, 1284, 1259, 1080, 652 cm^{-1}; **^{1}H NMR (600 MHz, CD_3OD)** δ 7.99 (d, *J*=7.7 Hz, 1 H), 7.59 (d, *J*=7.9 Hz, 1 H), 6.87 (d, *J*=9.7 Hz, 1 H), 6.40 (d, *J*=9.7 Hz, 1 H), 4.84 - 4.87 (m, 1 H), 3.86 - 3.90 (m, 1 H), 3.70 - 3.75 (m, 1 H), 3.61 (d, *J*=2.8 Hz, 1 H), 2.82 (d, *J*=12.8 Hz, 1 H), 2.66 (dd, *J*=13.0, 2.4 Hz, 1 H), 2.06 - 2.10 (m, 1 H), 2.02 - 2.05 (m, 2 H), 1.61 (q, *J*=11.9 Hz, 1 H), 1.33 (d, *J*=6.4 Hz, 3 H), 1.24 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD_3OD)** δ 206.94, 189.88, 183.63, 158.77, 146.24, 140.48, 139.88, 139.67, 134.93, 132.16, 120.02, 118.22, 115.36, 82.10, 78.65, 77.71, 76.11, 72.76, 71.84, 71.10, 53.26, 44.73, 35.35, 30.17, 17.71 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{25}H_{26}NaO_{10}$ 509.1424, found 509.1424.

S-But-3-ynyl 3,4-di-O-benzyl-L-olivoside donor (24). To *S*-but-3-ynyl 3,4-di-*O*-acetyl-L-olivoside[37] (150 mg, 0.50 mmol) was added 1.1 mL of 7.0 N NH_3 in MeOH and the resulting mixture was stirred at room temperature for 6 h. Solvent was removed under reduced pressure and the residue was azeotroped with toluene to produce crude diol. This diol was dissolved in 1.7 mL DMF and cooled at 0˚C. NaH (60% in mineral oil, 60 mg, 1.5 mmol) was added and the reaction mixture was stirred for 45 min at 0˚C. Next, benzyl bromide (0.15 mL, 1.25 mmol) was added and the resulting mixture was stirred for 12 h at room temperature before being quenched with water. The aqueous mixture was extracted with ethyl acetate and combined organic extracts were washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. Purification on flash column chromatography (hexanes/ethyl acetate, 10/1, v/v) provided 189 mg of corresponding *S*-but-3-ynyl 3,4-di-*O*-benzyl-L-olivoside donor (**24**) (96% yield for 2 steps). $[\alpha]_D^{23}$ = -82.3° (*c* = 0.3, $CHCl_3$); **FT-IR (thin film):** 3289, 3029, 2929, 2858, 1496, 1453, 1091, 734, 697 cm^{-1}; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.29 - 7.40 (ovrlp, 10 H, α and β), 5.41 (d, *J*=5.5 Hz, 1 H, H^1 α), 4.98 (ovrlp, 1 H, α and β), 4.57 - 4.73 (ovrlp, 3 H, α and β and 1H, H^3 β), 4.13 (dq, *J*=9.4, 6.2 Hz, 1 H, H^5 α), 3.91 (ddd, *J*=11.6, 8.6, 4.8 Hz, 1 H, H^3 α), 3.66 (ddd, *J*=11.2, 8.6, 5.1 Hz, 1 H, H^1 β), 3.39 (dq, *J*=9.3, 6.1 Hz, 1 H, H^5 β), 3.17 (ovrlp, 1 H, H^4 α and H^4 β), 2.91 - 2.97 (m, 1 H, β), 2.77 - 2.86 (m, 2 H, α), 2.67 - 2.73 (m, 1 H, β), 2.47 - 2.64 (ovrlp, 2 H, α and β), 2.41 (ddd, *J*=12.7, 5.1, 1.7 Hz, 1 H, H^2 β), 2.31 - 2.36 (m, 1 H, H^2 α), 2.02 - 2.09 (ovrlp, 1 H, α and β and 1 H, H^2 α), 1.71 - 1.79 (q, 1 H, H^2 β), 1.37 (d, *J*=6.1 Hz, 3 H, $C^6 – CH_3$ β), 1.33 (d, *J*=6.2 Hz, 3 H, $C^6 – CH_3$ α) ppm; **^{13}C NMR (150 MHz, $CDCl_3$)** δ 138.72, 138.64, 138.60, 138.47, 128.69, 128.66, 128.64, 128.61, 128.33, 128.21, 128.00, 127.95, 127.94, 127.90, 84.61, 83.65, 82.86, 80.73, 80.56, 79.88, 75.90, 75.61, 75.41, 72.04, 71.72, 69.75, 69.72, 67.93, 37.38, 36.30, 30.13, 29.96, 20.69, 20.16, 18.66, 18.31 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{24}H_{28}NaO_3S$ 419.1657, found 419.1667.

