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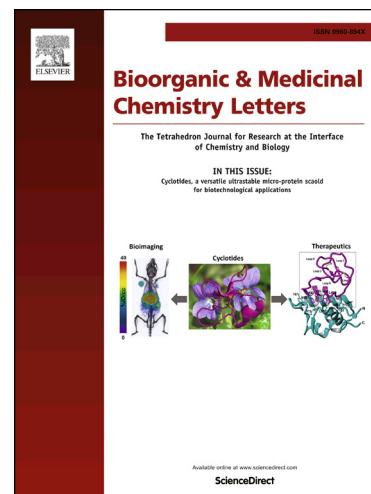
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Synthetic analogues of durantoside I from *Citharexylum spinosum* L. and their cytotoxic activity

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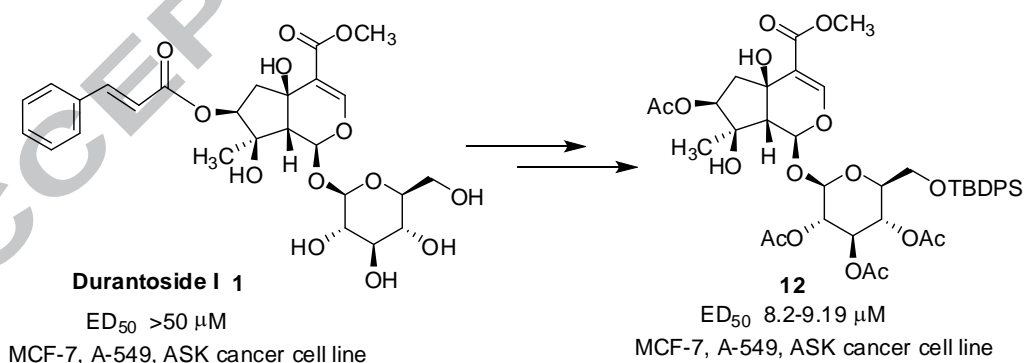
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Abstract

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.



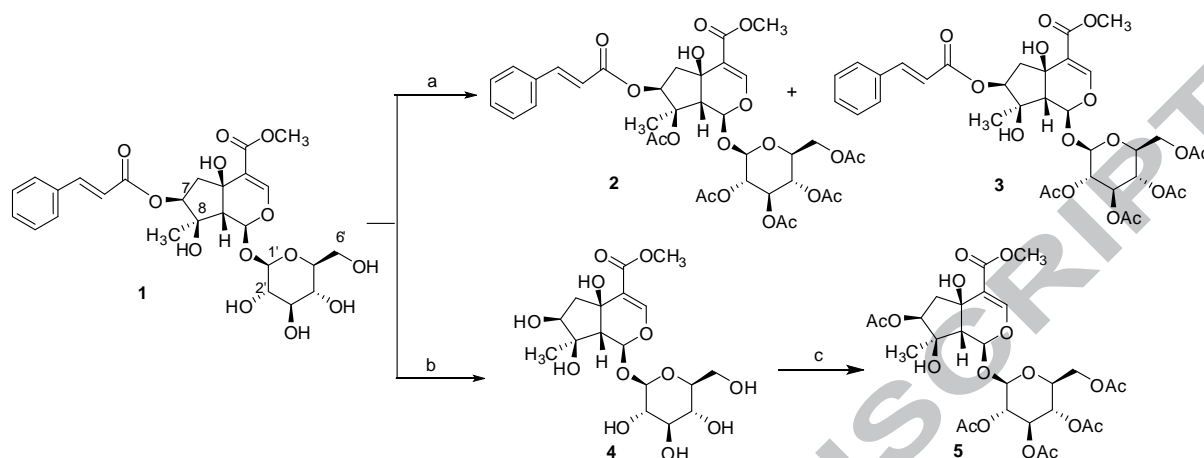
Keywords *Citharexylum spinosum* L., durantoside I, cytotoxic activity, iridoid glycoside

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-inflammatory¹, anticancer², anti-insects³, 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. Durantose I, is an iridoid glycoside present in some common and widely distributed Thailand plants within the family Verbenaceae and genera, *Duranta erecta*, *Duranta repens* 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 immunomodulatory¹⁹ and antifungal properties.²⁰

Durantose 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 Durantose I structure to silylether group to enhance activity of this natural compound.

