Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and *in vitro* investigation of potential antiproliferative monosaccharide–D-secoestrone bioconjugates

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ARTICLE INFO

Article history:
Received 17 January 2017
Revised 11 March 2017
Accepted 14 March 2017
Available online xxxx

Keywords:
Monosaccharide
D-Secoestrone
Oxime
Bioconjugate
CuAAC

ABSTRACT

The syntheses of monosaccharide–D-secoestrone conjugates are reported. They were prepared from 3-(prop-2-inyloxy)-D-secoestrone alcohol or oxime and monosaccharide azides via Cu(I)-catalyzed azide–alkyne cycloaddition reactions (CuAAC). The antiproliferative activities of the conjugates were investigated *in vitro* against a panel of human adherent cancer cell lines (HeLa, A2780 and MCF-7) by means of MTT assays. The protected D-glucose-containing D-secoestrone oxime bioconjugate (**24b**) proved to be the most effective with an IC_{50} value in the low micromolar range against A2780 cell line.

Literature precedents reveal that different synthetic modifications of estrone (**1**, Scheme 1) lead to anticancer compounds.[1] In order to suppress their hormonal action, substitutions at C-2, opening of ring D or inversion of configuration at C-13 are usually carried out.[2–7] We recently reported that 3-*O*-benzyl ethers of D-secoestrone alcohol or oxime (**3a** and **3b**, Scheme 1) display substantial *in vitro* antiproliferative action against certain cancer cell lines in the low micromolar range.[8,9]

The starting compounds bearing phenolic hydroxy groups (**2a** and **2b**) did not influence the proliferation of the investigated cell lines. The cytostatic potential of benzyl ethers (**3a** and **3b**) was successfully improved by the introduction of a 1,2,3-triazole moiety between the benzyl and the hydroxy groups. The heterocyclic ring was introduced to C-3 via a short oxymethylene group applying copper(I)-catalyzed alkyne–azide click reaction (CuAAC). The resulting 3-*O*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] derivatives (**5a** and **5b**) displayed submicromolar IC_{50} values against certain human reproductive adherent cancer cell lines.[10] There are already a number of literature examples of the synthesis of antiproliferative steroidal triazoles[9,11–13] and the triazole moiety is also used as a linker arm in bioconjugates owing to its high proteolytic and metabolic stability. On the other hand, making bioconjugates is a potential strategy to enhance the antiproliferative effect or to increase the selectivity of a compound. Preparation of natural product conjugates is a very promising approach, since the biological potency of the new hybrids may exceed that of the parent compounds.[14] In general, in the compounds constructed from diverse molecular entities, the components may result in synergic action, with better tolerability or the conjugate may possess more selective cellular uptake or may influence the pharmacokinetic properties.[14] Based on our previous observations and the potential advantages of modifying our most active D-secoestrones by making bioconjugates, here we aimed at synthesizing novel D-secoestrone bioconjugates, stemming from D-secoestrones, retaining the 3-*O*-[(1,2,3-triazol-4-yl)methyl] moiety, and introducing a monosaccharide unit instead of the previously used benzyl group on the triazole ring. The rationale behind this aim was the fact that although sugars *per se* have no therapeutic action in glycosylated steroid conjugates, they have a dramatic effect on the physical, chemical and biological properties of bioconjugates and the sugar moieties act as molecular elements that control the pharmacokinetics of a drug, such as absorption, distribution, metabolism and excretion.[15] As it was reported, the number, location and type of sugars in steroidal glycoalkaloids, even with identical aglycon, play an important role in the antiproliferative activity.[16] Similarly, subtle sugar modifications can dramatically, and independently,

