Enzymatic glucosylation of unnatural naphthols by a promiscuous glycosyltransferase from *Aloe arborescens*

Kebo Xie, Yujiao Zhang, Ridao Chen, Dawei Chen, Lin Yang, Xia Liu, Jungui Dai

PII: S0040-4039(17)30483-5
DOI: http://dx.doi.org/10.1016/j.tetlet.2017.04.041
Reference: TETL 48833

To appear in: *Tetrahedron Letters*

Received Date: 17 March 2017
Revised Date: 10 April 2017
Accepted Date: 13 April 2017

Please cite this article as: Xie, K., Zhang, Y., Chen, R., Chen, D., Yang, L., Liu, X., Dai, J., Enzymatic glucosylation of unnatural naphthols by a promiscuous glycosyltransferase from *Aloe arborescens*, *Tetrahedron Letters* (2017), doi: http://dx.doi.org/10.1016/j.tetlet.2017.04.041

Tetrahedron Letters
journal homepage: www.elsevier.com

Enzymatic glucosylation of unnatural naphthols by a promiscuous glycosyltransferase from *Aloe arborescens*

Kebo Xie[a] , Yujiao Zhang[b] , Ridao Chen[a] , Dawei Chen[a] , Lin Yang[b] , Xia Liu[a] and Jungui Dai[a,] *

[a]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street 1, Beijing 100050, PR China*
[b]*College of Life and Environmental Sciences, Minzu University of China, Beijing 10081, PR China*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Enzymatic glucosylation
Catalytic promiscuity
Unnatural aglycons
Naphthols
Bioactive glycoside

ABSTRACT

Enzymatic glucosylation of unnatural products by natural glycosyltransferases (GTs) has great potential in creating novel and bioactive glucosides. A new GT (AaGT3) from *Aloe arborescens* exhibited catalytic promiscuity and high efficiency to diverse unnatural naphthols. By combing the substrate flexibility and catalytic reversibility of AaGT3, a cost-effective enzymatic approach to novel and bioactive unnatural glucosides was established. These studies indicate the significant potential of promiscuous natural GTs in synthesis of unnatural bioactive glucosides in drug discovery.

Glycosylation is widely found in natural products, which can not only contribute to the diversity of structures but also affect the physicochemical and biological properties of the corresponding glycosides.[1] In bioactive unnatural products and even some chemical drugs, sugar moieties are also essential and their removal oftentimes results in the loss of biological activity[2]. However, chemical glycosylation in the synthesis of glycosides faces challenges in regioselectivity, stereoselectivity, and the protection and de-protection of functional groups, which limit the exploitation of diverse unnatural glycosides in drug discovery.[3] Glycosyltransferases (GTs) are generally recognized as powerful synthetic tools for bioactive glycosides, as they can transfer sugar moieties from donors to acceptors generating various glycosides.[4] However, most of the GTs only recognize natural products and few of them show activity to unnatural products with skeletal types different from natural products.[5] Thus, the discovery of GTs with catalytic promiscuity and novel specificity to diverse unnatural products is of necessity in practice.

Naphthols and their derivatives, as bioactive unnatural products, are ubiquitous in the field of medicine.[6] Glycosylation of naphthols can not only improve the water solubility but also affect their pharmacological activity, which might provide lead compounds for drug discovery. However, to the best of our knowledge, none of the known GTs is able to glycosylate various unnatural naphthols. In this work, we reported a new GT from *Aloe arborescens* with catalytic promiscuity in glucosylation of diverse naphthols generating novel and bioactive glucosides.

A. arborescens is a famous medical plant and a wide variety of structurally diverse glycosides have been isolated from its leaves.[7] The diverse glycosyated secondary metabolites imply the existence of corresponding GTs, which inspired us to mine GTs with novel catalytic properties. Thus, to clone novel GTs from *A. arborescens*, a degenerate PCR primer for 5′-RACE was designed based on the conserved PSPG (plant secondary product glycosyltransferases) motif of five plant GTs which are able to catalyze unnatural products (Table S1 and Fig. S1 in the Supplementary Material, SM).[8] Combined with 3′-RACE, nine new *A. arborescens* GT genes (*AaGTs*) were successfully cloned by RT-PCR amplification using the total RNA from *A. arborescens* leaves as a template and heterologously expressed in *Escherichia coli* as described in SM.

