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Synthetic analogues of durantoside I from *Citharexylum spinosum* L. and their cytotoxic activity

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ABSTRACT

New iridoid glycoside derivatives from durantoside I, the latter from the dried flowers and leaves of *Citharexylum spinosum*, were synthesized by variously modifying a sugar moiety by silylation or acetylation and/or removal of cinnamate group at C-7 position and subsequent screening for comparative cytotoxicity against several cancer cell lines. Addition of alkylsilane to durantoside I and removal of cinnamate group were most effective in improving cytotoxicity.

Natural products are an important source of therapeutic medicines in large part a function of their structural variability that allows for great diversity in synthetic compounds including new medical drugs. Indeed, almost half of all current medicines are either natural products or their semi-synthetic derivatives. Iridoid glycosides are a class of natural products, present in several plant species that possess a variety of medicinal properties including anti-inflammatory,[1] anticancer,[2] anti-insects,[3] antioxidant[4,5] and antimicrobial.[6] Their structure contains cyclopentanoid monoterpene-derived moieties with a sugar group attached at C1 position.[7,8] Several new iridoid glycosides have been isolated from plants but few with scaffold modifications suggestive of enhanced therapeutic properties.[9–11] This may reflect difficulties in isolation requiring several separation circles as well as stability of iridoid glycosides in chemical reactions. Durantoside I, is an iridoid glycoside present in some common and widely distributed Thailand plants within the family Verbenaceae and genera, *Durantaerecta*, *Durantarepens L.*[12–15] and *Citharexylum spinosum* L.[16,17] Extracts of one species, *C. spinosum* possess antiulcer, antihypertensive and hepatoprotective properties.[18] Moreover, the alcoholic extract and essential oil from this plant exhibited immunomodulatory[19] and antifungal properties.[20]

Durantoside I consists of a cyclopentane ring fused to a six-membered oxygen heterocycle with a cinnamic group at C-7 position of cyclopentane and glucose at position β on the iridoid. The present study designed and synthesized new cytotoxic compounds from a natural iridoid glycoside extracted from the dried flowers and leaves of *Citharexylum spinosum* L.[16,17] and measured their cytotoxic activity. Syntheses were designed through modifications of sugar moiety by silylation, acetylation and removal of cinnamate group at C-7 positions to obtain 13 analogues. Earlier, we demonstrated silicon in natural molecules possesses significant cytotoxic activity.[21,22] We discovered the addition of alkylsilane to andrographolide improved anticancer properties over those of the natural parent compound. Therefore, we converted the C-6 hydroxy group of sugar in Durantoside I structure to silylether group to enhance activity of this natural compound.

Synthesis protocols of natural durantoside I derivatives are outlined in Schemes 1 and 2. Acetylation reaction of hydroxy groups with acetic anhydride using pyridine was carried out at ambient temperature to produce 2″, 3″, 4″, 6″-tetraacetyl-durantoside **3** in 25% along with penta-acetylated product **2** in 12% yields, which hydroxyl group was acetylated at C8 position after continue stirring the reaction overnight (Scheme 1). Hydrolysis of cinnamate

