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Novel urease inhibitors from *Daphne oleoids*

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Abstract
Phytochemical investigations on the chloroform and ethyl acetate soluble fractions of the roots of the *Daphne oleoids* led to the isolation of the coumarin glycosides **1–6**. Compound **5** with IC_{50} values 22.05 and 26.30 μM repectively, was found to be the most active of these compounds when screened against *Bacillus pasteurii* and jack bean urease enzymes in a concentration-dependent fashion.

Keywords: Daphne oleoids, *thymelaeaceae, coumarin glycosides, urease, inhibition*

Introduction

The genus *Daphne* belongs to the family Thymelaeaceae which is an important source of coumarins and their dimers. It consists of about 70 species found in Europe, Mediterranean regions, temperate and subtropical Asia, Indo-Malaya, Philppines, Africa, Australia and Pacific [1,2]. The plants of the genus *Daphne* have been reported to possess valuable medicinal properties and find uses in traditional medicines for the treatment of reheumatism, ulcers, toothache and are also used as purgatives and abortificients [3]. *Daphne oleoids* is a xerophytic shrub which is found in the Northern areas of Pakistan. It is used by the local physicians for the treatment of kidney stones, ulcers and cancers. A methanolic extract of this plant showed significant inhibitory activity against the urease enzyme which prompted us to carry out phytochemical studies on this plant. As a result four dimeric coumarin glycosides (**1, 4, 5, 6**) and two trimeric coumarin glycosides (**2–3**) were isolated (Figure 1).

Ureases (E.C. 3.5.1.5) have been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health as well as for agriculture. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation [4,5]. It is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at the low pH of the stomach during colonization and, therefore, plays an important role in the pathogenesis of gastric and peptic ulcers (including cancer) [5]. Therefore strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease-producing bacteria. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving them of their essential nutrients and secondly by the toxicity associated with ammonia [6,7]. In the current study we have described the urease inhibitory activity of four dimeric and two trimeric coumarin glucosides which was isolated from *Daphne oleoids* and published previously by our research group [8, 9, 10, and 11].

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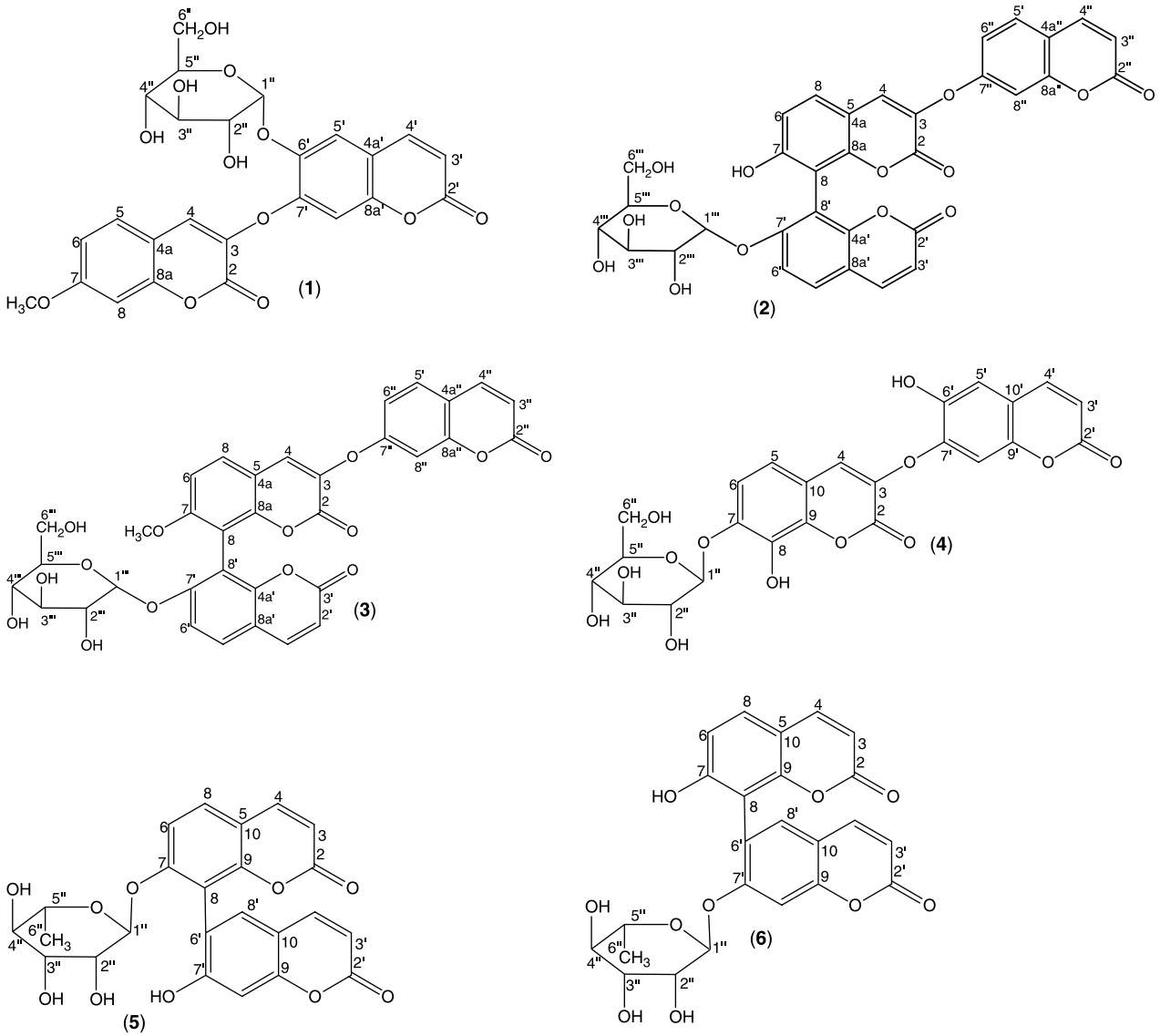