To screen the glucosylation capability of the recombinant AaGTs *in vitro*, UDP-glucose (UDP-Glc) along with 2-naphthol (**1**), which is a typical and basic structural unit of naphthols were used in enzymatic assays. The screening reactions (50 mM Tris-HCl, pH 7.4; 0.5 mM UDP-Glc; 0.25 mM aglycon; 500 μg of crude AaGTs; 30 °C, 6 h) were analyzed by HPLC-UV/MS (high-performance liquid chromatography with ultraviolet/mass spectrometry detection). Of the nine recombinant AaGTs, only AaGT3 showed glycosylation activity to 2-naphthol (**1**) with high conversion rate (Fig. 1). Control reactions lacking either enzyme or UDP-Glc confirmed that the reactions were dependent upon both the enzyme and the sugar donor. The glucosylated product (**1a**) was isolated from the preparative-scale reaction, and the

* Corresponding author. Tel.: +86-10-63165195; fax: +86-10-63017757; e-mail: jgdai@imm.ac.cn

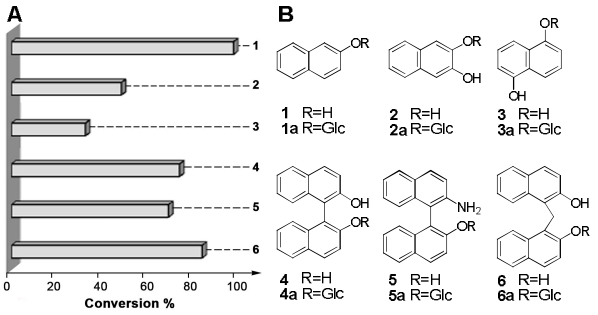


Figure 1. Catalytic promiscuity of AaGT3 in glucosylation of unnatural naphthols. (A) Percent conversions of glucosylated products catalyzed by AaGT3. The library numbers are listed based on the structural scaffolds shown in part B. (B) The structures of the aglycons and corresponding glucosylated products.

Table 1 Neuroprotective effects of 2-amino-2'-hydroxy-1,1'-binaphthalene (**5**) and its glucoside (**5a**) on the glutamate-induced toxicity in SK-N-SH cells

Compounds	Concentration (μM)	Cell viability (%)	Increased cell viability (%)
Control	0	100	0
L-Glutamic acid	2.5×10^4	62.08 ± 3.74	0
Resveratrol[a]	10	72.40 ± 4.13	16.69
5	10	63.33 ± 8.27	2.48
5a	10	72.08 ± 6.78	16.05*

[a] Resveratrol was used as a positive control.

* $P<0.05$

structure was characterized by MS, ^{1}H NMR and ^{13}C NMR spectroscopic data analyses (Figs. S6–S7 in SM). The observed large anomeric proton-coupling constant (J = 7.3 Hz, Table S2 in SM) indicated the formation of the β-anomer and an inverting mechanism for AaGT3. The cDNA sequence of *AaGT3* (1410 bp, GenBank accession number **KY662486**) contained an ORF encoding 469 amino acids, and the AaGT3 showed the highest identity to a predicted UGT88F-like glycosyltransferase from *Phoenix dactylifera*. Purification of recombinant His_6-AaGT3 was accomplished by His-tag affinity chromatography, and purified AaGT3 was analyzed by SDS-PAGE (Fig. S2 in SM).

To investigate the catalytic promiscuity of AaGT3 in recognizing diverse naphthols, a typical naphthols library including 2,3-dihydroxynaphthalene (**2**), 1,5-dihydroxynaphthalene (**3**), 1,1′-bi-2,2′-naphthol (**4**), 2-amino-2'-hydroxy-1,1'-binaphthalene (**5**) and 1,1'-methylenedi-2-naphthol (**6**) was employed for enzymatic assays (Fig. 1B). From the first-pass analysis with HPLC-UV/MS, AaGT3 was able to recognize all the naphthols in the library with high catalytic efficiency (Fig. 1). Of particular note is that all the acceptors are unnatural products, the structures of which have great differences to the natural products from *A. arborescens*. Generally, most of the reported GTs are capable of glucosylating acceptors with a typical phenol unit. However, AaGT3 was able to glucosylate structurally diverse unnatural naphthols, which indicated the catalytic promiscuity of this plant enzyme. The broad substrate spectrum and high efficiency make AaGTs being a potential tool enzyme in enzymatic synthesis of unnatural glycosides. To confirm the structures and evaluate their pharmacological activities, glycosylated products of **4**, **5** and **6** were prepared from the scale up reactions and identified by MS and NMR (Figs. S8–S15 in SM). All the prepared glucosides **4a**, **5a** and **6a** are

