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Vindogentianine, a hypoglycemic alkaloid from *Catharanthus roseus* (L.) G. Don (Apocynaceae)

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ABSTRACT

Vindogentianine, a new indole alkaloid together with six known alkaloids, vindoline, vindolidine, vindolicine, vindolinine, perivine and serpentine were isolated from leaf extract (DA) of *Catharanthus roseus* (L.) G. Don. Their structures were elucidated by spectroscopic methods; NMR, MS, UV and IR. Vindogentianine is a dimer containing a vindoline moiety coupled to a gentianine moiety. After 24 h incubation, vindogentianine exhibited no cytotoxic effect in C2C12 mouse myoblast and β-TC6 mouse pancreatic cells (IC_{50} > 50 μg/mL). Real-time cell proliferation monitoring also indicated vindogentianine had little or no effect on C2C12 mouse myoblast cell growth at the highest dose tested (200 μg/mL), without inducing cell death. Vindogentianine exhibited potential hypoglycemic activity in β-TC6 and C2C12 cells by inducing higher glucose uptake and significant *in vitro* PTP-1B inhibition. However, *in vitro* α-amylase and α-glucosidase inhibition assay showed low inhibition under treatment of vindogentianine. This suggests that hypoglycemic activity of vindogentianine may be due to the enhancement of glucose uptake and PTP-1B inhibition, implying its therapeutic potential against type 2 diabetes.

1. Introduction

Catharanthus roseus (L.) G. Don (Apocynaceae) is a medicinal plant used in many traditional practices for the treatment of diabetes in several countries such as Malaysia [1], India, China, South Africa and Mexico [2]. The study of oral hypoglycemic activity led to the discovery of vinblastine and vincristine, the first natural anticancer agents used clinically [3]. This drive the attention on this plant towards anticancer and neglecting the antidiabetes activity from this plant, discovery of more than 130 alkaloids [4]. Even though with the extensive alkaloids constituent evaluation conducted onto this plant, still new indole alkaloids were discovered and reported recently. [5–8].

Therefore, several studies have reported the *in vitro* and *in vivo* activity supporting the antidiabetic activity of this plant. The extract of *C. roseus* from twig and leaves showed increase glucose utilization in cells, reduced blood glucose in animal model such as rabbits and rats [9,10]. A significant increase of glucokinase activity in the liver of rats treated with extract of this plant was also reported [11]. Some chemical constituents

Abbreviations: RTCA, real-time cell proliferation assay; TE, trolox equivalent; 2-NBDG, 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline.

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of this plant had been showed to exhibit hypoglycemic activity such as vindoline, tetrahydroalstonine, catharanthine, lochericine, leurosine, vindolinine, adenosine, β-sitosterol, quercetin, ursolic acid and tannic [12–14].

However, there are still many other chemical constituents of this plant especially alkaloids, that have never been evaluated for their hypoglecemic activity. Recently, the hypoglycemic activity of several known indole alkaloids from *C. roseus* has been reported, with vindolicine (Fig. 1) showing potential as a novel protein-tyrosine phosphatase 1B (PTP-1B) inhibitor that may serve as an "insulin sensitizer" in the management of type 2 diabetes [15].

In this communication, a dimer containing a vindoline substructure coupled with a gentianine moiety, vindogentianine (**1**) was isolated from *Catharanthus roseus* (Fig. 1). This paper communicates the isolation and structure elucidation of **1**. We evaluated the *in vitro* hypoglycemic ability of **1** by glucose uptake assay, PTP-1B and α-amylase inhibition. In addition, cytotoxicity testing and antioxidant of **1** was also evaluated.

2. Results and discussion

Vindogentianine, **1** was obtained as a yellowish brown amorphous solid and was positive to Dragendoff's reagent with purity of about 90% (Fig. 1). **1** was purified from the alkaloid crude (DA) obtained from the dichloromethane (DE) leave extract of *Catharanthus roseus*. DE also showed hypoglycemic activity by exhibiting higher glucose uptake in β-TC6, mouse pancreatic cell, as compared to untreated cells [15]. Chemical investigation on the active extract led to the isolation of **1**.

