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Synthesis, characterization and biological studies of diosgenyl analogues

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ABSTRACT

A series of optical amino acid diosgenyl esters and diosgenyl salicylate conjugates were designed and synthesized to develop new anticancer and anti-inflammatory agents. The analogue **9c** that contains an 6-aminohexanoic acid residue at C-3 of diosgenin exhibits higher potency against all three tumor cell lines with IC_{50} values ranging from 4.7 μM in C26 cells to 14.6 μM in Hep G2 cells. In addition, seven of newly synthesized compounds significantly inhibit xylene-induced ear edema and exhibit comparable or better anti-inflammatory activities than those of diosgenin and aspirin. Furthermore, preliminary structure–activity relationship studies demonstrate that diosgenyl salicylate conjugates have stronger anti-inflammatory activities than amino acid diosgenyl esters.

Diosgenin (3β-hydroxy-5-spirostene, Fig. 1), which is a major steroidal sapogenin that is extracted from *Dioscorea zingiberensis* C.H. Wright, is a main precursor for the synthesis of sex hormones, corticosteroids, and other steroid products.[1] Recent studies indicate that diosgenin may be an active constituent that contributes to a variety of biological activities, for example, by exhibiting anti-inflammatory[2] and anti-thrombosis effects,[3] inducing megakaryocytic differentiation in human erythroleukemia cells,[4] enhancing regulatory T-cell immunity,[5] and inducing apoptosis in DU-145 prostate cancer cells[6] and HCT-116 human colon carcinoma cells.[7] In recent years, diosgenyl esters have received considerable attention due to their favorable anticancer properties.[8] Kvasnica[9] designed and synthesized twelve steroidal platinum (II) complexes with steroidal esters (i.e., cholesterol, diosgenin, and cholestanol) of L-histidine and L-methionine; several of the compounds showed significant activities against T-lymphoblastic leukemia CEM cell line with IC_{50} values in the range of 14–25 μM.

Aspirin, which is a synthetic antipyretic, analgesic, and anti-inflammatory agent, has been known for over 110 years. Today, it is still one of the most widely consumed nonsteroidal anti-inflammatory drugs (NSAIDs) in the world. Inflammation is a symptom of various common diseases, and it can be an early phase of serious diseases such as cancer.[10] In 1988, Kune and his collaborators reported a 50% reduction in the risk of colorectal cancer in patients who regularly took aspirin, compared with controls.[11] Subsequent studies have shown that long-term aspirin use aids in the prevention of vascular events and may reduce the risk of cancer, and it potentially leads to a reduced death rate from cancer.[12–15] Since the relationship between inflammation and carcinogenesis has been examined in so many case-controlled studies,[16] the synthesis of new complexes containing anti-inflammatory drugs such as aspirin or salicylic acid is a strategy that is employed for the development of potential anti-tumor drugs.[17] However, long-term administration of aspirin or high doses of aspirin could block the production of prostaglandin E2; this result in the risk of aspirin-induced gastrointestinal bleeding increases with age and dose.[18] A variety of approaches have been investigated to avoid aspirin-induced gastrointestinal effects including decreasing the acidity and increasing the pharmacological activity of the drugs, which can be achieved by preparing amino acid salts and ester derivatives of aspirin.[19]

In recent years, our group has focused on studying the extraction and applications of *Dioscorea zingiberensis* C.H. Wright,[20,21] the acute toxicity and sub-chronic toxicity of diosgenyl saponins,[22] and preliminary structure–activity relationships of steroidal saponins.[23] In addition, we have reported the anti-thrombosis effects of diosgenin,[3] the anticancer activity and anti-angiogenesis of deltonin,[24,25] the protective effects of diosgenin in human vascular endothelial cells against hydrogen peroxide-induced apoptosis,[26] etc. Clinical studies are underway and preliminary results indicate that NSAID/aspirin and steroidal sapogenin/diosgenin share some similar pharmacological activities, including anti-inflammatory and anti-thrombosis effects as well as anticancer properties. Herein, we report the synthesis of diosgenyl esters coordinated to aspirin and diosgenin via a series of amino acids, including

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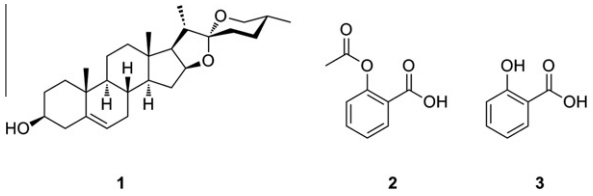


Figure 1. Structures of diosgenin (**1**), aspirin (**2**), and salicylic acid (**3**).