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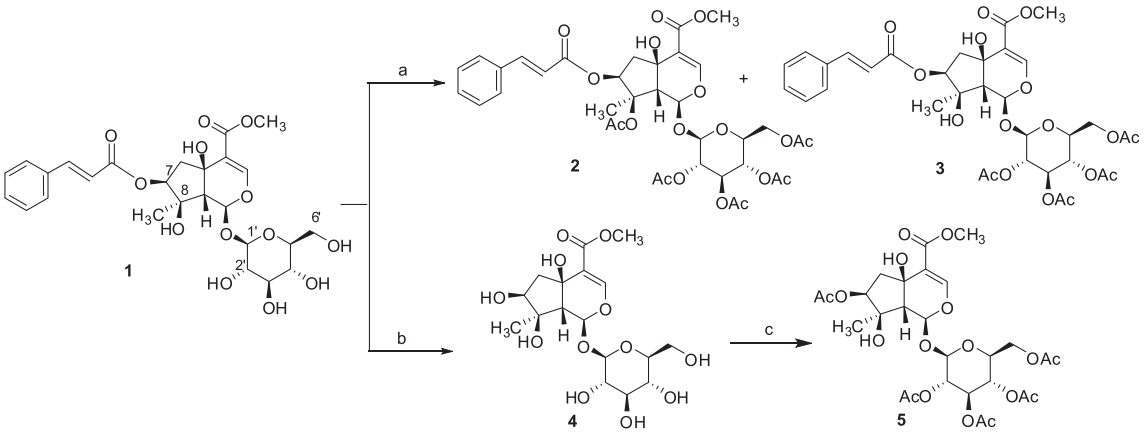
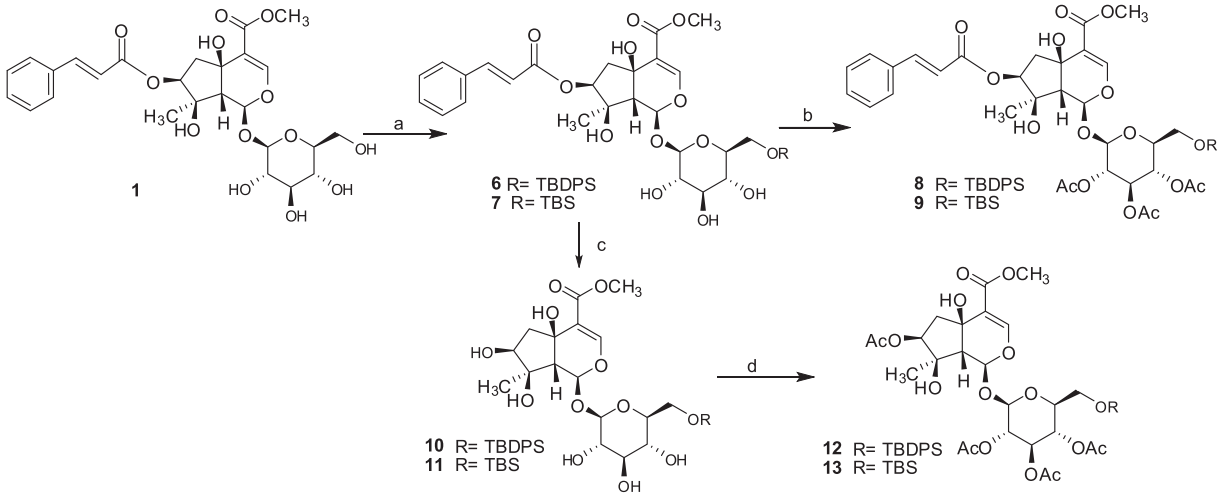


Scheme 1. Reagents and conditions: (a) pyridine, 10 equiv. Ac_2O, rt., overnight (b) 3.2 equiv. $Ba(OH)_2$, MeOH, rt. 40 min. (c) pyridine, excess Ac_2O, rt., 4 h.



Scheme 2. Reagents and conditions: (a) 2 equiv. TBDPSCl or TBSCl, pyridine, rt., 2 h. (b) pyridine, 8 equiv. Ac_2O, rt., 4 h. (c) 2 equiv. $Ba(OH)_2$, MeOH, 60 °C, 20 min. (d) pyridine, 10 equiv. Ac_2O, rt., 4 h.

group at C-7 using $Ba(OH)_2$ in MeOH was performed to give **4** followed by acetylation using pyridine and Ac_2O for 4 h to produce compounds 2″, 3″, 4″, 6″-acetyl-durantoside **5** in 32% overall yield in two steps.