Mesylate (26). A mixture of *S*-but-3-ynyl 3,4-di-*O*-benzyl-L-olivoside donor **24** (33.6 mg, 0.085 mmol) and partially protected β-*C*-arylglycoside acceptor **12**[32] (50 mg, 0.057 mmol) was azeotroped with 2 mL benzene and kept in high vacuum for 30 minutes. To this mixture were sequentially added 23 mg of freshly activated 4 Å molecular sieves, silver triflate (1.5 mg, 0.0057 mmol) and a freshly prepared solution of gold catalyst in dry CH_2Cl_2 (prepared by dissolving 2.0 mg (0.0028 mmol) of chloro[tris(*para*-trifluoromethylphenyl)phosphine]gold(I) in 0.56 mL CH_2Cl_2). The resulting mixture was stirred at room temperature for 1 h before being quenched with a pinch of solid $NaHCO_3$. The reaction mixture was diluted with CH_2Cl_2, filtered through small pad of Na_2SO_4, concentrated under vacuo, and purified using preparative TLC (hexanes/ethyl acetate, 2/1, v/v, with 1% MeOH) to afford 61.5 mg (91% yield) of desired disaccharide as a mixture of inseparable anomers (α/β, 2/1). To a mixture of these anomers (50 mg, 0.042 mmol) dissolved in 1.6 mL of EtOAc/MeOH (1/1, v/v) was added 10% palladium on carbon (45 mg, 0.042 mmol). The resulting mixture was evacuated and filled with hydrogen for five times and stirred at room temperature under positive hydrogen pressure (40 psi) for 40 h. The reaction mixture was diluted with CH_2Cl_2/MeOH (10/1, v/v), filtered through celite, concentrated, and purified via preparative TLC (CH_2Cl_2/MeOH, 10/1, v/v) to give 11.2 mg (36% yield) of desired α-disaccharide. To a solution of this α-disaccharide (15.5 mg, 0.021 mmol) in 0.45 mL DMF cooled at 0 °C was added Cs_2CO_3 (8.2 mg, 0.025 mmol) followed by addition of 22 μL stock solution of benzyl bromide in DMF (0.031 mmol, 1.5 eq.) (Note: the stock solution was prepared by adding 40 μL of benzyl bromide in 200 μL DMF). The reaction mixture was stirred at room temperature for 7 h and quenched with a pinch of solid ammonium chloride. DMF was removed by air flow and the residue was purified by using preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to furnish 11.1 mg (63% yield) of the desired mesylate (**26**). $[\alpha]_D^{23}$ = -69.3° (*c* = 0.1, $CHCl_3$); **FT-IR (thin film):** 3391, 2923, 2856, 1721, 1330, 1041, 973, 908, 529 cm^{-1}; **^{1}H NMR (600 MHz, CD_3OD)** δ 7.88 (d, *J*=8.8 Hz, 1 H), 7.62 (d, *J*=8.8 Hz, 1 H), 7.47 - 7.50 (m, 2 H), 7.41 - 7.45 (m, 2 H), 7.35 - 7.39 (m, 1 H), 5.04 - 5.08 (m, 2 H), 4.99 (d, *J*=3.1 Hz, 1 H), 4.89 - 4.92 (m, 2 H), 3.90 - 3.95 (m, 1 H), 3.82 - 3.87 (m, 2 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 3.56 - 3.66 (m, 2 H), 3.27 - 3.30 (m, 1 H), 3.18 - 3.21 (m, 3 H), 3.13 (t, *J*=9.0 Hz, 1 H), 2.97 (t, *J*=9.2 Hz, 1 H), 2.80 (d, *J*=12.7 Hz, 1 H), 2.59 (dd, *J*=12.7, 2.8 Hz, 1 H), 2.22 (dd, *J*=12.6, 3.6 Hz, 1 H), 1.93 - 2.04 (m, 3 H), 1.55 - 1.65 (m, 2 H), 1.31 (d, *J*=6.1 Hz, 3 H), 1.28 (d, *J*=6.2 Hz, 3 H), 1.20 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD_3OD)** δ 207.17, 151.68, 151.52, 151.37, 138.92, 134.78, 131.26, 129.72, 129.67, 129.66, 129.25, 128.92, 126.15, 124.59, 123.52, 120.45, 95.16, 82.07, 79.25, 79.17, 79.08, 79.03, 78.08, 77.89, 76.66, 76.10, 73.10, 69.44, 69.34, 63.60, 62.05, 51.52, 43.04, 39.39, 38.31, 37.98, 30.97, 30.49, 18.81, 18.24 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{41}H_{52}NaO_{16}S$ 855.2874, found 855.2869.