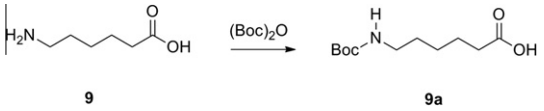
glycine, alanine, glutamic acid, and 6-aminohexanoic acid with the aim of increasing the anticancer activities and anti-inflammatory properties of these compounds and eliminating the gastrointestinal side effects of aspirin. These complexes were also investigated to elucidate how the amino acids affect the cytotoxic activity of the compounds against human breast cancer MDA-MB-231 cells, murine colon carcinoma C26 cells, and human hepatocellular carcinoma Hep G2 cells. Further, preliminary structure–activity relationships of these compounds were analyzed via their anti-inflammatory activities using a xylene-induced ear edema model.

In this present study, a series of diosgenyl derivatives, including four amino acid diosgenyl esters (**6c**–**9c**) and six diosgenyl salicylate compounds (**5**, **6d**, **6e**, **7e**, **8d** and **9e**), were synthesized. The pyridine-promoted esterification of diosgenin and 2-acetoxybenzoyl chloride[27] mainly provided the desired compound **5** (Scheme 1). With increased reaction time or temperature, more diosgenyl acetate **5a** was generated and the yield of compound **5** decreased from 65% to 40%.

N-tert-Butyloxycarbonates are stable toward a variety of experimental conditions and easily convert to the corresponding primary amines under mildly acidic conditions. In this study, all N-protected amino acids used were commercially obtained except for compound **9a**, which was synthesized via the reaction of 6-aminohexanoic acid with $(Boc)_2O$ in an acetone–water mixed solvent in the presence of triethylamine (Scheme 2).[28] Unfortunately, the target compound **9a** could not be obtained using the method reported by Chankeshwara without basic catalytic compounds.[29] Therefore, the alkalinity provided by triethylamine (Et_3N) is important for *N-tert*-butyloxycarbonylation.

Amino acid diosgenyl esters **6c**–**9c**, were prepared by an esterification reaction involving DCC/DMAP, the corresponding *N-tert*-butoxycarbonyl amino acids, and diosgenin in dry dichloromethane followed by deprotection of the *N-tert*-butoxycarbonyl group using trifluoroacetic acid at room temperature. Meanwhile, didiosgenyl ester **7c** was obtained when *N*-(*tert*-butoxycarbonyl)-D-glutamic acid was used as the reagent.

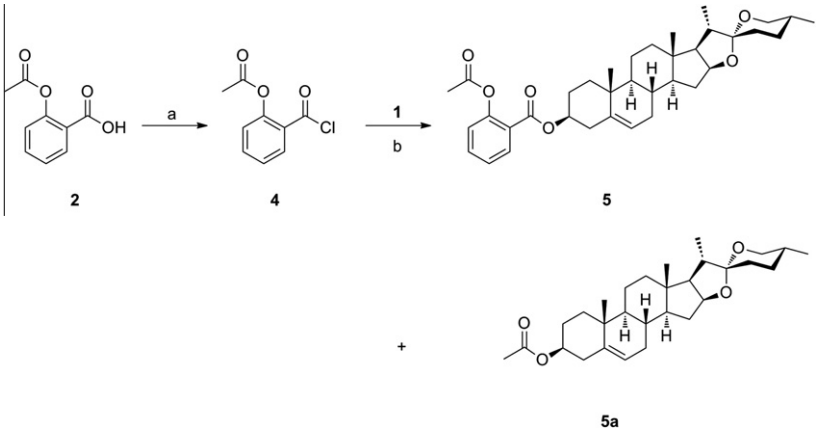
The diosgenyl salicylate compounds were synthesized. The coupling of amino acid diosgenyl esters **6c**–**9c** with 2-acetoxybenzoyl chloride in the presence of triethylamine afforded **6d**, **6e**, **7e**, **8d** and **9e** in good yields. However, the target products **6e**, **7e**, and **9e** by losing of the acetyl group at 2-acetoxybenzoyl part were respectively obtained after their flash chromatography on silica gel (Schemes 3–5). The synthesis of diosgenyl salicylate compound from **8d** which features a glycine bridge was unsuccessful. This fact is very interrogative. The loss of acetyl group was probably due to mildly basic details made by excess of Et_3N and hydrous condition of post-processing reaction, the phenolic acetyl group was hydrolyzed. As steric hindrance of diosgenyl salicylate compounds decreased, occurrence of hydrolysis reaction increased. Therefore, longer amino acid bridging chains might lead to easier lose of acetyl group of the 2-acetoxybenzoyl group. The structures of compounds **6e**, **7e**, and **9e** were confirmed by ^1H NMR, ^{13}C NMR, and HR-MS spectral data.