Figure 1. Structures of compounds **1**–**6**.

Materials and methods

Urease assay and inhibition

Reaction mixtures, comprising 25 μl of enzyme solution (Jack bean and *Bacillus pasteurii* ureases in buffer) were incubated for 30 min with 5 μl test compounds **1–6** dissolved in DMSO at 30°C for 15 min in 96-well plates and then 55 μl of buffers containing 100 mM urea were incubated for 15 min. All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M LiCl). Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [12] [indophenol reagents: 45 μl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well]. The absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μl. The results were processed using SoftMax Pro software (Molecular Device, USA). Percentage inhibitions were calculated from the formula 100-[(Optical $\text{Density}_{\text{testwell}}$/Optical $\text{Density}_{\text{control}}$) × 100]. Thiourea was used as the standard inhibitor of urease.

Results and discussion

Urease is an enzyme that is present in many plants and in soil. It catalyzes the hydrolysis of urea to ammonium and carbonate ions, which decompose to ammonia and carbon dioxide respectively. The active site contains two nickel (II) atoms which, as shown by X-ray analysis, are linked by a carbonate bridge; furthermore, two imidazole nitrogen atoms are bound to each nickel atom, and a carboxylate group and a water molecule fill the remaining coordination sites of

Table I. *In vitro* inhibition (IC_{50}) of ureases by compounds **1–6**.

Compound	IC_{50} ± Sem (Bacillus pasteurii urease)	IC_{50} ± Sem (Jack bean urease)
1	55.27 ± 1.53	61.91 ± 1.12
2	24.17 ± 1.96	29.39 ± 1.31
3	37.62 ± 0.35	40.00 ± 0.07
4	29.01 ± 0.22	31.60 ± 1.33
5	22.05 ± 1.66	26.30 ± 2.02
6	90.25 ± 1.16	93.67 ± 1.06
Thiourea(Std)	15.06 ± 0.72	21 ± 0.11

Standard mean error of 3–5 assays. All the IC_{50} values are μM.

the metal ion. The coordination geometry of the first nickel atom is pseudo tetrahedral, while that of second is roughly trigonal bipyramidal [4]. All the coumarins **1–6** showed concentration-dependent activity with IC_{50} values ranging between 26.30–93.67, and 22.05–90.25 μM against Jack bean and *Bacillus pasteurii* ureases (Table I), respectively. Compounds **2, 4** and **5** displayed potent inhibitory potential, while compounds **1, 3** and **6** exhibited moderate inhibition against both the urease enzymes. These compounds are not all that potent but are good lead compounds for further studies on design of urease inhibitors.

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