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Syntheses and structure–activity relationship studies of N-substituted-β-D-glucosaminides as selective cytotoxic agents

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ABSTRACT

Twenty-four diosgenyl saponins bearing cinnamoyl, carbamido and thiosemicarbazone groups were synthesized concisely. The cytotoxicities of the synthetic compounds on six human caner cell lines were evaluated employing MTT method. Structure–activity relationship could be observed, and two of the synthesized compounds (**5c** and **5f**) exhibited selective inhibition on HeLa and MCF-7 cells, while three of them (**5d**, **5f** and **5h**) showed strong inhibition against HT1080.

Saponins which are composed of a saccharide moiety and the aglycone have attracted much attention due to their cytotoxic activity. Among the saponins isolated from nature, either steroidal or triterpenoidal, the presenting saccharides attached on the aglycon are usually β-D-glucopyranose, α-L-rhamnopyranose, and β-D-galactopyranose. Though it is quite common as a constituent of many natural poly- or oligosaccharides, glucosamine rarely emerged in natural saponin, however they are presenting strong in vitro cytotoxicity towards different tumor cell lines.[1,2] The potent cytotoxic property indicates that the sugar chains of glucosamine dramatically enhance the bioactivity of their aglycons. Therefore, it is a quite rational design that some modifications are performed on the glucosamine. Up to date, most researches are focused on the isolation of glucosaminides, fewer works are done with the structural modification of those saponins.[3,4] The earliest reference could date back to the year of 2003 in which 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride was synthesized, and in combination with cladribine it can increase the number of apoptotic B cells isolated from B-CLL patients.[5,6] Later in 2008 and 2009, modifications on the amino group of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside were initially reported, which showed that the substitutents on amino groups had great influences on their cytotoxicity against several tumor cell lines.[7,8]

Inspired by the previous results, we assumed that the introduction of some cytotoxic pharmaphores onto glucosamine saponin might be a rational method to produce more potent lead structures. Since *trans*-Cinnamic acid has been demonstrated to possess a variety of biological activities including anti-cancer and used to induce a reversal of malignant properties of several human tumor cells in vitro.[9–12] While both carbamido and thiosemicarbazone groups are quite common substructures in the anti-cancer compounds. Especially for thiosemicarbazone, it has been researched for decades, and believed that the antitumor activity seems to be due to an inhibition of DNA synthesis.[13–15] Based on these researches, three series of diosgenyl glucosaminide with substituents of cinnamoyl, carbamido and thiosemicarbazone on their amino groups were designed and synthesized to test their in vitro cytotoxicities against different cancer cell lines and find the SARs of these compounds (Fig. 1).

As parts of our research on saponins, the representative aglycons of steroidal saponins diosgenin was selected as scaffolds to prepare the N-substituted-β-D-glucosaminide derivatives (Scheme 1). 2,2,2-Trichloroethoxycarbonyl group (Troc) was used to protect amino group in glycosyl imidate, considering the toleration and deprotection conditions of the protecting group.[16] The synthetic route began with the glycosylation between diosgenin and glycosyl imidate catalyzed by TMSOTf as showing in Scheme 1. The configuration of newly formed glucosidic bond was β (demonstrated by the 8.2 Hz coupling constant of $J_{1\text{-}2}$ in ^{1}H NMR), due to Troc group's neighboring group participating capacity. Intermediate **2** was prepared by following known procedures.[7,8] After deprotection of the Troc group, the amine **3** was condensed with substituted cinnamoyl chloride, which were prepared via Knoevenagel reaction from commercially available substituted benzaldehydes and malonic acid in the presence of pyridine to afford intermediates **4a–4n**,[17]