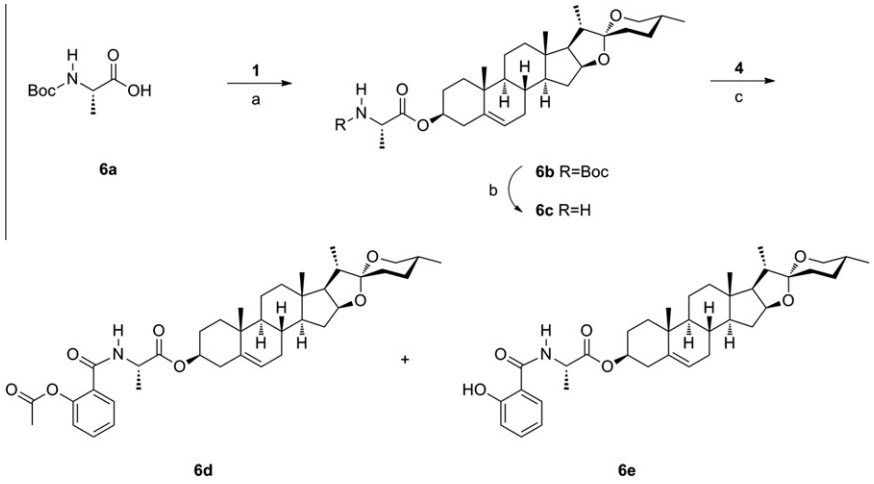
Scheme 2. Reagents and conditions: $(Boc)_2O$, Et_3N, CH_3COCH_3:H_2O (v:v, 1:1), rt, 3 h, 98%.

In this work, a series of diosgenyl derivatives were synthesized and the cytotoxic activities of these compounds were evaluated through a MTT assay[24,25] in the following tumor cell lines: human breast cancer MDA-MB-231 cells, murine colon carcinoma C26 cells, and human hepatocellular carcinoma Hep G2 cells. The IC_{50} values of deltonin[24,25] were also included in the study for comparison. The data presented in Table 1 show that compound **9c** demonstrates significantly higher inhibition against all three cancer cell lines than either aspirin or diosgenin. Also, **9c** demonstrates stronger activity than **6c**, **7c**, and **8c**, and has a better activity than its salicylate conjugates **9e**.

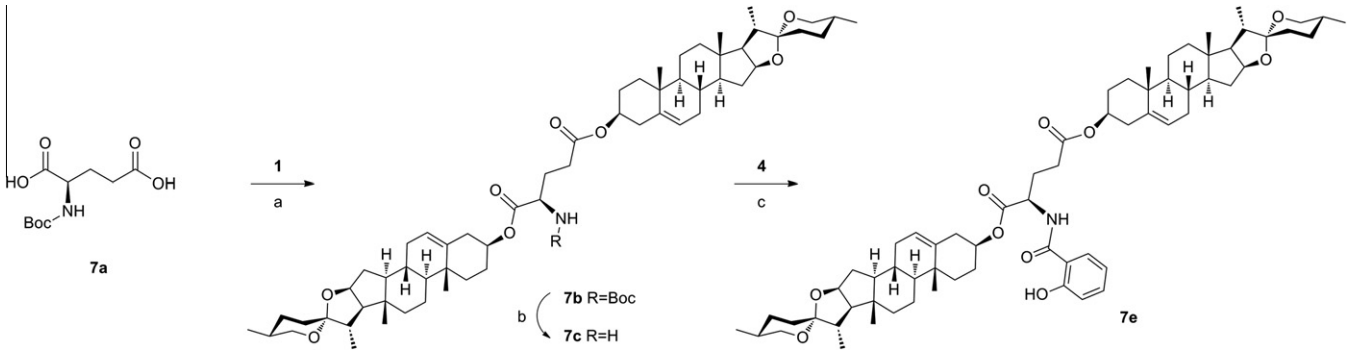
Evidence from a wide range of epidemiological studies suggests that long-term aspirin or related NSAIDs use is associated with a 30–50% reduction in the risk of cancer.[12–15] However, aspirin and the salicylate conjugates (**6e**, **7e**, **8d**, and **9e**) did not have a significant effect against any of the three tumor cell lines after 48 h of incubation (i.e., the IC_{50} values were greater than 50 μM). Only amino acid diosgenyl esters **8c** and **9c** showed significant tumor cell growth inhibition. It is possible that the introduction of various amino acid residues to the diosgenyl has a different impact on cytotoxic activities. 6-Aminohexanoic acid (**9**), which is an analog of the amino acid lysine, is used to treat excessive post-operative bleeding, and block the binding of plasminogen to fibrin and its activation to plasmin.[30,31] Compound **9c**, which contains an



