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Arylated gymnemic acids from *Gymnema sylvestre* R.Br. as potential α-glucosidase inhibitors

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ABSTRACT

A mixture of gymnemic acids was precipitated from the water extract of leaves of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (Asclepiadaceae) by acidification with mineral acid. The chromatographic separation of gymnemic acids mixture afforded four new arylated gymnemic acids (**1–4**) along with a gymnemasaponin (**5**). The compounds were characterized on the basis of extensive spectral data analysis. The arylated compounds (**1–4**) showed dose dependent inhibition of α-glucosidase that was found to be comparable to acarbose (IC_{50} 265 μg/ml). Maximum α-glucosidase inhibition was achieved with compound **2** (IC_{50} 145 μg/ml) followed by **3** (IC_{50} 220 μg/ml), **1** (IC_{50} 230 μg/ml) and **4** (IC_{50} 310 μg/ml). The results revealed that the overall pattern of acyl/aryl substitution and glycosylation of compounds affected their inhibitory activity. The bidesmosidic glycosides (**2** and **3**) showed improved potency than the monodesmosidic glycosides (**1** and **4**) possibly because the additional glucose unit in the former facilitated stronger hydrogen bonding at the catalytic site. The current study provides relatively direct evidence of effectiveness of *G. sylvestre* against hyperglycemia.

1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by relative or absolute insulin absence or insulin insensitivity resulting in elevated blood glucose levels, and subsequent disturbed lipid and protein metabolism (Alberti and Zimmet, 1998). Hyperglycemia is the most important risk factor in the onset and development of its co-morbidities (Dong et al., 2012). The elevated blood glucose levels can be the cause of several complications associated with diabetes, such as retinopathy, nephropathy, neuropathy and impaired wound healing (Ban and Twigg, 2008). α-Glucosidase is primarily an enteric enzyme located on the epithelial wall of the small intestine, and its inhibition would delay the digestion as well as absorption of carbohydrates, to consequently suppress PPHG (Krentz and Bailey, 2005). Common side effects associated with the currently used α-glucosidase inhibitors (acarbose, miglitol and voglibose) are abdominal distension, flatulence, meteorism and diarrhoea. These side effects are partly ascribed to the non-specific inhibition of both the α-glucosidase and α-amylase enzymes by these agents. Selective α-glucosidase inhibitors can be expected to bring about stricter glycemic control while keeping the pancreatic (insulin) pathway undisturbed (Sheliya et al., 2015). Recently several triterpenes have aroused greater interest in tackling disturbances in carbohydrate and lipid metabolism. They are reported to inhibit the formation of advanced glycation end products that are implicated in the pathogenesis of diabetic nephropathy, neuropathy or atherosclerosis (Nazaruk and Borzym-Kluczyk, 2015). The curative potential of triterpenoids is very high yet still poorly recognised.

Gymnema sylvestre (Retz.) R.Br. ex Sm. (Asclepiadaceae) is a widely distributed medicinal herb in India. It is locally known as *Gur-mar* as it supresses the ability to taste sweetness. *Sushruta* – an ancient compilation of Indian medicinal plants – refers to *G. sylvestre* as a destroyer of *Madhumeha* (glycosuria). It is believed, therefore, that it might neutralize the excess sugar present in diabetes (Nadkarni, 1986). The plant is popular in Indian systems of traditional medicine, such as Siddha,

Abbreviations: COSY, correlation spectroscopy; DAGA, deacyl gymnemic acid; DEPT, distortionless enhancement by polarization transfer; DMSO, dimethyl sulfoxide; FTIR, fourier transform infra red; HRES-MS, high resolution electrospray mass spectrometry; MPLC, medium pressure liquid chromatography; MW, molecular weight; NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multinuclear quantum correlation; PNPG, *p*-nitrophenyl-α-D-glucopyranoside; PPHG, postprandial hyperglycaemia; TLC, thin layer chromatography; TMS, tetramethyl silane