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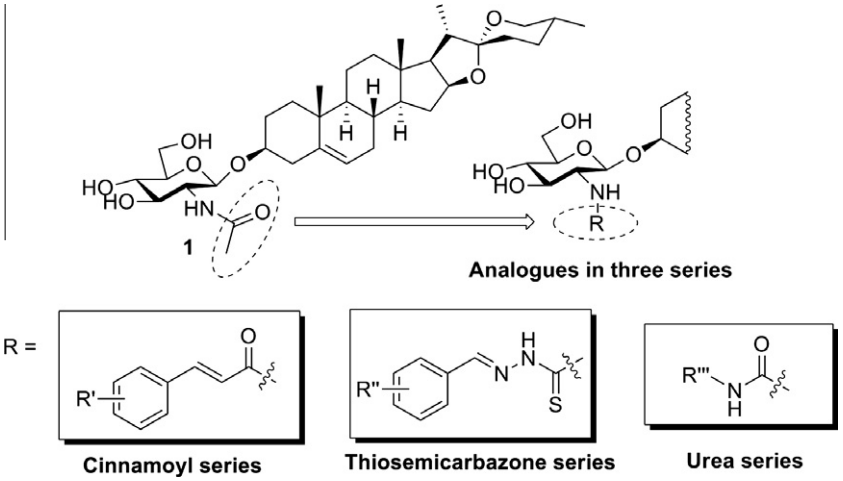
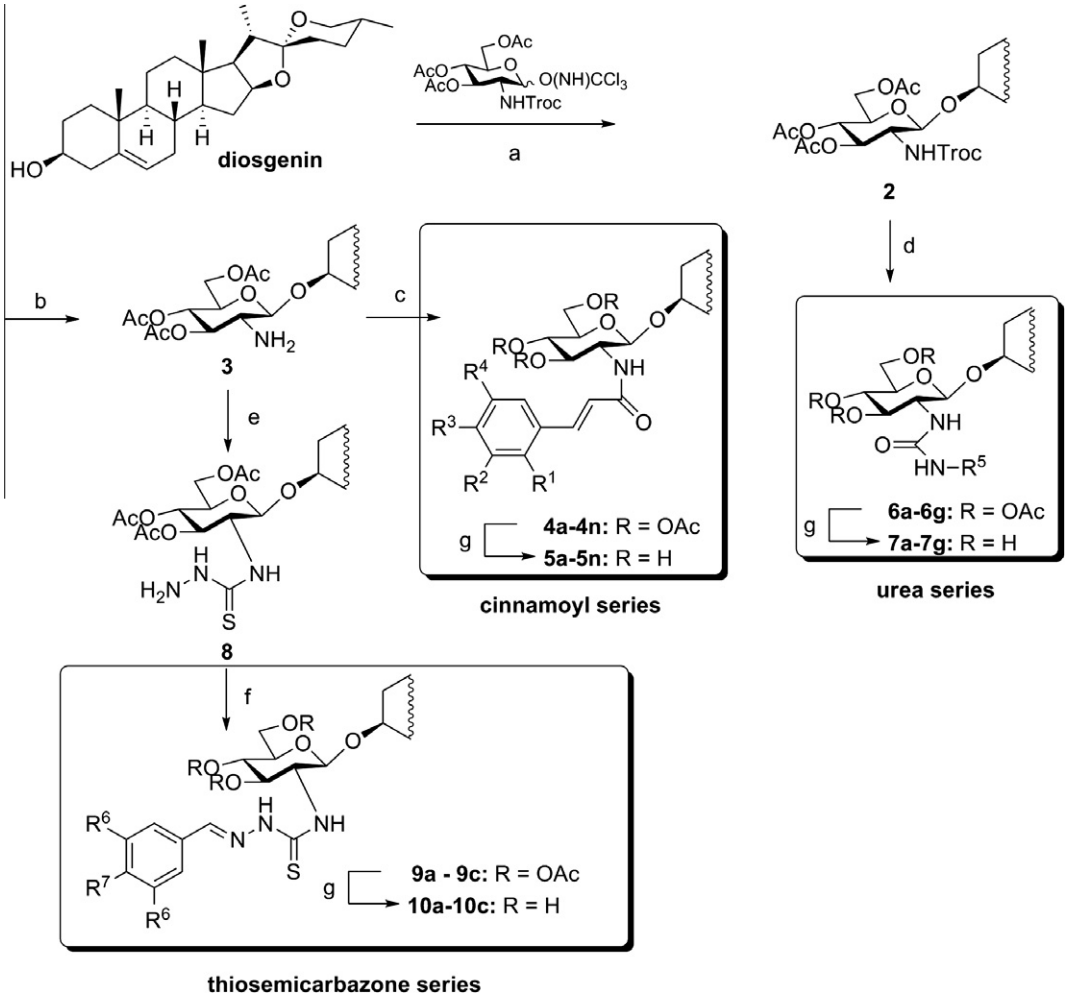


Figure 1. Structures of N-substituted-β-D-glucosaminide.