The molecular formula of **1** was determined as $C_{35}H_{41}N_3O_8$ based on ESI-IT-TOFMS [*m/z* 632.3060 ($M + H^+$), Δ + 8.9 mmu] and [*m/z* 676.2897 ($M + HCOO^-$), Δ + 2.7 mmu]. IR absorptions implied the presence of hydroxyl (3458 cm^{-1}) and ester carbonyl (1741 cm^{-1}) functionalities. UV spectral absorbance's of λ_{max} (log ε): 216 (4.46), 259 (4.11) and 310 (4.01) nm suggested the presence of a dihydroindole moiety [16].

The ^{13}C NMR spectrum showed the presence of 35 carbons; four sp^3 methine, six sp^3 methylene, six methyl, six sp^2 methine and thirteen quaternary carbons. Based on comparisons of ^{13}C and ^1H NMR spectra to other earlier reported alkaloids from this plant, **1** consisted of two subunits; A and B, in which subunit A is the vindoline while subunit B is gentianine-related moeity (Fig. 1). Both units are connected through a C-10 and C-9′ linkage (Fig. 2).

The subunit A has 25 carbons consisting of three sp^3 methines, four sp^3 methylenes, five methyls, four sp^2 methines and nine quaternary that were observed in ^{13}C and DEPT 135° NMR spectra. The ^1H NMR spectrum indicated the presence of two *cis*-olefinic protons, H-14(δ_H 5.84, dd, 1H, J = 4.6, 10.1 Hz) and H-15(δ_H 5.20, d, 1H, J = 10.1 Hz). Two methoxyls attached to C-22 and C-11 resonated as two 3H singlets at δ_H 3.74 and δ_H 3.72 respectively. The presence of an acetate functionality was confirmed by the resonance of the carbonyl carbon at δ_C171.0 with the corresponding methyl carbon resonating at δ_C21.2. Two aromatic protons H-9 and H-12, appeared as singlets at δ_H 6.57 and δ_H 6.00 respectively, indicating that C-10 and C-11 were substituted. The methyl of an ethyl moiety attached to C-20 resonated as a triplet at δ_H 0.49. All the carbons and protons of the subunit A were assigned through extensive analysis of HMBC, HMQC and COSY spectra (Table 1 & Fig. 2). All chemical shifts were consistent with the reported values for vindoline.

The 10 remaining carbons were assigned to subunit B; one sp^3 methine, two sp^3 methylenes, one methyl, two sp^2 methine and four quaternary carbons. Subunit B has six degrees of unsaturation, consistent with the presence of a six membered lactone fused with a pyridine ring to give the gentianine-related moiety [11]. Two highly deshielded aromatic protons were observed as 1H singlet at δ_H 9.06 (H-3′) and δ_H 8.58 (H-

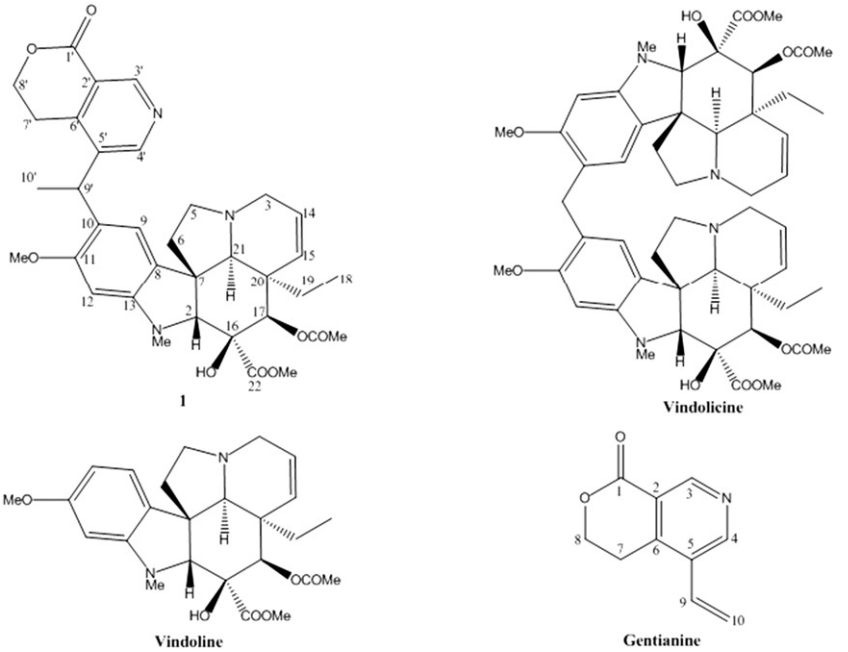

Fig. 1. Chemical structure of **1**, vindolicine, vindoline and gentianine.