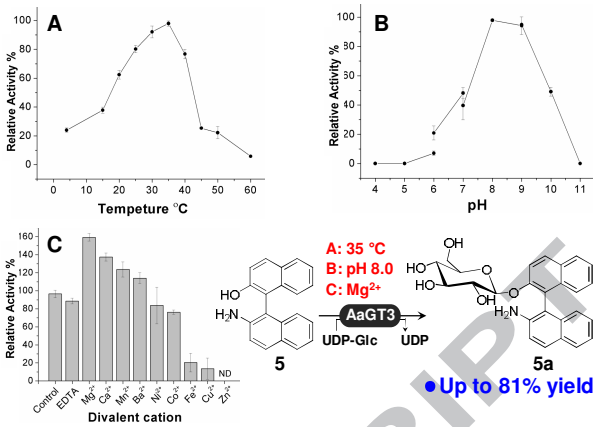


Figure 2. Enzymatic glucosylation of 2-amino-2'-hydroxy-1,1'-binaphthalene (**5**) by AaGT3 with UDP-Glc as a sugar donor. Relative activities of AaGT3 at different tempetures (A), pH buffers (B) and with divalent cations (C) are shown. ND: not detected. The yield of glucosylated product **5a** was up to 81% when the reaction was performed at 35 °C and in pH 8.0 buffer with Mg^{2+}.

novel compounds, and all anomers are in the β-configuration due to anomeric protons with large coupling constants (J > 7.0 Hz, Table S2 in SM). It is important to note that most glucosylated products exhibited improved water-solubility and the glucoside form **5a** had a more potent neuroprotective effect than the corresponding aglycon **5** toward the glutamate-induced toxicity in SK-N-SH cells,[9] which might be a potential drug lead for neurodegenerative disease (Table 1).

In order to improve the yield of bioactive product **5a**, the biochemical characteristics of AaGT3 were investigated with **5** as an acceptor and UDP-Glc as a sugar donor, respectively. The optimum temperature and pH of purified AaGT3 were determined to be 35 °C and pH 8.0, and this enzyme was independent of metal ions, while Mg^{2+} could greatly enhance the activity (Fig. 2). Thus the yield of **5a** was up to 81% under the optimum reaction conditions (50 mM Tris-HCl, pH 8.0; 0.5 mM UDP-Glc; 0.25 mM aglycon **5**; 0.5 mg of purified AaGT3; 5 mM $MgCl_2$; 35 °C, 6 h). Although the yield of **5a** has been improved to be more than 80%， the cost of this method is still not effective due to the high price of UDP-Glc.

In addition to the capability of glycosylating unnatural products, AaGT3 was also able to catalyze the deglycosylation of unnatural products such as 4-nitrophenyl-β-D-glucopyranoside (**7**), which means the glycosylation catalyzed by AaGT3 was reversible. As UDP-Glc was an expensive sugar donor, we thus exploited the reversibility of AaGT3 to provide sugar donors for generating bioactive glycosides through one-pot reaction (Fig 3). In the coupled reactions, UDP-Glc was generated by AaGT3-catalyzed deglycosylation of **7** with UDP, and the sugar moiety was intermediately transferred to the targeted aglycon acceptor (**5**) through the glycosylation catalyzed by AaGT3. The relative higher concentration of sugar donor (**7**) drives the one-pot reaction toward the deglycosylation of **7** and glycosylation of **5**. Thus, the high yield (62%) of **5a** occurred in the presence of only a catalytic amount of UDP (1/100 of **7**), which revealed that UDP was under cyclic utilization throughout the coupled reactions (Figs. 3A and S5 in SM). To further explore the substrate spectrum toward the NDP moieties, AaGT3 was subsequently probed with **5** and four other commercially available NDPs in one-pot reactions. Interestingly, AaGT3 displayed broad substrate specificity, recognizing ADP, TDP, CDP and GDP, as well as their corresponding activated glucoses, albeit with lower