Analogue (6). To a solution of mesylate **26** (10 mg, 0.012 mmol) in 0.76 mL acetonitrile cooled at 0 °C was added 48 μL of stock solution of cerium ammonium nitrate in water (0.036 mmol, 3 eq.) was added (Note: the stock solution was prepared by adding 174 mg of cerium ammonium nitrate in 400 μL water). The reaction mixture was stirred at 0 °C for 30 min before being diluted with 2 mL ethyl acetate. 0.5 mL of ice cooled saturated $NaHCO_3$ was added and the resulting mixture was stirred for 5 minutes. The organic layer was separated and passed through a small pad of Na_2SO_4, concentrated under reduce pressure, and kept in vacuum for 10 minutes. This crude material was dissolved in 0.15 mL of mixed solvents (EtOAc/MeOH, 1:1, v/v) and 10% palladium on carbon (2.6 mg, 0.0024 mmol) was added. The mixture was evacuated and filled with hydrogen for three times. After being stirring at room temperature under positive hydrogen pressure for 1 h, the reaction mixture was diluted with methanol, filtered through celite, and concentrated under reduced pressure. The residue was purified through preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to afford 6.2 mg of analogue C (**6**) as dark red solid (84% yield for 2 steps). $[\alpha]_D^{23}$ = 99.3° (*c* = 0.1, CH_3OH); **FT-IR (thin film):** 3380, 2958, 2921, 2854, 1725, 1636, 1260, 1055, 476 cm^{-1}; **^{1}H NMR (600 MHz, CD_3OD)** δ 7.86 (d, *J*=7.9 Hz, 1 H), 7.58 (d, *J*=7.9 Hz, 1 H), 6.87 (d, *J*=9.7 Hz, 1 H), 6.40 (d, *J*=9.7 Hz, 1 H), 5.04 (d, *J*=3.1 Hz, 1 H), 4.85 - 4.87 (m, 1 H), 3.91 - 3.97 (m, 1 H), 3.85 (ddd, *J*=11.6, 9.0, 5.0 Hz, 1 H), 3.73 - 3.79 (m, 1 H), 3.45 - 3.51 (m, 1 H), 3.11 - 3.18 (m, 1 H), 2.96 (t, *J*=9.3 Hz, 1 H), 2.82 (d, *J*=12.8 Hz, 1 H), 2.67 (dd, *J*=13.0, 2.6 Hz, 1 H), 2.56 (ddd, *J*=12.7, 4.8, 1.7 Hz, 1 H), 1.95 - 2.08 (m, 3 H), 1.61 - 1.67 (m, 1 H), 1.38 (d, *J*=6.1 Hz, 3 H), 1.27 (d, *J*=6.1 Hz, 3 H), 1.24 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD_3OD)** δ 206.91, 189.82, 183.58, 158.75, 146.29, 140.46, 139.89, 139.13, 134.37, 132.26, 120.02, 118.19, 115.43, 95.33, 82.09, 79.00, 78.64, 77.83, 77.79, 77.68, 76.65, 72.32, 69.42, 69.39, 53.26, 44.72, 39.40, 37.55, 30.76, 30.17, 18.79, 18.16 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{31}H_{36}NaO_{13}$ 639.2054, found 639.2084.