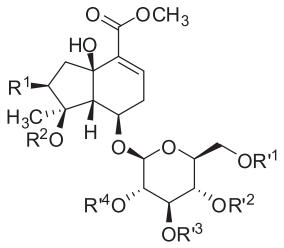
Selective silylation of the hydroxy group at C-6″ of the sugar ring to silyl ethers in natural durantoside I was carried out at ambient temperature *via* reactions with *tert*-butyldiphenylsilylchloride (TBDPSCl) or *tert*-butyldimethylsilylchloride (TBSCl) and pyridine to produce silyl-analogues **6** and **7** in yields of 69% and 62%, respectively. Acetylation of the three remaining hydroxy groups on sugar with acetic anhydride and pyridine was performed at ambient temperature for 4 h to produce durantoside derivatives **8** and **9** in good yield (75 and 69% respectively). Hydrolysis of cinnamate group of compounds **6** and **7** at C-7 position using barium hydroxide was conducted in MeOH to give **10** and **11** followed by acetylation in step two without separation of **10** and **11**. The process was conducted at room temperature and the substances were stirred for 4 h producing yields of iridoid glycoside compounds **12** and **13** of 55 and 27%, respectively (Scheme 2).

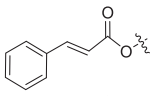
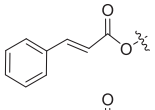
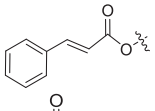
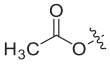
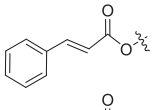
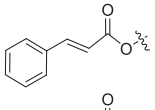
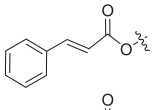
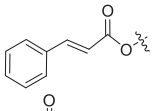
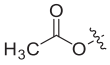
Cytotoxic activity of dutantoside I and synthetic analogues

Cytotoxic activities of all synthetic analogues were evaluated using *in vitro* screening against selected cancer cell lines by *in vitro* screening: P-388 (murine leukaemia), KB (human epidermoid carcinoma of the mouth), HT-29 (human colorectal adenocarcinoma), MCF-7 (human breast cancer), A549 (human lung adenocarcinoma) and ASK (rat glioma Table 1). Sulforhodamine B (SRB) assay was carried out to evaluate the cytotoxic activities of synthesized compounds. All tested analogues were dissolved in DMSO (less than 0.05%). Ellipticine, a potent anti-cancer agent was used as a positive control. Cells at concentration of 10^4 cells/well were seeded in 96-well plates with varying concentrations of compounds (0.1–50 μM). At the end of treatment period, cells were washed, fixed with trichloroacetic acid (TCA) and were stained with 0.4% SRB dissolved in 1% acetic acid. The cellular protein-bound dye was extracted for the determination at optical density (515 nm) with a 96 well microliter plate reader. The amount of bound dye can be extrapolated to measure cell viability. The cytotoxic potency was expressed as IC_{50}, the concentration that inhibit 50% of cell viability.

Cytotoxic activities of durantoside analogues indicated the importance of the substituted group of the hydroxy function at C-6 on the sugar ring. Acetyl sugar durantoside I analogues **2**, **3**, **4** and **5** showed no cytotoxic activity in all cell lines similar to the parent compound 1. However, with the introduction of a *tert*-butyldiphenylsilyl (TBDPS) group on the C-6 of sugar ring (compound **6**), cytotoxic activity increased significantly in all cell lines in the range 9–27 μM. Analog **6** displayed stronger activity

Table 1
Cytotoxic activity of synthetic Durantoside I analogues.