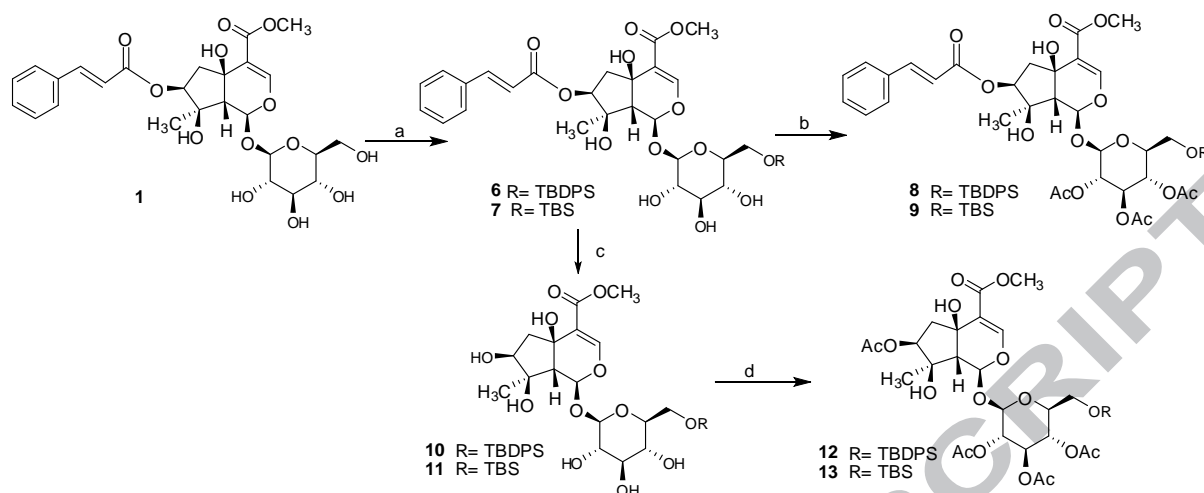
Synthesis protocols of natural durantose I derivatives are outlined in Scheme 1 and 2. Acetylation reaction of hydroxy groups with acetic anhydride using pyridine was carried out at ambient temperature to produce 2'', 3'', 4'', 6''-tetraacetyl-durantose **3** in 25% along with penta-acetylated product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



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

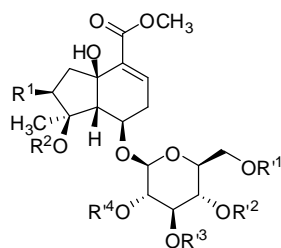
Selective silylation of the hydroxy group at C-6'' of the sugar ring to silyl ethers in natural durantoside **1** was carried out at ambient temperature *via* reactions with *tert*-butyldiphenylsilylchloride (TBDPSCl) or *tert*-butyldimethylsilylchloride (TBSCl) and pyridine to produce silyl-analogues **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).



Scheme 2 Reagents and conditions: (a) 2 equiv. TBDPSCl or TBSCl, pyridine, rt., 2h. (b) pyridine, 8 equiv. Ac₂O, rt., 4h. (c) 2 equiv. Ba(OH)₂, MeOH, 60°C, 20 min. (d) pyridine, 10 equiv. Ac₂O, rt., 4h.

2.2 Cytotoxic activity of Dutantoides 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 10⁴ 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 IC₅₀, the concentration that inhibit 50% of cell viability.

Table 1 Cytotoxic activity of synthetic Durantoside I analogues

Compound	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	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

^aIC₅₀ values (the concentration that inhibit 50% of cell viability) in μM.^bPositive control

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 than the parent Durantoside I and especially on MCF-7 and A-549 cancer cell lines with the IC₅₀<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 analogue **12** exhibited high cytotoxic activity with an IC_{50} below 10 μ M 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 analogs 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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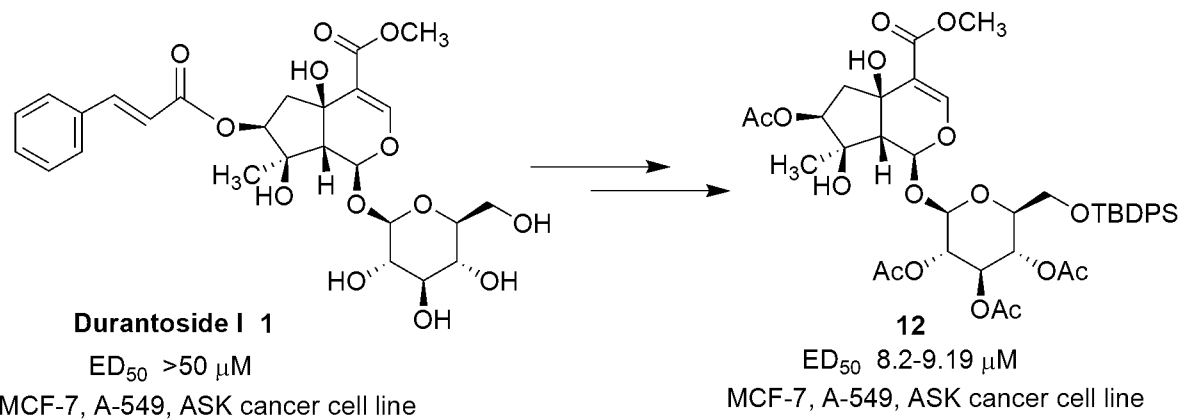
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Highlights

- Structure modifications of natural durantose I from dried flowers and leaves of *Citharexylum spinosum* L. were carried out.
- New synthetic iridoid analogues were screened for cytotoxicity against six cancer cells.
- Silyl analog without cinnamate group showed generally higher cytotoxicity than compound which contained cinnamate at C-7.