S-but-3-ynyl 3,4-di-O-benzyl-L-olioside donor (25). To *S*-but-3-ynyl 3,4-di-*O*-acetyl-L-olioside[37] (120 mg, 0.40 mmol) was added 0.86 mL of 7.0 N NH_3 in MeOH and the resulting mixture was stirred at room temperature for 12 h. Solvent was removed under reduced pressure and the residue was azeotroped with toluene to produce crude diol. This diol was dissolved in 1.3 mL DMF and cooled at 0 °C. NaH (60% in mineral oil, 48 mg, 1.2 mmol) was added and the reaction mixture was stirred for 45 min at 0 °C. Next, benzyl bromide (0.12 mL, 1.0 mmol) was added and the resulting mixture was stirred for 12 h at room temperature before being quenched with water. The aqueous mixture was extracted with ethyl acetate and combined organic extracts were washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. Purification on flash column chromatography (hexanes/ethyl acetate, 10/1, v/v) provided 139 mg of corresponding *S*-but-3-ynyl 3,4-di-*O*-benzyl-L-olioside donor (**25**) (88% yield for 2 steps). $[\alpha]_D^{22}$ = -76.5° (*c* = 0.3, $CHCl_3$); **FT-IR (thin film):** 3291, 3030, 2931, 1496, 1454, 1362, 1059, 733, 697 cm^{-1}; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.29 - 7.45 (ovrlp, 10 H, α and β), 5.55 (d, *J*=5.7 Hz, 1 H, H^1 α), 4.97 - 5.04 (ovrlp, 1 H, α and β), 4.70 - 4.77 (ovrlp, 1 H, α and β), 4.54 - 4.69 (ovrlp, 2 H, α and β and 1H, H^1 β), 4.17 (q, *J*=6.5 Hz, 1 H, H^5 α), 3.88 (ddd, *J*=12.2, 4.4, 2.5 Hz, 1 H, H^3 α), 3.64 (s, 1 H, H^4 α), 3.60 (ddd, *J*=11.6, 4.6, 2.6 Hz, 1 H, H^3 β), 3.55 - 3.58 (m, 1 H, H^4 β), 3.41 - 3.48 (m, 1 H, H^5 β), 2.96 (ddd, *J*=13.3, 8.5, 6.6 Hz, 1 H, β), 2.77 - 2.86 (ovrlp, 1 H, α and β), 2.66 - 2.74 (m, 1 H, α), 2.50 - 2.62 (ovrlp, 2 H, α and β and 1 H, H^2 α), 2.21 (q, *J*=11.8 Hz, 1 H, H^2 β), 2.07 - 2.14 (m, 1 H, H^2 β), 2.04 - 2.06 (ovrlp, 1 H, α and β), 2.01 - 2.04 (m, 1 H, H^2 α), 1.25 (d, *J*=6.4 Hz, 3 H, $C^6 – CH_3$ β), 1.22 (d, *J*=6.4 Hz, 3 H, $C^6 – CH_3$ α) ppm; **^{13}C NMR (150 MHz,**

CDCl$_3$) δ 138.86, 138.77, 138.38, 128.52, 128.49, 128.43, 128.42, 128.39, 128.35, 128.24, 128.21, 128.16, 127.70, 127.66, 127.59, 127.47, 127.36, 127.31, 82.95, 82.77, 81.15, 80.13, 78.87, 75.91, 75.01, 74.47, 74.46, 74.25, 70.48 , 70.19, 69.37, 69.27, 67.20, 32.05, 30.93, 29.95, 29.44, 20.42, 19.93, 17.65, 17.21 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{24}H_{28}NaO_3S$ 419.1657, found 419.1655.