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http://dx.doi.org/10.1016/j.bmcl.2017.03.029
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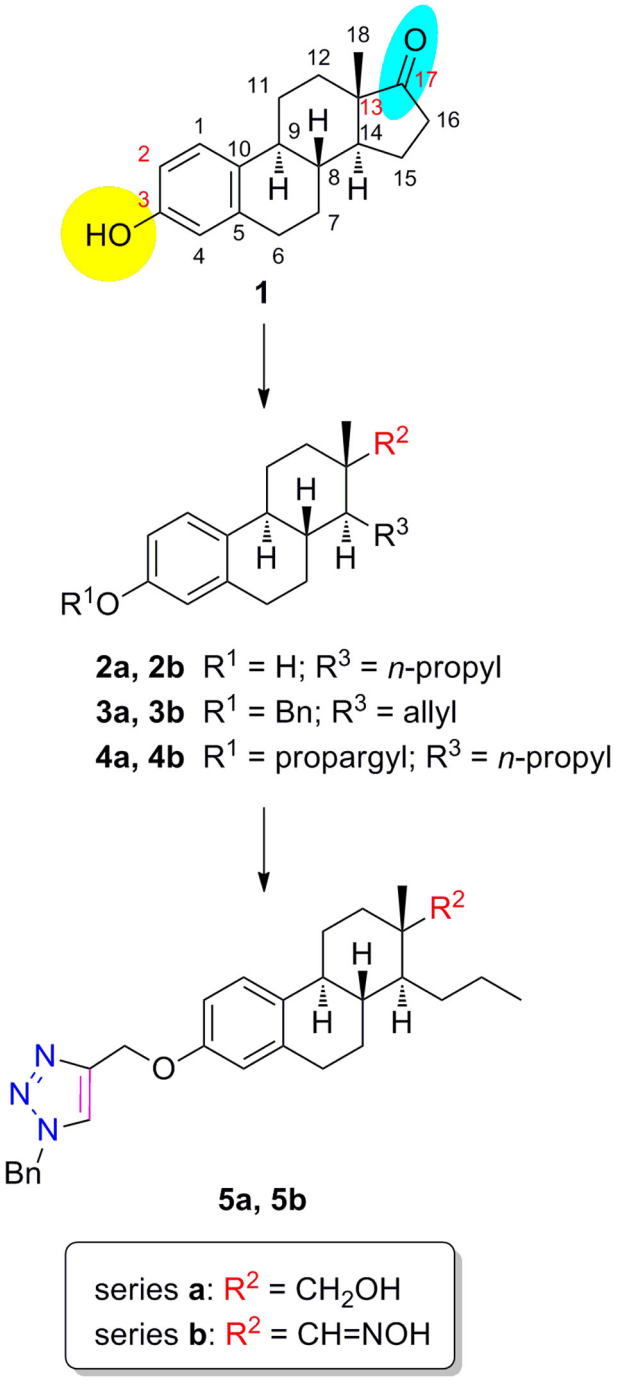


Scheme 1. The synthesis of estrone derivatives **2**–**5** obtained earlier.

modulate both the cytotoxic properties and the Na^+/K^+-ATPase inhibitory properties of cardiac glycosides.[17] In this vein, we planned to perform CuAAC reactions of steroidal alkynes (**4a** and **4b**) with protected monosaccharide azides and to investigate the *in vitro* antiproliferative activities of these bioconjugates by means of MTT assays against a panel of human adherent cancer cell lines (HeLa, MCF-7 and A2780).

As our aim was to synthesize carbohydrate–D-secoestrone bioconjugates from our previously reported 3-*O*-propargyl D-secoestrones using CuAAC, this conjugation reaction required the synthesis of azide-containing carbohydrate building blocks and their CuAAC reaction with propargylated D-secoestrones (**4a** and **4b**, Scheme 1).

Some of the most abundant monosaccharides in the nature, D-glucose, D-mannose, D-galactose and D-ribose were chosen to prepare these building blocks. We aimed at synthesizing their azide derivatives in which the azide group is built into their glycosidic position or in place of their primary hydroxy groups (position 6 in hexopyranoses and position 5 in ribofuranose). The most common ways for the preparation of glycosyl azides are either an S_N2 substitution of a protected glycosyl halide by sodium azide at high temperature in DMF[18] or an S_N1 substitution of a peracetylated carbohydrate under mild conditions using a Lewis acid catalyst and trimethylsilyl azide.[19–21] We have chosen the latter method as all of our sugars contained a neighbouring participation group at position 2 (*O*-acetyl or *O*-benzoyl) that can ensure the desired stereoselectivity. The primary hydroxy groups of the monosaccharides can be replaced to azides in a two-step procedure[22] involving the introduction of a good leaving group (*e.g.* a tosyl) to the primary hydroxy and a subsequent azide substitution of the tosylate by sodium azide in DMF.