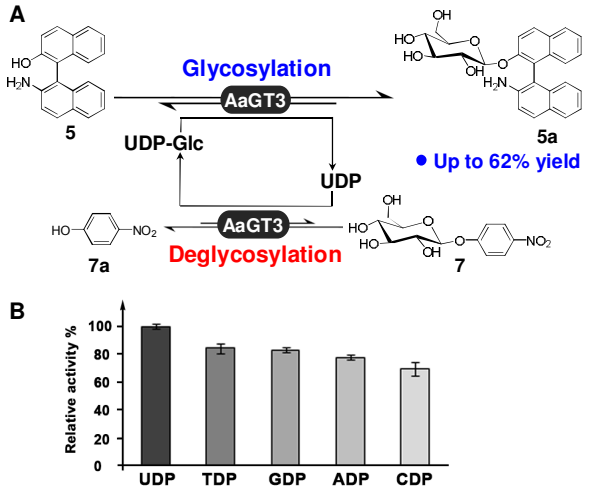


Figure 3. One-pot reaction catalyzed by AaGT3. (A) The bioactive glucoside (**5a**) was generated from a simple sugar donor (**7**) with a catalytic amount of UDP. (B) Besides UDP, other NDPs (TDP, GDP, ADP and CDP) can also be used in the one-pot reaction.

conversion rates than that of UDP (Fig. 3B). This feature allows AaGT3 to be applied in producing bioactive glucosides from a simple sugar donor *in vivo* with different NDPs of host strains.[10] Above all, the coupled reactions mediated by AaGT3 generating bioactive unnatural glucosides from abundant unnatural glucosides without adding activated sugars establishes a cost-effective enzymatic method for synthesizing diverse bioactive glucosides.

In summary, enzymatic glucosylation of unnatural naphthols for generating novel and bioactive unnatural glucosides was achieved by a natural GT AaGT3 from *A. arborescens*. AaGT3 exhibited robust glucosylation activity toward simple basic units and structurally diverse derivatives of naphthols. Moreover, the catalytic reversibility of AaGT3 coupled with its catalytic promiscuity was exploited as a powerful biocatalyst for the enzymatic synthesis of novel and bioactive unnatural glucosides. This study not only demonstrates the application prospect of natural GTs in synthesis of target unnatural glycosides with pharmacological activities but also provides a potential tool in glycorandomization of diverse unnatural products.

Acknowledgments

This work is financially supported by the National Natural Science Foundation of China (Grant No. 81602999) and CAMS Innovation Fund for Medical Sciences (CIFMS-2016-I2M-3-012).