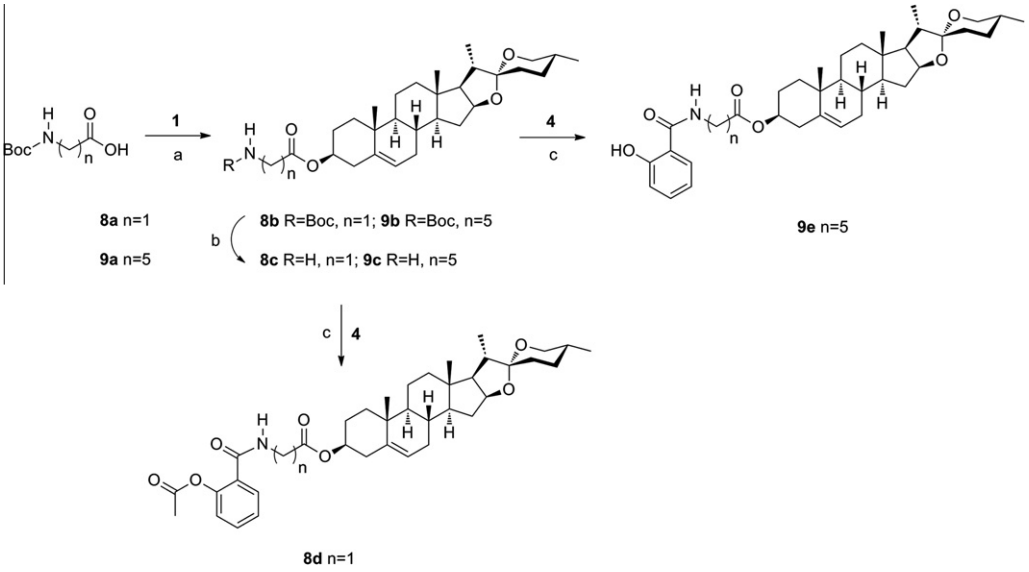
Scheme 1. Reagents and conditions: (a) $SOCl_2$, DMF, 70–75 °C, 3 h; (b) Py, CH_2Cl_2, 0 °C, 1 h, 65%.



Scheme 3. Reagents and conditions: (a) DMAP, TsOH, DCC, CH_2Cl_2, rt, 94%; (b) CF_3COOH, CH_2Cl_2, rt, 90%; (c) Et_3N, CH_2Cl_2, 0 °C, flash chromatography, 57% for **6d** and 17% for **6e**.



Scheme 4. Reagents and conditions: (a) DMAP, TsOH, DCC, CH_2Cl_2, rt, 92%; (b) CF_3COOH, CH_2Cl_2, rt, 86%; (c) Et_3N, CH_2Cl_2, 0 °C, flash chromatography, 93%.



Scheme 5. Reagents and conditions: (a) DMAP, TsOH, DCC, CH_2Cl_2, rt; (b) CF_3COOH, CH_2Cl_2, rt; (c) Et_3N, CH_2Cl_2, 0 °C, flash chromatography, 73% for **8d** and 82% for **9e**.

aminocaproic acid residue, shows a strong activity against the proliferation of MDA-MB-231 (IC_{50} 7.6 μM) and C26 (IC_{50} 4.7 μM). This result suggests that the aminohexanoic residue is optimal for the anti-tumor effect. In summary, 6-aminohexanoic acid diosgenyl ester (**9c**) is a potential new anticancer drug.