First, the hexoses studied were peracetylated according to a literature method[23] to yield compounds **6**–**8** (Scheme 2). Next, the glycosidic *O*-acetyl groups were replaced with the azide group using tin tetrachloride as a Lewis acid catalyst and trimethylsilyl azide as the source of the nucleophilic azide ion to afford compounds **9**–**11**.

The S_N1 type substitution resulted in only 1,2-*trans* products due to the neighbouring group participation of the *O*-acyl group at position 2. For D-glucose and D-galactose the β-anomer, for D-mannose the α-anomer formed in this way in 72–79% yield. The purity of the azide products and their quantities were sufficient for the subsequent conjugation reactions.

In order to introduce the azide group to positions 6 (hexopyranoses) and 5 (pentofuranose), first the methyl glycosides of D-glucose, D-mannose and D-ribose (**12**–**14**, Scheme 3) were selectively tosylated in pyridine on their primary hydroxy groups without the protection of the secondary hydroxy groups. This distinction was allowed by the higher reactivity of the primary hydroxy groups over the secondary ones.

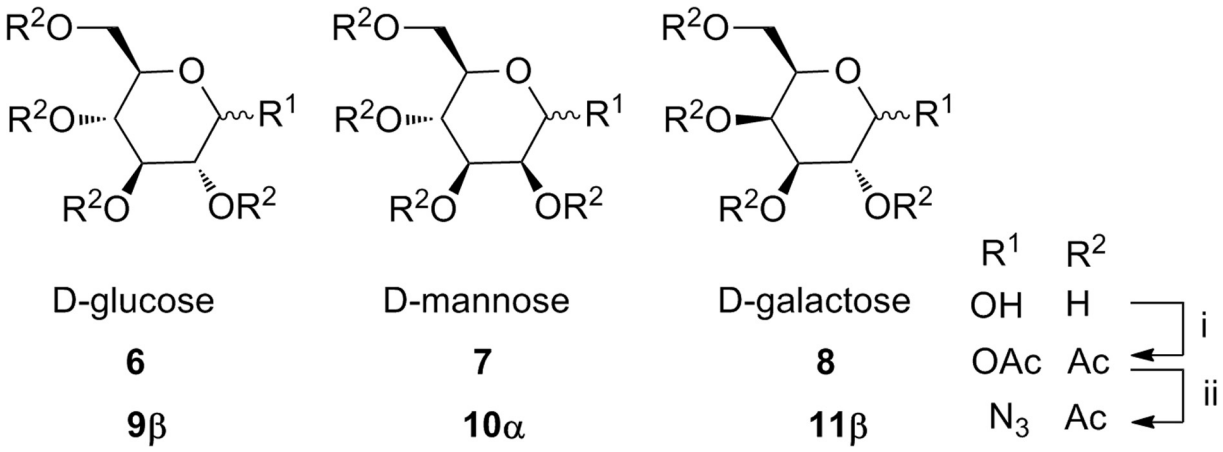
Unfortunately, the direct replacement of the tosyl group with azide in compounds **15**–**17** was not successful probably due to solubility reasons, therefore the secondary hydroxy groups were benzoylated first, then the tosyl–azide exchange has successfully occurred in all the fully protected monosaccharides **18**–**20** and resulted in the fully protected 6-azido-6-deoxy- and 5-azido-5-deoxymonosaccharides (**21**–**23**).

The azide group-containing monosaccharides (**9**–**11** and **21**–**23**) were coupled to the alkyne-containing D-secoalcohol (**4a**) and D-secooxime (**4b**) using similar CuAAC conditions that we used previously (Scheme 4) applying copper(I) iodide, triphenylphosphine and DIPEA as a base with a slight access of propargyl-D-secosteroid in toluene at boiling temperature until TLC showed quantitative conversions.[24]