Mesylate (27). A mixture of *S*-but-3-ynyl 3,4-di-*O*-benzyl-L-olioside donor **25** (33.6 mg, 0.085 mmol) and partially protected β-*C*-arylglycoside acceptor **12**[32] (50 mg, 0.057 mmol) was azeotroped with 2 mL benzene and kept in high vacuum for 30 minutes. To this mixture were sequentially added 23 mg of freshly activated 4 Å molecular sieves, silver triflate (1.5 mg, 0.0057 mmol) and a freshly prepared solution of gold catalyst in dry CH_2Cl_2 (prepared by dissolving 2.0 mg (0.0028 mmol) of chloro[tris(*para*-trifluoromethylphenyl)phosphine]gold(I) in 0.56 mL CH_2Cl_2). The resulting mixture was stirred at room temperature for 1.5 h before being quenched with a pinch of solid $NaHCO_3$. The reaction mixture was diluted with CH_2Cl_2, filtered through small pad of Na_2SO_4, concentrated under vacuo, and purified using preparative TLC (hexanes/ethyl acetate, 2/1, v/v, with 1% MeOH) to afford 56.6 mg (84% yield) of desired disaccharide as a mixture of inseparable anomers (α/β, 2/1). To a mixture of these anomers (50 mg, 0.042 mmol) dissolved in 1.6 mL of EtOAc/MeOH (1/1, v/v) was added 10% palladium on carbon (45 mg, 0.042 mmol). The resulting mixture was evacuated and filled with hydrogen for five times and stirred at room temperature under positive hydrogen pressure (40 psi) for 66 h. The reaction mixture was diluted with CH_2Cl_2/MeOH (10/1, v/v), filtered through celite, concentrated, and purified via preparative TLC (CH_2Cl_2/MeOH, 10/1, v/v) to give 17.4 mg (56% yield) of desired α-disaccharide. To a solution of this α-disaccharide (17.3 mg, 0.023 mmol) in 0.5 mL DMF cooled at 0 °C was added Cs_2CO_3 (9.1 mg, 0.028 mmol) followed by addition of 25 μL stock solution of benzyl bromide in DMF (0.031 mmol, 1.5 eq.) (Note: the stock solution was prepared by adding 40 μL of benzyl bromide in 200 μL DMF). The reaction mixture was stirred at room temperature for 5 h and quenched with a pinch of solid ammonium chloride. DMF was removed by air flow and the residue was purified by using preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to furnish 11 mg (57% yield) of the desired mesylate (**27**) (α only). $[\alpha]_D^{23}$ = -89.7° (*c* = 0.1, $CHCl_3$); **FT-IR (thin film):** 3393, 2976, 2938, 1718, 1329, 1167, 1040, 699, 639, 529 cm^{-1}; **^{1}H NMR (600 MHz, CD$_3$OD)** δ 7.87 (d, *J*=9.0 Hz, 1 H), 7.61 (d, *J*=8.8 Hz, 1 H), 7.47 (d, *J*=7.2 Hz, 2 H), 7.40 - 7.45 (m, 2 H), 7.37 (d, *J*=7.3 Hz, 1 H), 5.05 (dd, *J*=16.0, 4.2 Hz, 3 H), 4.90 (d, *J*=4.6 Hz, 2 H), 4.20 (d, *J*=6.8 Hz, 1 H), 4.04 (dd, *J*=12.1, 1.8 Hz, 1 H), 3.84 (d, *J*=19.1 Hz, 1 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 3.63 - 3.66 (m, 1 H), 3.55 - 3.61 (m, 2 H), 3.27 - 3.30 (m, 1 H), 3.19 (s, 3 H), 3.10 - 3.15 (m, 1 H), 2.79 (d, *J*=12.7 Hz, 1 H), 2.59 (dd, *J*=12.7, 2.8 Hz, 1 H), 2.23 (dd, *J*=12.38, 3.6 Hz, 1 H), 2.00 - 2.04 (m, 1 H), 1.92 - 1.96 (m, 1 H), 1.91 (d, *J*=3.7 Hz, 1 H), 1.67 (dd, *J*=12.7, 5.0 Hz, 1 H), 1.57 (q, *J*=12.5 Hz, 1 H), 1.30 (d, *J*=6.1 Hz, 3 H), 1.25 (d, *J*=6.6 Hz, 3 H), 1.18 (s, 3 H) ppm; **^{13}C NMR (150 MHz, CD$_3$OD)** δ 207.15, 151.65, 151.50, 151.35, 138.87, 134.77, 131.22, 129.66, 129.64, 129.25, 128.90 , 126.16 , 124.58 , 123.51 , 120.44 , 95.34 , 82.04 , 79.25, 79.13 , 79.06 , 77.84 , 77.77, 76.69, 76.08 , 73.06, 72.40 , 71.52 , 67.67, 66.70 , 63.60 , 62.07 , 51.50 , 43.01, 38.32 , 37.94, 33.47, 30.96 , 30.49, 18.82, 17.26 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{41}H_{52}NaO_{16}S$ 855.2874, found 855.2867.