The anti-inflammatory activities of the diosgenyl esters were evaluated using a xylene-induced ear edema model assay.[32–34] The results shown in Table 2 reveal that diosgenin (at a dose of 100 mg/kg, inhibition 32.2%) has an anti-inflammatory activity that is comparable to that of the standard reference drug, aspirin

Table 1
Cytotoxic activity (IC_{50}, μM) of synthetic diosgenyl esters[a,b]

Compound	Cancer cell line		
	MDA-MB-231	C26	Hep G2
Aspirin	1510	1740	3841
Diosgenin	>50	>50	>50
5	>50	>50	>50
6c	>50	>50	>50
6e	>50	>50	>50
7c	>50	>50	>50
7e	>50	>50	>50
8c	48.2	>50	>50
8d	>50	>50	>50
9c	7.6	4.7	14.6
9e	>50	>50	>50
Deltonin	1.58[24]	1.22[25]	7.66

[a] IC_{50} is the concentration in μM that inhibits cell growth by 50% in 48 h compared to cells that remained untreated.
[b] Means obtained from a minimum of $n = 3$ independent MTT assays for each compound.

Table 2
Anti-inflammatory activities of diosgenyl esters against xylene-induced ear edema in mice[a,b]

Agents	Edema weight (X ± SD mg)	Inhibition (%)
CMC	10.73 ± 3.01	
Aspirin	7.22 ± 2.50 [d]	31.9
Diosgenin	7.18 ± 2.57 [c]	32.2
5	5.67 ± 2.23 [d]	46.5
6c	7.57 ± 2.75 [c]	28.6
6e	6.81 ± 2.53 [d]	35.8
7c	8.58 ± 2.18 [c]	19.1
7e	7.41 ± 2.92	30.1
8c	7.36 ± 2.59 [c]	30.6
8d	6.46 ± 2.09 [d]	39.1
9c	6.51 ± 3.02 [c]	38.6
9e	6.08 ± 2.91 [c]	42.6

[a] All compounds were dissolved in 0.5% CMC and administered orally at the dose of 100 mg/kg except Aspirin group at the dose of 50 mg/kg.
[b] Ten animals were used in each group ($n = 10$) and data were represented as mean ± SD.
[c] Compare to CMC group, $p < 0.05$.
[d] Compare to CMC group, $p < 0.01$.

(at a dose of 50 mg/kg, inhibition 31.9%). It was noted that the salicylate conjugates (i.e., **5**, **6e**, **8d**, and **9e**) possess higher anti-inflammatory activities than both diosgenin and aspirin, especially compounds **5** and **9e**, which have inhibition rates of 46.5% and 42.6%, respectively. Moreover, compound **9c** also showed significant suppression with an inhibition rate of 38.6%. However, the anti-inflammatory activities of the other amino acid diosgenyl esters (i.e., **6c**: 28.6% and **8c**: 30.6%) were slightly less, and no significant effect was found for compound **7c**, which had an inhibition rate of only 19.1%.

Most of the diosgenyl compounds significantly inhibited xylene-induced rat ear edema (Table 2), and exhibited comparable or superior anti-inflammatory activities than the reference drugs aspirin and diosgenin. Structure–activity relationship studies reveal that salicylate conjugate **5** has better anti-inflammatory activity than salicylate complexes **6e**, **7e**, **8d**, and **9e**, which have amino acid bridges between aspirin and diosgenin. In addition, diosgenyl esters **8c** and **9c**, which have straight amino acid chains, demonstrate significantly higher anti-inflammatory effects than compounds **6c** and **7c**, which have branched amino acid chains.

We synthesized several amino acid diosgenyl esters and diosgenyl salicylate compounds with different amino acid bridging chains. 6-Aminohexanoic acid diosgenyl ester (**9c**) is highly active towards inhibiting the proliferation of MDA-MB-231 and C26. However, no significant effects were found for diosgenyl salicylate conjugates against any of the three tumor cell lines investigated. Meanwhile, structure–activity relationship studies indicate that compound **9c** and all salicylate conjugates exhibit desirable anti-inflammatory activity compared to aspirin and diosgenin. The incorporation of an aminocaproic residue with diosgenin is optimal for the anti-tumor and anti-inflammatory activities.

Further investigations on the synthesis and biological behavior of diosgenyl esters with long, straight amino acid chains to elucidate their anticancer effects and anti-inflammatory properties are forthcoming.

Acknowledgments

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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.2012.10.086. These data include MOL files and InChiKeys of the most important compounds described in this article.

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