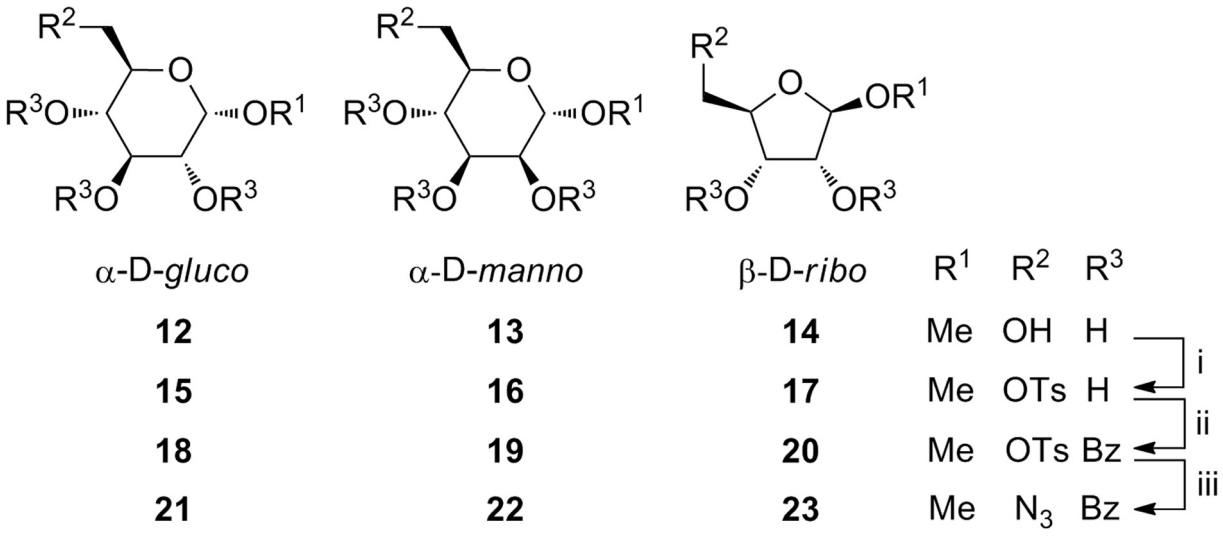
In case of two glucose-containing bioconjugates (**24a** and **24b**), which showed the best biological activities, the acetyl protecting groups were removed by the Zemplén's method[25] using sodium methylate in methanol to obtain their unprotected derivatives **30a** and **30b**.

The antiproliferative properties of the D-secoestrone–carbohydrate conjugates (**24**–**30**) were characterized *in vitro* on a panel of human adherent cancer cell lines (HeLa, A2780 and MCF-7) by means of MTT assays (Table 1).

The antiproliferative properties of some of the presented compounds proved to comparable to that of reference agent cisplatin that is utilized clinically in the treatment of certain gynaecological malignancies.[26,27] The most potent compounds (**24b**, **25b** and **26b**) exhibited remarkable activities with IC_{50} values in the range 5.3–20.5 μM, exerting their best effects against A2780 cells. Among



Scheme 2. Synthesis of protected glycosyl azides **9**–**11**. Reagents: (i) Ac_2O, NaOAc; (ii) $SnCl_4$, Me_3SiN_3, CH_2Cl_2.