Scheme 1. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 84%; (b) Zinc dust, AcOH, overnight; (c) substituted cinnamoyl chloride, pyridine, CH_2Cl_2, 5 h; (d) R-NH_2, DIEA, DMSO, 70 °C; (e) thiocarbonyl chloride, $CaCO_3$, CH_2Cl_2, H_2O, rt, then 80% hydrazine hydrate, ethanol, rt, 52.4% in two steps; (f) substituted benzaldehydes, THF, reflux, 1 h; (g) NH_3, MeOH, overnight, yields and compound details of cinnamoyl series see Table 1; **7a**: R_5 = benzyl, 62.7%; **7b**: R_5 = 4-flurobenzyl, 55.4%; **7c**: R_5 = 4-methoxybenzyl, 68.3%; **7d**: R_5 = cyclopropyl, 71.2%; **7e**: R_5 = *t*-butyl, 69.4%; **7f**: R_5 = heptyl, 54.6%; **7g**: R_5 = piperidyl, 75.1%; **10a**: R_6 = R_7 = H, 48.4%; **10b**: R_6 = H, R_7 = OMe, 52.5%; **10c**: R_6 = CF_3, R_7 = H, 53.0%.

followed by a fully deprotection in NH_3-MeOH to provide the *N*-cinnamoyl-β-D-glucosaminide **5a–5n**. All the synthetic compounds' structural details and yields were summerized in Table 1. For the synthesis of urea series, the key intermediate **2** was directly condensed with commercially available amines in the presence of DIEA in DMSO at 70 °C as shown in Scheme 1, then

Table 1
Structural details and yields of the cinnamoyl series compounds

Entry	R^1	R^2	R^3	R^4	Yield[a] (%)
5a	H	H	H	H	48.8
5b	H	H	F	H	69.2
5c	H	H	Me	H	59.0
5d	OMe	H	H	H	61.4
5e	H	OMe	H	H	62.8
5f	H	H	OMe	H	65.7
5g	OMe	OMe	H	H	58.6
5h	OMe	H	OMe	H	60.0
5i	OMe	H	H	OMe	49.5
5j	H	OMe	OMe	H	45.4
5k	H	OMe	OMe	OMe	59.5
5l	H	Cl	H	H	63.3
5m	H	Br	H	H	45.3
5n	NO_2	H	H	H	47.9

[a] Yields based on intermediate **2**

the condensation products **6a–6g** were deacetylated in NH_3-MeOH solution to give the urea-based products **7a**–**7g** in 54.6% to 75.1% yields. To synthesize the thiosemicarbazone series, the free amino group in intermediate **3** was firstly converted to isosulfocyanide, which was then reacted with hydrazine affording **8** in ethanol. After condensation with substituted benzaldehydes in THF, all the protective acetyl groups were cleaved to yield **10a**–**10c**.

With all the synthetic glucosaminides with diversified substituents on their amino groups in hand, the cytotoxic effect against HeLa, MCF-7, A549, HepG2, HCT116 and HT1080 were determined employing MTT assay, and the IC_{50}s were illustrated in Table 2. Compound **1** with acetyl on the amino group and 5-fluorouracil were chosed as positive controls. The preliminary in vitro screening indicated that some saponins belonging to the cinnamoyl series showed moderate to excellent antiproliferative activities against cell lines tested comparing to the positive control **1** and 5-FU. Whereas introducing carbamido and thiosemicarbazone groups neither provided any competitive compounds comparing to the positive control, nor generated obvious structure–activity relationships and inhibition selectivity.

As shown in Table 2 (from entries 1 to 15), some compounds in cinnamoyl series showed selective inhibitions against several cancer cell lines. This selectivity referred to two kinds of diversities, including different sensitivities of varied cancer cell lines to the same samples in transverse comparison and influences of different samples with tiny modifications in their structures on the cytotoxicity against the same cancer cell lines in longitudinal compraison. For most of the synthetic saponins belonging to the cinnamoyl series, they generally showed more active in HeLa and MCF-7 than in A549, HepG2 and HCT116, which matched the selectivity tendency of positive control **1**. The cytotoxicity diversities were significantly relied on the types of cinnamoyl groups introduced. For instances, cinnamoyl series derivatives **5a** showed a modest increases of cytotoxicities comparing to the positive control **1** against all the cancer cell lines tested, however **5c**, **5f** which was slightly modified from **5a** showed excellent activity against HeLa and MCF-7 than **5a** and positive control **1**, while changes of their cytotoxicities against A549, HepG2 and HCT116 were not obvious. Most of the samples showed remarkable activity increases against HT1080 comparing to the positive control **1**, nevertheless tiny modifications on their structures still could significantly affect their cytotoxicities. The detailed structure–activity relationships were discussed as follows.