References and notes

1. (a) Weymouth-Wilson, A. C. *Nat. Prod. Rep.* **1997**, 14, 99–110; (b) Luzhetskyy, A.; Bechthold, A. *Appl. Microbiol. Biotechnol.* **2008**, 80, 945–952; (c) Chang, A.; Singh, S.; Phillips, G. N.; Thorson, J. S. *Appl. Microbiol. Biotechnol.* **2008**, 80, 945–952; (d) Elshahawi, S. I.; Shaaban, K. A.; Kharel, M. K.; Thorson, J. S. *Chem. Soc. Rev.* **2015**, 44, 7591–7697.
2. (a) Kren, V.; Martínková, L. *Curr. Med. Chem.* **2001**, 8, 1303–1328; (b) Belkhadem, F.; Othman, A. A. *Mol. Diversity* **2016**, 21, 115–124; (c) Meng, W.; Ellsworth B. A.; Nirschl, A. A.; McCann, P. J.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Morrison, E. P.; Biller, S. A.; Zahler, R.; Deshpande, P. P.; Pullockaran, A.; Hagan, D. L.; Morgan, N.; Taylor, J. R.; Obermeier, M. T.; Humphreys, W. G.; Khanna, A.; Discenza, L.; Robertson, J. G.; Wang, A.; Han, S.; Wetterau, J. R.; Janovitz, E. B.; Flint, O. P.; Whaley, J. M.; Washburn, W. N. *J. Med. Chem.* **2008**, 51, 1145–1149.
3. (a) Blanchard, S.; Thorson, J. S. *Curr. Opin. Chem. Biol.* **2006**, 10, 263–271; (b) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, 93, 1503–1531; (c) Cox, D. J.; Smith, M. D.; Fairbanks, A. J. *Org. Lett.* **2010**, 12, 1452–1455; (d) Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, 8, 497–510.
4. (a) Lim, E. K. *Chem. Eur. J.* **2005**, 11, 5486–5494; (b) Luzhetskyy, A.; Méndez, C.; Salas, J. A.; Bechthold, A. *Curr. Top. Med. Chem.* **2008**, 8, 680–709; (c) Gantt, R. W.; Peltier-Pain, P.; Thorson, J. S. *Nat. Prod. Rep.* **2011**, 28, 1811–1853.
5. (a) Minami, A.; Uchida, R.; Eguchi, T.; Kakinuma, K. *J. Am. Chem. Soc.* **2005**, 127, 6148–6149; (b) Gantt, R. W.; Goff, R. D.; Williams, G. J.; Thorson J. S. *Angew. Chem. Int. Ed.* **2008**, 47, 8889–8892; (c) Xie, K.; Chen, R.; Li J.; Wang, R.; Chen, D.; Dou, X.; Dai, J. *Org. Lett.* **2014**, 16, 4874–4877; (d) Sun, L.; Chen, D.; Chen, R.; Xie, K.; Liu, J.; Yang, L.; Dai, J. *Tetrahedron Lett.* **2016**, 57, 1518–1521; (e) Chen, D.; Chen, R.; Wang, R.; Li, J.; Xie, K.; Bian, C.; Sun, L.; Zhang, X.; Liu, J.; Yang, L.; Ye, F.; Yu, X.; Dai, J. *Angew. Chem. Int. Ed.* **2015**, 54, 12678–12682; (f) Xie, K.; Chen, R.; Chen, D.; Li, J.; Wang, R.; Yang, L.; Dai, J. *Adv. Synth. Catal.* **2017**, 359, 603–608; (g) Feng, J.; Zhang, P.; Cui, Y.; Li, K.; Qiao, X.; Zhang, Y.; Li, S.; Cox, R. J.; Wu, B.; Ye, M.; Yin, W. *Adv. Synth. Catal.* **2017**, DOI:10.1002/adsc.201601317.
6. (a) Das, B.; Reddy, C. R.; Kashanna, J.; Mamidyala, S. K.; Kumar, C. G. *Med. Chem. Res.* **2012**, 21, 3321–3325; (b) Todd, P. A.; Clissold, S. P. *Drugs* **1990**, 40, 91–137; (c) Moore, B. R.; Laman, M.; Salman, S.; Batty, K. T.; Page-Sharp, M.; Hombhanje, F.; Manning, L.; Davis, T. M. *Drugs* **2016**, 76, 789–804.
7. (a) Chen, W.; Van Wyk, B.; Vermaak, I.; Viljoen, A. M. *Phytochem Lett.* **2012**, 5, 1–12; (b) Beppu, H.; Kawai, K.; Shimpo, K.; Chihara, T.; Tamai, I.; Ida, C.; Ueda, M.; Kuzuya, H. *Biochem. Syst. Ecol.* **2004**, 32, 783–795.
8. (a) Vogt, T.; Jones, P. *Trends Plant Sci.* **2000**, 5, 380–386; (a) Gachon, C. M.; Langlois-Meurinne, M.; Saindrenan, P. *Trends Plant Sci.* **2005**, 10, 542–549.
9. Ahmed, I.; Bose, S. K.; Pavese, N.; Ramlackhansingh, A.; Turkheimer, F.; Hotton, G.; Hammers, A.; Brooks, D. J. *Brain* **2011**, 134, 979–986.
10. Williams, G. J.; Yang, J.; Zhang, C.; Thorson, J. S. *ACS Chem. Biol.* **2011**, 6, 95–100.

Supplementary Material

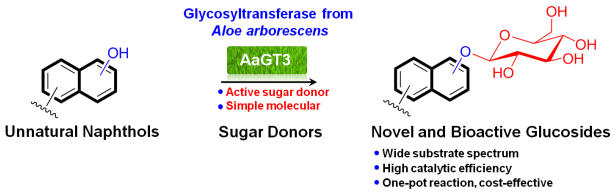
Supplementary material (experimental operations, including gene cloning, expression, reaction analysis and products purification protocols, LC/MS, HRESIMS and NMR characterization data and spectra of glucosylated products) associated with the article can be found, in the online version, at http:xxx

Graphical Abstract

Enzymatic glucosylation of unnatural naphthols by a promiscuous glycosyltransferase from *Aloe arborescens*

Leave this area blank for abstract info.

Kebo Xie, Yujiao Zhang, Ridao Chen, Dawei Chen, Lin Yang, Xia Liu, Jungui Dai*



Highlights

A new GT from *Aloe arborescens* exhibited catalytic promiscuity and high efficiency to diverse unnatural naphthols.

A cost-effective enzymatic approach to novel and bioactive unnatural glycosides was established.

This work indicates the significant potential of natural GTs in synthesis of unnatural bioactive glycosides.