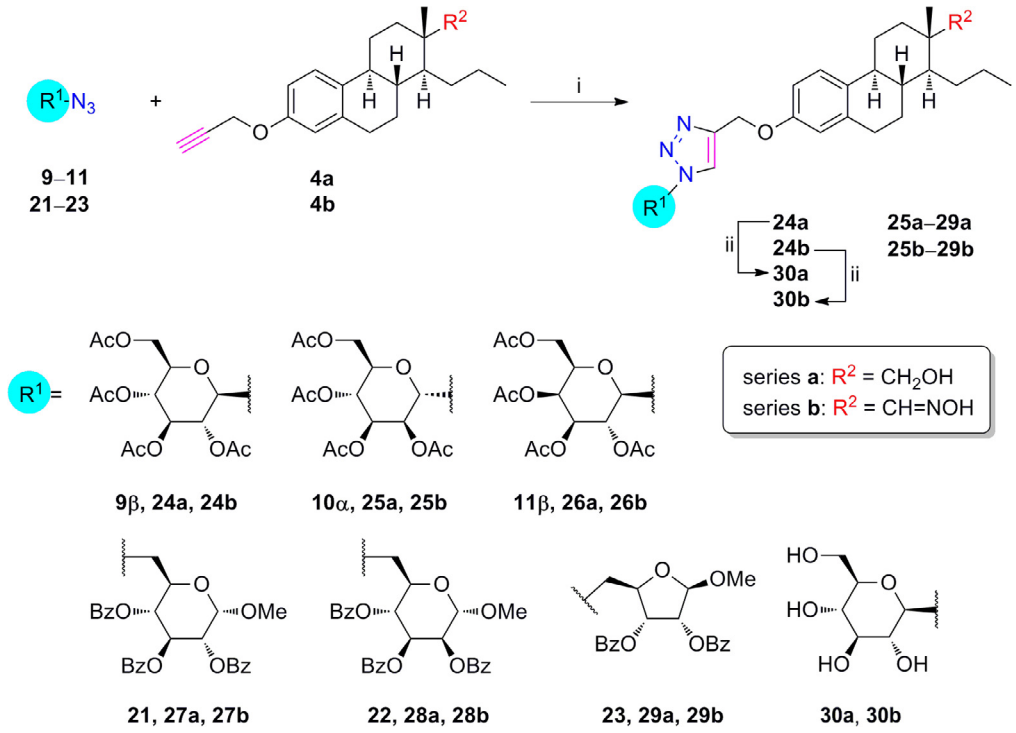


Scheme 3. Synthesis of 6-azido-6-deoxy-D-hexopyranose (**21**, **22**) and 5-azido-5-deoxy-D-ribofuranose (**23**) derivatives. Reagents: (i) TsCl, pyridine; (ii) BzCl, pyridine; (iii) NaN_3, LiBr, DMF.

the potent compounds the glucoside conjugate (**24b**) displayed the highest, the mannoside derivative (**25b**) the lowest cell-line selectivity. Considering the results of Table 1, important structure–activity relationships appear. The antiproliferative activities of the compounds greatly depend on the attachment site of the monosaccharide unit, and on the nature of the functional group at C-13, but do not really depend on the type of monosaccharide attached. It can be stated that the glycoside derivatives (**24**–**26**) are more potent than their methyl glycoside analogues (**27**–**29**) which were attached to the triazole ring at the 6′- or 5′-positions of the carbohydrate units. The removal of the protecting groups from the most potent **24b** compound results in a compound (**30b**) with decreased antiproliferative properties showing the importance of the nonpolar property of the moiety attached to the triazole ring for the bioactivity. Comparison of the results for acetylated conjugates formed from the D-secooxime and the D-secoalcohol reveals that the presence of the oxime function generally improves the growth-inhibitory properties of the conjugates. Extending the discussion on our recent results,[9] it seems that both the polarity and the size of the fragment at C-3 position greatly influence the antiproliferative properties. The presence of the less polar, but bulky benzyl function is advantageous over the phenolic hydroxy group, and further enhancement in the activity is achieved by incorporation of a triazole ring between the 3-hydroxy and the benzyl group. An additional determining factor is the nature (type, attachment position and polarity) of the substituent on the triazole ring. The benzyl to monosaccharide exchange decreases the cell growth-inhibition. Even the most potent hexose conjugates (**24b**, **25b** and **26b**) displayed one order of magnitude higher IC_{50} values than certain recently described 3-*O*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] derivatives (**5a** and **5b**). Cancer selectivity is a critical parameter determining the fate of a potential drug candidate. A viability assay on mouse fibroblasts cannot substitute the toxicological evaluation. However, it seems advantageous that our most potent compounds (**24b**, **25b** and **26b**) exert substantially less growth inhibiting action on fibroblasts than on cancerous cell lines.[9]

Conclusions

Fourteen monosaccharide–D-secoestrone bioconjugates were prepared by CuAAC reaction and their antiproliferative properties were investigated. Although the antiproliferative activities of the bioconjugates were not as high as the activities of the parent compounds, the structure–activity relationships provided by the



Scheme 4. The synthesis of monosaccharide–D-secoestrone bioconjugates **24**–**30**. Reagents: (i) Cu(I), $(Ph)_3P$, DIPEA, toluene, reflux; (ii) NaOMe, MeOH.