A diverse electron withdrawing groups and electron donating groups were introduced to the different position of phenyl ring in **5a** for investigation of the structure–activity relationships (SAR). As shown in Table 2, compound **5c** and **5f** bearing methyl and methoxy groups at *para*-position were significantly more active in HeLa and MCF-7 than **5a** and **5b**, which contains no or electron withdrawing groups at the same positions. The similar tendency could be observed between derivatives bearing

Table 2
The in vitro cytotoxicities of three series saponins

Entry	compound	IC_{50}[a,b] (μM)					
		HeLa	MCF-7	A549	HepG2	HCT116	HT1080
1	**5a**	12.8±2.2	16.0±1.3	41.8±0.9	36.3±1.3	39.1±3.7	29.8±3.2
2	**5b**	26.9 ± 1.1	21.2 ± 2.0	33.8 ± 1.2	39.8 ± 2.7	35.0 ± 2.5	34.9 ± 2.5
3	**5c**	6.5 ± 1.1	5.3 ± 0.7	41.6 ± 2.4	35.5 ± 0.9	40.4 ± 1.7	23.5 ± 1.5
4	**5d**	27.5 ± 5.5	23.1 ± 3.2	23.1 ± 1.3	29.5 ± 1.2	38.9 ± 2.6	4.3 ± 0.3
5	**5e**	27.0 ± 3.0	24.1 ± 4.4	26.9 ± 0.9	>50	>50	26.6 ± 1.5
6	**5f**	6.1 ± 1.2	0.5 ± 0.2	24.4 ± 4.2	30.7 ± 2.5	29.5 ± 2.2	3.5 ± 0.8
7	**5g**	36.2 ± 0.2	44.4 ± 3.7	43.4 ± 0.7	45.6 ± 3.7	47.4 ± 1.3	21.2 ± 2.1
8	**5h**	22.3 ± 8.1	26.7 ± 3.3	27.1 ± 3.1	41.3 ± 4.0	42.9 ± 3.5	5.8 ± 0.1
9	**5i**	32.2 ± 2.7	>50	47.8 ± 0.8	31.7 ± 0.8	35.6 ± 4.7	>50
10	**5j**	26.1 ± 1.8	31.3 ± 2.4	30.3 ± 2.7	25.9 ± 1.7	29.0 ± 0.6	>50
11	**5k**	>50	>50	>50	>50	>50	>50
12	**5l**	>50	>50	>50	>50	>50	>50
13	**5m**	>50	>50	>50	>50	>50	>50
15	**5n**	>50	>50	>50	>50	>50	36.5 ± 0.5
16	**7a**	27.0 ± 3.0	23.9 ± 1.8	28.5 ± 1.5	>50	>50	>50
17	**7b**	>50	>50	>50	>50	>50	42.0 ± 2.2
18	**7c**	>50	>50	>50	>50	>50	>50
19	**7d**	43.5 ± 3.7	>50	>50	>50	>50	>50
20	**7e**	27.6 ± 6.0	26.8 ± 2.9	>50	>50	>50	42.3 ± 2.3
21	**7f**	>50	>50	>50	>50	>50	43.0 ± 3.0
22	**7g**	>50	>50	>50	>50	>50	35.1 ± 1.4
23	**10a**	36.2 ± 9.3	48.2 ± 4.0	49.7 ± 0.1	29.5 ± 3.9	40.0 ± 3.4	>50
24	**10b**	34.8 ± 9.0	>50	37.3 ± 2.2	28.2 ± 2.6	38.2 ± 1.8	>50
25	**10c**	>50	>50	>50	>50	>50	>50
26	**1**	21.5 ± 1.1[c]	18.3 ± 0.6[c]	>50	>50	>50	>50
27	**5-FU**	63.4	78.6	88.9	51.5	36.4	15.2

[a] Concentration inhibiting fifty percent of cell growth for 48 h exposure period of tested samples. Data represent mean values ± standard deviation for independent experiments.
[b] IC_{50}s more than 50 μM are not shown their exact value.
[c] IC_{50}s reported in Ref. [8]

substituents with different electron properties at the same position (**5d** comparing with **5n**, and **5e** comparing with **5l** and **5m**). Accordingly, we can conclude that electron donating groups in the cinnamoyl groups were preferred. Meanwhile comparing the cytotoxicities against HeLa and MCF-7 of those saponins bearing methoxy groups (entries 4–11), it is clear that mono-substituted at the *para*-position is much more active, while introduction of methoxy groups at *ortho*- or *meta*-position leads to a decline of cytotoxicity.

