β-Glucuronidase Inhibiting Constituents from *C. casimiriana* Duthie and Prain ex Prain. and *Corydalis govaniana* Wall

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**Abstract**

*Corydalis casimiriana* Duthie and Prain ex Prain. and *Corydalis govaniana* Wall. have been used in the treatment of scrofula, cutaneous infections, syphilis, along with diarrhea and dysentery. In the present work, phytochemical investigation of these two medicinal plants resulted the isolation of one tetrahydroprotoberberine type alkaloid, govaniadine (1); and six other alkaloids, caseadine (2), caseamine (3), protopine (4), stylopine (5), apocavidine (6), and fagarine I (7). β-glucuronidase enzyme plays a major role in generation of toxic metabolites, which may cause cancer in the intestine. Compound 1 showed a good β-glucuronidase (IC₅₀ = 41.9 ± 3.1 µM) inhibition, better than D-saccharic acid 1, 4-lactone (IC₅₀ = 45.8 µM). Similarly, compound 2 showed significant inhibitory effect against β-glucuronidase (IC₅₀ = 71.6 ± 4.3 µM). Therefore, these compounds can be considered for further research for drug development to prevent tumors in intestine.

**Keywords:** *Corydalis*, govaniadine, caseadine, β-glucuronidase inhibition, alkaloids

**Introduction**

Nepal is rich in biodiversity and hundreds of species are known to have medicinal values. The genus *Corydalis* comprises 470 species, which is native in China, Nepal, India and Pakistan and also found in mountainous regions of Eastern Africa. *Corydalis govaniana* Wall and *C. casimiriana* Duthie and Prain ex Prain are the important herb and has been used to cure scrofula, syphilis, diarrhea and dysentery. Also, secondary metabolite of these plant has been showing inhibitory effect against hepatitis virus, amoeba, tumors, liver cancer, as well as acrosodyne and sedative, improved immunological function, hepatocirrhosis, ascites, etc [1]. The excellent bioactivity profile and ethno-botanical uses of these plants attract us to isolate fully characterized pure compounds and for bioassay screening of these compounds. β-Glucuronidase (EC 3.2.1.31) is an exoglycosidase hydrolase enzyme that catalyzes the cleavage of glucuronosyl-O-bonds. It plays a major role in the generation of toxic and carcinogenic metabolites in the large intestine [2]. The activity of this enzyme has been detected both in the intestinal tissues and intestinal bacteria. In the large intestine, bacterial species such as *Escherichia coli*, *Klebsiella spp, Clostridium spp*, and *Bacillus spp*, etc. possess glucuronidase activity [3,4] and generate toxic and carcinogenic materials which may promote tumors [5]. Many synthetic and natural compounds are used clinically to inhibit the activity of β-glucuronidase enzyme for the treatment of related diseases [6]. So, inhibitors of β-glucuronidase are of great pharmacological importance for better anti-tumor, antioxidant effects and reduction of systemic toxicity by excretion of toxic xenobiotics.

**Materials and Methods**

**Plant Collection and Extraction**

The whole plant of both *C. govaniana* and *C. casimiriana* were collected from Langtang, Rasuwa, Nepal, and identified by Mr. Sanjiv Kumar Rai, Taxonomist, Department of Plant Resources, Thapathali, Kathmandu, Nepal. A voucher specimen, CG-207, has been deposited in Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Air-dried whole plant powder was soaked and extracted with methanol. After evaporation under reduced pressures, the residue was stirred with 7% citric acid for five hours and filtered and neutralized with ammonia solution and extracted with chloroform. The chloroform extract was subjected to column chromatography over silica-gel column by using acetone/hexanes with a few drops of diethylamine with increasing polarity, which afforded the compounds 1-7.
**Structure elucidation of pure compounds**: Details of structure elucidation of compounds 1-4 has already published in our previous paper [1]. Structure of compounds 5-7 were deduced from different UV, IR, mass and NMR techniques. All the physical and spectral data of compounds 5-7 were found to be similar with reported compounds from same genus [6, 9].

### β-Glucuronidase Inhibition Assay

β-Glucuronidase activity was determined by the spectrophotometric method measuring the absorbance at 405 nm of p-nitrophenol formed from the substrate. The total reaction volume was 250 µL. The reaction mixture contained 185 µL of 0.1 M acetate buffer, 5 µL of test compound solution, 10 µL of enzyme solution was incubated at 37 °C for 30 min. The plates were read on a multiplate reader (SpectraMax plus 384, Molecular Devices, CA, USA) at 405 nm after the addition of 50 µL of 0.4 mM p-nitrophenyl-β-D-glucuronide [10].

### Statistical Analysis

All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance) were processed by using SoftMax Pro software (Molecular Devices, CA, USA) and then by MS Excel. The % of inhibition was calculated as:

\[ \% \text{Inhibition} = 100 - \left( \frac{\text{OD of test sample}}{\text{OD of the control}} \right) \times 100 \]

Results are presented as means ± S.E.M. as indicated in Table 1-3. IC₅₀ values were determined by using EZ-FIT (enzyme kinetics software by Perrella Scientific, Inc., Amherst, USA).

### Results and Discussion

All compounds except stylopine (5) and fagarine I (8) exhibited over 50% inhibition while screening at 500 µM. Then these compounds 1-4 and 7 were further investigated to find their exact IC₅₀ values, which is shown in Table 1. Govaniadine exhibited the promising inhibition against β-glucuronidase and slightly better than the standard D-Saccharic acid 1, 4-lactone.

#### Table 1: β-glucuronidase inhibition activity compound 1-5, 7 and 8

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>% Inhibition</th>
<th>IC₅₀ ± S.E.M. (µM)</th>
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<tbody>
<tr>
<td>Govaniadine (1)</td>
<td>73.1</td>
<td>41.9±3.1</td>
</tr>
<tr>
<td>Caseadine (2)</td>
<td>59.7</td>
<td>71.6±4.3</td>
</tr>
<tr>
<td>Caseamine (3)</td>
<td>70.5</td>
<td>282.3±2.2</td>
</tr>
<tr>
<td>Protopine (4)</td>
<td>71.6</td>
<td>113.5±2.6</td>
</tr>
<tr>
<td>Stylopine (5)</td>
<td>43.4</td>
<td>-</td>
</tr>
<tr>
<td>Apocavidine (6)</td>
<td>71.6</td>
<td>255.3±3.2</td>
</tr>
<tr>
<td>Fagarine I (7)</td>
<td>30.2</td>
<td>-</td>
</tr>
<tr>
<td><strong>D-Saccharic acid 1, 4-lactone</strong></td>
<td><strong>89.4</strong></td>
<td><strong>45.8±2.2</strong></td>
</tr>
</tbody>
</table>

*a*Initial screening at 500 µM

*b*The standard inhibitorytortakenforthsressearchforcomparison

S.E.M. = Standard Error of Mean at n = 3

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### References