Table 1
Anticancer activity of monosaccharide–D-secoestrone conjugates **24–30** against different cell lines.

Compd. No. or name[a]	Monosaccharide configuration	Attachment site in monosaccharide	Concn. (μM)	Inhibition (%) ± SEM [calculated IC_{50} (μM)][b]			
				HeLa	A2780	MCF-7	NIH/3T3 (mouse fibroblast)
24a	β-D-Glc*p*	1′	10	34.5 ± 0.7	21.0 ± 1.6	27.4 ± 2.8	
			30	23.9 ± 0.9	19.5 ± 1.4	45.5 ± 1.5	
24b	β-D-Glc*p*	1′	10	20.4 ± 1.8	69.5 ± 0.9	46.7 ± 1.3	<20
			30	40.0 ± 2.2	76.7 ± 0.8	67.7 ± 1.6	28.4 ± 0.9
			[IC_{50}]	[> 30]	[5.3]	[10.7]	
25a	α-D-Man*p*	1′	10	51.9 ± 1.0	34.9 ± 2.3	26.3 ± 2.2	
			30	41.5 ± 1.7	36.5 ± 1.4	54.3 ± 0.6	
25b	α-D-Man*p*	1′	10	52.4 ± 1.7	69.5 ± 0.6	53.9 ± 0.9	27.3 ± 0.5
			30	89.6 ± 0.5	86.4 ± 0.8	65.9 ± 1.2	34.8 ± 0.3
			[IC_{50}]	[8.9]	[6.6]	[9.4]	
26a	β-D-Gal*p*	1′	10	23.9 ± 1.1	<20	<20	
			30	31.8 ± 1.2	32.7 ± 1.0	61.9 ± 0.9	
26b	β-D-Gal*p*	1′	10	31.5 ± 2.0	59.3 ± 0.9	59.1 ± 2.2	<20
			30	61.9 ± 2.0	85.2 ± 0.4	71.5 ± 1.5	25.5. ± 1.4
			[IC_{50}]	[20.5]	[8.8]	[8.0]	
27a	α-D-Glc*p*	6′	10	<20	<20	<20	
			30	<20	<20	<20	
27b	α-D-Glc*p*	6′	10	<20	28.4 ± 0.8	<20	
			30	<20	45.7 ± 2.1	<20	
28a	α-D-Man*p*	6′	10	<20	<20	<20	
			30	<20	<20	<20	
28b	α-D-Man*p*	6′	10	<20	<20	<20	
			30	<20	<20	<20	
29a	β-D-Rib*f*	5′	10	<20	<20	<20	
			30	<20	<20	<20	
29b	β-D-Rib*f*	5′	10	24.3 ± 2.6	<20	<20	
			30	<20	<20	<20	
30a	β-D-Glc*p*	1′	10	<20	<20	<20	
			30	<20	<20	<20	
30b	β-D-Glc*p*	1′	10	<20	<20	<20	
			30	31.5 ± 0.4	57.9 ± 0.6	<20	
			[IC_{50}]	[>30]	[27.4]	[>30]	
Cisplatin	–	–	10	42.6 ± 2.3	83.6 ± 1.2	66.9 ± 1.8	94.2 ± 0.4
			30	99.9 ± 0.3	95.0 ± 0.3	96.8 ± 0.4	96.4 ± 0.2
			[IC_{50}]	[12.4]	[1.3]	[5.8]	[3.2]

[a] For structures see Scheme 4. The compound series **a** contain hydroxymethyl, series **b** oxime moieties at C-13 position of the steroid skeleton.
[b] Mean value from two independent determinations with five parallel wells; standard deviation <15%.

results deliver very important information for the design of antiproliferative estrone derivatives in the future.

Acknowledgments

The authors thank the Hungarian Scientific Research Fund – Hungary (OTKA K113150 and K109293), the New Hungary Development Plan (TÁMOP-4.2.2.A-11/1/KONV-2012-0047) for financial support. This research was also supported by the European Union and the State of Hungary, co-financed by the European Social Fund – Belgium and Hungary (TÁMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program').

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.03.029.

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