Compound	R^1	R^2	R'^1	R'^2	R'^3	R'^4	IC_{50}[a] ± SD (μM)					
							P-388	KB	HT-29	MCF-7	A-549	ASK
1		H	H	H	H	H	>50	>50	>50	>50	>50	>50
2		Ac	Ac	Ac	Ac	Ac	>50	>50	>50	>50	>50	>50
3		H	Ac	Ac	Ac	Ac	>50	>50	>50	>50	>50	>50
5		H	Ac	Ac	Ac	Ac	>50	>50	>50	>50	>50	>50
6		H	TBDPS	H	H	H	20.61 ± 3.68	14.63 ± 1.49	16.35 ± 1.61	9.47 ± 0.25	9.63 ± 0.43	26.85 ± 0.26
7		H	TBS	H	H	H	>50	>50	>50	>50	>50	>50
8		H	TBDPS	Ac	Ac	Ac	6.11 ± 2.07	>50	37.17 ± 1.63	>50	>50	>50
9		H	TBS	Ac	Ac	Ac	3.11 ± 0.53	>50	>50	43.33 ± 5.92	>50	>50
12		H	TBDPS	Ac	Ac	Ac	3.67 ± 0.38	14.74 ± 2.67	35.04 ± 1.45	9.01 ± 0.20	8.20 ± 0.28	9.19 ± 0.30
13		H	TBS	Ac	Ac	Ac	41.02 ± 1.46	>50	>50	>50	>50	>50
Ellipticine[b]							1.84 ± 0.082	2.15 ± 0.02	2.00 ± 0.084	1.99 ± 0.039	1.96 ± 0.033	2.02 ± 0.280

[a] IC_{50} values (the concentration that inhibit 50% of cell viability) in μM.
[b] Positive control.

than the parent Durantoside I and especially on MCF-7 and A-549 cancer cell lines with the IC_{50} <10 μM. Acetylation of the remaining hydroxy group on sugar of **6** to **8**, resulted in a dramatic reduction in potency of all cancer cells except P-388. Compound **8** exhibited selective cytotoxic activity against P-388 cells. Removal of the cinnamate group at C-7 of compound **8** led to analog **12,** the most active compound of this study. SilylTBDPS analog **12** exhibited high cytotoxic activity with an IC_{50} below 10 μM on P-388, MCF-7, A-549 and ASK cancer cell lines. This indicated the structural presence of cinnamate group was not important with respect to enhancing cytotoxic activity of iridoid glycoside. Replacement of silyl-TBDPS group at C-6 of sugar with other silyl derivatives such as silyl-TBS (compound **7**) did not enhance cytotoxic activity compared to that of silyl-TBDPS analogues indicating the importance of the type of silyl-substituted group. However, compounds bearing silyl-TBS (compounds **9** and **13**) showed selective cytotoxicity against P-388 over other cell lines.

In conclusion, Durantoside I was isolated from dried flowers and leaves of *Citharexylum spinosum* L. Molecular structure was modified by chemical reactions including silylation, acetylation, and decinnamoylation to produce new analogues of iridoid glycoside. Semi-synthetic compounds **6** and **12** exhibited higher cytotoxic activity than the original natural durantoside I in all cell lines. Silyl-TBDPS analog **12** without cinnamate group showed generally higher cytotoxicity than compound **6** which contained cinnamate at C-7. This supports the design and synthesis of new iridoid analogues with smaller molecular units without cinnamate as potentially important cytotoxic agents. To our knowledge, this is the first report on structure modifications of natural durantoside I and their respective cytotoxic activities against a series of cancer cell lines.

Conflicts of interest

We declare that we have no conflict of interest.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.03.068.