Analogue (7). To a solution of mesylate **27** (15.2 mg, 0.0183 mmol) in 1.2 mL acetonitrile cooled at 0 °C was added 73 μL of stock solution of cerium ammonium nitrate in water (0.055 mmol, 3 eq.) was added (Note: the stock solution was prepared by adding 174 mg of cerium ammonium nitrate in 400 μL water). The reaction mixture was stirred at 0 °C for 30 min before being diluted with 2 mL ethyl acetate. 0.5 mL of ice cooled saturated $NaHCO_3$ was added and the resulting mixture was stirred for 5 minutes. The organic layer was separated and passed through a small pad of Na_2SO_4, concentrated under reduce pressure, and kept in vacuum for 10 minutes. This crude material was dissolved in 0.23 mL of mixed solvents (EtOAc/MeOH, 1:1, v/v) and 10% palladium on carbon (3.9 mg, 0.0037 mmol) was added. The mixture was evacuated and filled with hydrogen for three times. After being stirring at room temperature under positive hydrogen pressure for 1 h, the reaction mixture was diluted with methanol, filtered through celite, and concentrated under reduced pressure. The residue was purified through preparative TLC in CH_2Cl_2/MeOH (10/1, v/v) to afford 8.4 mg of analogue D (**7**) as dark red solid (75% yield for 2 steps). $[\alpha]_D^{23}$ = 41.3° (*c* = 0.1, CH_3OH); **FT-IR (thin film):** 3389, 2974, 2927, 1723, 1637, 1436, 1284, 1082, 1056, 598 cm^{-1}; **^{1}H NMR (600 MHz, CD$_3$OD)** δ 7.85 (d, *J*=7.7 Hz, 1 H), 7.57 (d, *J*=7.9 Hz, 1 H), 6.87 (d, *J*=9.9 Hz, 1 H), 6.40 (d, *J*=9.7 Hz, 1 H), 5.07 (d, *J*=3.3 Hz, 1 H), 4.85 (s, 1 H), 4.21 (q, *J*=6.5 Hz, 1 H), 4.01 - 4.05 (m, 1 H), 3.72 - 3.79 (m, 1 H), 3.55 - 3.57 (m, 1 H), 3.45 - 3.51 (m, 1 H), 3.10 - 3.16 (m, 1 H), 2.80 - 2.88 (m, 1 H), 2.67 (dd, *J*=12.9, 2.7 Hz, 1 H), 2.55 (ddd, *J*=12.7, 4.8, 1.7 Hz, 1 H), 2.00 - 2.08 (m, 2 H), 1.93 (td, *J*=12.5, 3.9 Hz, 1 H), 1.68 (dd, *J*=12.7, 5 Hz, 1 H), 1.38 (d, *J*=6.1 Hz, 3 H), 1.23 - 1.25 (m, 7 H) ppm; **^{13}C NMR (150 MHz, CD$_3$OD)** δ 206.91, 189.82, 183.57, 158.74, 146.29, 140.45, 139.88, 139.14, 134.35, 132.23, 120.03, 118.19, 115.41, 95.50, 82.08, 78.62, 77.76, 77.69, 77.50, 76.70, 72.43, 72.32, 67.75, 66.70, 53.26, 44.71, 37.49, 33.48, 30.18, 18.81, 17.18 ppm; **ESI-HRMS [M+Na]$^+$** calculated for $C_{31}H_{36}NaO_{13}$ 639.2054, found 639.2068.

Biological studies

Synthetic aquayamycin (**3**), analogues (**4**-**7**) and previously prepared derhodinosylurdamycin A (**2**) were submitted to the Development Therapeutics Program of the National Cancer Institute of National Institutes of Health for the NCI-60 Human Tumor Cell Lines Screening using standard protocols. These known 60-cell panel contains a variety of human cell lines of leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer. Detailed data are provided in the Supporting Information.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

We are very grateful to The University of Toledo for supporting this research. This work is also supported in part by a grant from National Science Foundation (CHE-1213352). We thank the Development Therapeutics Program of the National Cancer Institute of National Institutes of Health for evaluating our synthetic molecules for their anticancer activity using the NCI-60 Human Tumor Cell Lines Screen. We also thank Ms. Myoung Soo Choi, Mr. Hee Bum Lee, and Mr. Christopher Lopez for experimental assistance.

Notes and references

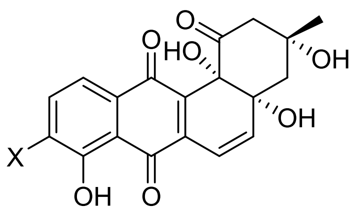
1 J. Rohr and R. Thiericke, *Nat. Prod. Rep.*, 1992, **9**, 103-137.
2 K. Krohn and J. Rohr, *Top. Curr. Chem.*, 1997, **188**, 127-195.
3 M. K. Kharel, P. Pahari, M. D. Shepherd, N. Tibrewal, S. E. Nybo, K. A. Shaaban and J. Rohr, *Nat. Prod. Rep.*, 2012, **29**, 264-325.
4 S. I. Elshahawi, K. A. Shaaban, M. K. Kharel and J. S. Thorson, *Chem. Soc. Rev.*, 2015, **44,** 7591-7697.