The selective inhibition against HT1080 of cinnamoyl series can also be found, and the selectivity relies on the types and position of the substituents. As shown in Table 2 (HT1080 datas in Entries 4, 6 and 8), methoxy group is exclusive and any introduction of other groups leads to the decline of activity. *Ortho*- and *para*-position is preferred, and introduction of the methoxy group at *meta*-position leads to a significant decline of activity.

In summary, a series of N-substituted-β-D-glucosaminides were synthesized from commercially available starting materials in high yields. Cytotoxic effects of all the synthetic saponins were evaluated against six human cancer cell lines. Preliminary SAR research showed that diosgenyl saponins bearing an electron-donating substituent at parasite of cinnamoyl group is preferable against HeLa and MCF-7, while diosgenyl saponins bearing methoxy groups at metasite or parasite of cinnamoyl group is selective active against HT1080. Further study based on the selective inhibition on HeLa, MCF-7 and HT1080 cells will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.075.

Reference and notes

1. Maged, A.-K.; Jeannine, H.; John, M. B.; Randy, E.; James, S. M.; Jan, H. W.; Stephen, W. M.; James, M. D.; David, G. I. K. *J. Nat. Prod.* **2001**, *64*, 536.
2. Youngwan, S.; Jeannine, H.; Maged, A.-K.; Malone, S.; Derveld, I.; Hermus, A.; Werkhoven, M. C. M.; Jan, H. W.; Stephen, W. M.; James, M. D.; David, G. I. K. *J. Nat. Prod.* **2002**, *65*, 170.
3. Wang, P.; Wang, J.; Guo, T. T.; Li, Y. X. *Carbohydr. Res.* **2010**, *345*, 607.
4. Yan, M.-C.; Liu, Y.; Chen, H.; Ke, Y.; Xu, Q.-C.; Cheng, M.-S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4200.
5. Henryk, M.; Dorota, B.; Maria, N.; Wieslaw, K. *Carbohydr. Res.* **2003**, *338*, 133.
6. Dorota, B.; Wieslaw, K.; Henryk, M.; Lilianna, S.; Zygfryd, S.; Andrzej, Z. *Carbohydr. Res.* **2000**, *328*, 249.
7. Matthew, J. K.; Mary, L. T.; Mac, M.; Stacey, L. T.; Roxanne, P.; John, T.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2009**, *17*, 7670.
8. Matthew, J. K.; Mary, L. T.; John, T.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2008**, *16*, 3209.
9. Yen, G.-C.; Chen, Y.-L.; Sun, F.-M.; Chiang, Y.-L.; Lu, S.-H.; Weng, C.-J. *Eur. J. Pharm. Sci.* **2011**, *44*, 281.
10. Parthasarathi, P.; Manjuvani, A.; Meenubharathi, N.; Mary, B. C.-P.; Subbu, S. V.; Zaher, M. A. J. *Eur. J. Med. Chem.* **2012**, *53*, 1.
11. Cem, E.; Josef, F.; Wolfgan, M. *Cancer Lett.* **1998**, *128*, 137.
12. Yukihiro, A.; Hiroe, M.; Kenji, M.; Kenji, O.; Kumi, N.; Takashi, S.; Yoko, A.; Satoshi, M.; Yoshinori, N. *Biol. Pharm. Bull.* **2003**, *26*, 1057.
13. Danuta, S. K.; Patricia, Q.; Des, Q. R. R. *Future. Med. Chem.* **2009**, *1*, 1143.
14. Yu, Y.; Danuta, S. K.; Zaklina, K.; Aritee, R. S.; Patric, J. J.; Christian, S.; David, B. L.; Philip, C. S.; Paul, V. B.; Des, R. R. *J. Med. Chem.* **2009**, *52*, 5271.
15. Kesavan, K.; Kumari, P.; Venkatesan, J.; Arijit, B.; Nibha, M.; Bingsen, Z.; Shuya, H.; Yun, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6248.
16. Seiichi, I.; Koichi, F.; Shoichi, K. *Tetrahedron Lett.* **2001**, *42*, 7613.
17. Edson, F. S.; Marilene, M. C.-C.; Viviane, R. B.; Braz Lea, C.-F.; Leonor, L. L.; Aurea, E. *Eur. J. Med. Chem.* **2002**, *37*, 979.