References

1. Zhang L, Zhu T, Qian F, et al. Iridoid glycosides isolated from *Scrophulariadentata* Royle ex Benth. and their anti-inflammatory activity. *Fitoterapia*. 2014;98:84–90.
2. Rakotondramasy VC, Mouriès C, Cachet X, et al. A novel series of cytotoxic iridoidglucosides derived from aucubin: Design, synthesis and structure activity relationships. *Eur J Med Chem*. 2010;45:2314–2320.
3. Dobler S, Petschenka G, Pankoke H. Coping with toxic plant compounds – The insect's perspective on iridoidglycosides and cardenolides. *Phytochemistry*. 2011;72:1593–1604.
4. Abdel-Mageed WM, Backheet EY, Khalifa AA, Ibraheim ZZ, Ross SA. Antiparasitic antioxidant phenylpropanoids and iridoid glycosides from *Tecomamollis*. *Fitoterapia*. 2012;83:500–507.
5. Mar A, Pripdeevech P. Chemical composition and antibacterial activity of essential oil and extracts of *Citharexylumspinosum* flowers from Thailand. *Nat Prod Commun*. 2014;9:707–710.
6. Lelono RAA, Tachibana S, Itoh K. Isolation of antifungal compounds from *Gardenia jasminoides*. *Pak J Biol Sci*. 2009;12:949–956.
7. Dinda B, Debnath S, Harigaya Y. Naturally occurring iridoids. A review, part 1. *Chem Pharm Bull*. 2007;55:159–222.
8. Ren L, Xue X, Zhang F, et al. Studies of iridoid glycosides using liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2007;21:3039–3050.
9. Misra A et al. Modified iridoid glycosides as anti-implantation agents: inhibition of cell adhesion as an approach for developing pregnancy interceptive agents. *Bioorg Med Chem*. 2001;9:2763–2772.
10. Takeda Y, Tanigawa N, Sunghwa F, et al. Morroniside cinnamic acid conjugate as an anti-inflammatory agent. *Bioorg Med Chem Lett*. 2010;20:4855–4857.
11. Pandeti S, Sharma K, Bathula S, Tadigoppula N. Synthesis of novel anticancer iridoid derivatives and their cell cyclearrest and caspase dependent apoptosis. *Phytomedicine*. 2014;21:333–339.
12. Rimpler H, Timm H. Iridoideaus *Durantarepens* L. *Z Naturforsch*. 1974;29C:111–115.
13. Kuo Y-H, Chen Z-S, Lin Y-L. Chemical components of the leaves of *Durantarepens* Linn. *Chem Pharm Ball*. 1996;44:429–436.
14. Mohammed MHH, Hamed ANE-S, Khalil HE, Kamel MS. Phytochemical and pharmacological studies of *Citharexylum quadrangulare* Jacq. Leaves. *J Med Plants Res*. 2016;10:232–241.
15. Takeda Y, Morimoto Y, Matsumoto T, et al. Iridoid glucosides from the leaves and stems of *Durantaeracta*. *Phytochemistry*. 1995;39:829–833.
16. Bablzs B, Toth G, Duddeck H, Soliman HSM. Iridoid and lignan glycosides from *Citharexylum spinosum* L. *Nat Prod Res*. 2006;20:201–205.
17. Ayeb-Zakhama AE, Beyaoui A, Salem SB, et al. Phytochemical and phytotoxic investigation of the flowers from *Citharexylum spinosum* L. *Ind Crops Prod*. 2015;76:653–659.
18. Khalifa TI, El-Gindi OD, Ammar HA, El-Naggar DM. Iridoid glycosides from *Citharexylum quadrangular*. *Asian J Chem*. 2002;14:197–202.
19. Shalaby NMM, Bahgat M. Phytochemistry and some biological studies of *Citharexylum quadrangular* Jacq. *Bull Fac Pharm Cairo Univ*. 2003;41:219–228.
20. Mansour MMA, Salem MZM, Khamis MH, Ali HM. Natural Durability of *Citharexylum spinosum* and *Morusalba* woods against three mold fungi. *Bioresources*. 2015;10:5330–5344.
21. Sirion U, Kasemsook S, Suksen K, Piyachaturawat P, Suksamrarn A, Saeeng R. New substituted C-19-andrographolide analogues with potent cytotoxic activities. *Bioorg Med Chem Lett*. 2012;22:49–52.
22. Kasemsook T, Piyachaturawat P, Bunthawong R, et al. One-pot three steps cascade synthesis of novel isoandrographolide analogues and their cytotoxic activity. *Eur J Med Chem*. 2017;138:952–963.