5 H. Drautz, H. Zähner, J. Rohr and A. Zeeck, *J. Antibiot.*, 1986, **39**, 1657-1669.
6 J. Rohr and A. Zeeck, *J. Antibiot.*, 1987, **40**, 459-467.
7 J. Rohr, *J. Antibiot.*, 1989, **42**, 1482-1488.
8 E. Kuenzel, B. Faust, C. Oelkers, U. Weissbach, D. W. Bearden, G. Weitnauer, L. Westrich, A. Bechthold and J. Rohr, *J. Am. Chem. Soc.*, 1999, **121**, 11058-11062.
9 U. Rix, L. L. Remsing, D. Hoffmeister, A. Bechthold and J. Rohr, *ChemBioChem*, 2003, **4**, 109-111.
10 M. Sezaki, S. Kondo, K. Maeda, H. Umezawa and M. Ohno, *Tetrahedron*, 1970, **26**, 5171-5190.
11 T. Uchida, M. Imoto, Y. Watanabe, K. Miura, T. Dobashi, N. Matsuda, T. Sawa, H. Naganawa, M. Hamada and T. Takeuchi, *J. Antibiot.*, 1985, **38**, 1171-1181.
12 S. Omura, H. Tanaka, R. Ōiwa, J. Awaya, R. Masuma and K. Tanaka, *J. Antibiot.*, 1977, **30**, 908-916.
13 A. Kawashima, Y. Kishimura, M. Tamai and K. Hanada, *Chem. Pharm. Bull.*, 1989, **37**, 3429-3431.
14 T. Henkel, T. Ciesiolka, J. Rohr and A. Zeeck *J. Antibiot.*, 1989, **42**, 299-311.
15 A. Trefzer, D. Hoffmeister, E. Künzel, S. Stockert, G. Weitnauer, L. Westrich, U. Rix, J. Fuchser, K. Bindseil, J. Rohr and A. Bechthold, *Chem. Biol.*, 2000, **7**, 133-142.
16 Y. Hayakawa, K. Furihata, H. Seto and N. Ōtake, *Tetrahedron Lett.*, 1985, **26**, 3475-3478.
17 M. Sezaki, T. Hara, S. Ayukawa, T. Takeuchi, Y. Okami, M. Hamada, T. Nagatsu and H. Umezawa, *J. Antibiot.*, 1968, **21**, 91-97.
18 K. Krohn, P. Frese and U. Florke, *Chem. Eur. J.*, 2000, **6**, 3887-3896.
19 M. Yamaguchi, T. Okuma, A. Horiguchi, C. Ikeura and T. Minami, *J. Org. Chem.*, 1992, **57**, 1647-1649.
20 V. A. Boyd and G. A. Sulikowski, *J. Am. Chem. Soc.*, 1995, **117**, 8472-8473.
21 K. Kim, V. A. Boyd, A. Sobti and G. A. Sulikowski, *Isr. J. Chem.*, 1997, **37**, 3-22.
22 G. Matsuo, Y. Miki, M. Nakata, S. Matsumura and K. Toshima, *Chem. Comm.*, 1996, 225-226.
23 G. Matsuo, Y. Miki, M. Nakata, S. Matsumura and K. Toshima, *J. Org. Chem.*, 1999, **64**, 7101-7106.
24 K. Krohn, A. Agocs and C. Baeuerlein, *J. Carbohydr. Chem.*, 2003, **22**, 579-592.
25 A. Kirschning, G. Chen, G. Dräger, I. Schuberth and L. F. Tietze, *Bioorg. Med. Chem.*, 2000, **8**, 2347-2354.
26 S. Kusumi, H. Nakayama, T. Kobayashi, H. Kuriki, Y. Matsumoto, D. Takahashi and K. Toshima, *Chem. Eur. J.*, 2016, **22**, 18733–18736.
27 T. Matsumoto, H. Yamaguchi, T. Hamura, M. Tanabe, Y. Kuriyama and K. Suzuki, *Tetrahedron Lett.*, 2000, **41**, 8383-8387.
28 H. Yamaguchi, T. Konegawa, M. Tanabe, T. Nakamura, T. Matsumoto and K. Suzuki, *Tetrahedron Lett.*, 2000, **41**, 8389-8392.
29 T. Matsumoto, H. Yamaguchi, M. Tanabe, Y. Yasui and K. Suzuki, *Tetrahedron Lett.*, 2000, **41**, 8393-8396.
30 F. M. Hauser and R. P. Rhee, *J. Org. Chem.*, 1978, **43**, 178-180.
31 D. Mal and P. Pahari, *Chem. Rev.*, 2007, **107**, 1892–1918.
32 H. R. Khatri, H. Nguyen, J. K. Dunaway and J. Zhu, *Chem. Eur. J.*, 2015, **21**, 13553-13557.
33 M. A. Tius, J. Gomez-Galeno, X. Q. Gu and J. H. Zaidi, *J. Am. Chem. Soc.*, 1991, **113**, 5775-5783.
34 V. A. Boyd, B. E. Drake and G. A. Sulikowski, *J. Org. Chem.*, 1993, **58**, 3191-3193.
35 K. Toshima, H. Nagai, Y. Ushiki and S. Matsumura, *Synlett*, **1998**, 1007-1009.
36 See Experimental and Supporting Information for details.
37 S. Adhikari, K. N. Baryal, D. Zhu, X. Li and J. Zhu, *ACS Catal.*, 2013, **3**, 57-60.
38 E. A. Hillard, F. C. de Abreu, D. C. M. Ferreira, G. Jaouen, M. O. F. Goulart and C. Amatore, *Chem. Commun.*, 2008, 2612-2628.
39 S. M. Wuerzberger, J. J. Pink, S. M. Planchon, K. L. Byers, W. G. Bornmann, and D. A. Boothman, *Cancer Res.*, 1998, **58**, 1876-1885.
40 T. Rodrigues, M. Werner, J. Roth, E. H. G. da Cruz, M. C. Marques, P. Akkapeddi, S. A. Lobo, A. Koeberle, F. Corzana, E. N. da Silva Júnior, O. Werz and G. J. L. Bernardes, *Chem. Sci.*, 2018, **9**, 6899-6903.
41 N. Kongkathip, B. Kongkathip, P. Siripong, C. Sangma, S. Luangkamin, M. Niyomdecha, S. Pattanapa, S. Piyaviriyagul and P. Kongsaeree, *Bioorg. Med. Chem.*, 2003, **11**, 3179-3191.
42 E. L. Bonifazi, C. Ríos-Luci, L. G. León, G. Burton, J. M. Padrón and R. I. Misico, *Bioorg. Med. Chem.*, 2010, **18**, 2621-2630.
43 P. R. Duchowicz, D. O. Bennardi, D. E. Bacelo, E. L. Bonifazi, C. Rios-Luci, J. M. Padrón, G. Burton and R. I. Misico, *Eur. J. Med. Chem.*, 2014, **77**, 176-184.
44 T. V. Baiju, R. G. Almeida, S. T. Sivanandan, C. A. de Simone, L. M. Brito, B. C. Cavalcanti, C. Pessoa, I. N. N. Namboothiri and E. N. da Silva Júnior, *Eur. J. Med. Chem.*, 2018, **151**, 686-704.
45 R. M. De Lederkremer and C. Marino, *Adv. Carbohydr. Chem. Biochem.*, 2008, **61**, 143–216.
46 A. Kirschning, A. F.-W. Bechthold and J. Rohr, *Top. Curr. Chem.*, 1997, **188**, 1-84.

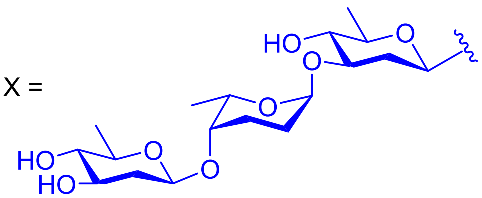
Synthesis and antitumor activities of aquayamycin and analogues of derhodinosylurdamycin A†

Padam P. Acharya, Hem Raj Khatri, Sandip Janda, and Jianglong Zhu*

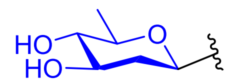
An analogue without the sugar moiety of natural derhodinosylurdamycin A is better.



derhodinosylurdamycin A (**2**) X =



aquayamycin (urdamycinone A) (**3**) X =



analogue (**4**) X = **H** (active agaisnt a wide range of human cancer cell lines, GI_{50} values range 0.23 - 18.3 μM)