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Unani, and Ayurveda, and is listed in the Indian pharmaceutical codex as a remedy for diabetes (Singh et al., 2008). The leaf extract is reported to reduce hyperglycaemia in humans (Khare et al., 1983) and experimental animal models (Srivastava et al., 1985; Shanmugasundaram et al., 1983, 1990). The blood glucose-lowering effect is postulated to be mediated through the stimulation of insulin release (Persaud et al., 1999), regeneration of β-cells (Ahmed et al., 2010) or inhibition of glucose uptake in the intestine (Shimizu et al., 1997). The anti-sweet constituents of the plant are loosely referred to as gymnemic acids. A number of triterpenoidal derivatives have been isolated from the plant. The anti-sweet activity of the extracts and some of the saponins has also been reported (Liu et al., 1992; Sahu et al., 1996; Yoshikawa et al., 1989a,b, 1991; Ye et al., 2000, 2001). The literature reports concerning the anti-diabetic or antisweet effects of triterpenoids from *G. sylvestre* have been compiled in an exhaustive review by Fabio et al. (2014). There are only a few reports on the gut glucosidase inhibition by individual gymnemic acids (Daisy et al., 2009; Kimura, 2006).

As part of our ongoing research on the preparative isolation of bioactive natural compounds from traditional herbs, this article describes the isolation and characterization of new arylated gymnemic acids from the leaves of *G. sylvestre* and the delineation of their α-glucosidase inhibitory activity.

2. Results and discussion

A mixture of gymnemic acids was precipitated from the water extract of leaves of *G. sylvestre* by acidification with mineral acid. The residue was dissolved in minimal amount of methanol, fractionated over silica gel (50–60 μm) column using MPLC and purified by prep-TLC. This resulted in the isolation of four new arylated gymnemic acids **1–4** and a gymnemasaponin **5**. Their structures were elucidated by the interpretation of spectral data. The chemical structures of compounds **1–5** are presented in Fig. 1. The orientation of glucopyranosyl and glucuronopyranosyl residues was determined to be β on the basis of ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2). Furthermore, D-configuration of these sugar residues was established by TLC comparison of the acid hydrolysate with the reference standards.

Compound **1** was obtained as a brownish sticky mass from ethyl acetate eluents. Its FTIR spectrum exhibited absorption bands for hydroxyl (3393 cm^{-1}), aromatic (1997, 1635 cm^{-1}), and carbonyl (1719, 1669 cm^{-1}) functionalities. Its molecular formula was derived to be $C_{50}H_{74}O_{13}$ (MW 882) on the basis of mass and ^{13}C/DEPT NMR spectra (Fig. S2–S4, see Supplemental material). Its HR-ES-MS displayed a fragment ion peak at *m/z* 824.5737 $[C_{48}H_{72}O_{11}]^+$ (calc. 824.5075) arising due to the loss of two methoxy groups from the parent ion. The mass spectrum also exhibited a retro-Diels Alder fragment ion peak at *m/z* 435 $[C_{28}H_{35}O_4]^+$ suggesting the presence of four hydroxyls in rings D/E (besides two methoxy groups in the parent compound). Another fragment ion peak at *m/z* 162 $[C_6H_{10}O_5]^+$ indicated the presence of a glucose unit, the presence of which was also confirmed by the comparison of TLC of the acid hydrolysate of **1** with the reference standard of D-glucose.

The ^{1}H NMR spectrum exhibited signals for a benzoyl unit [δ 7.74 (dd, J = 2.2, 8.1 Hz, H-2a), 7.26 (dd, J = 2.4, 8.0 Hz, H-3a), 6.65 (br d, J = 8.8 Hz, H-4a), 6.75 (dd, J = 2.6, 8.0 Hz, H-5a) and 7.64 (dd, J = 2.2, 8.0 Hz, H-6a)] and a tigloyl moiety [δ 7.04 (q, J = 7.0 Hz, H-3b), 1.85 (d, J = 7.0 Hz, H_3-4b) and 2.04 (s, H_3-5b)]. It also displayed a signal for an olefinic proton (H-12 at δ 5.35), characteristic of oleanenes (Fig. S1, see Supplemental material). The ^{13}C/DEPT NMR spectra of **1** revealed the presence of four oxygenated methine and two oxygenated methylene carbons [δ 70.4 (C-3), 69.6 (C-16), 78.4 (C-21), 71.9 (C-22), 72.8 (C-23) and 57.4 (C-28)] associated with its genin part. Comparison of NMR data of **1** with those of gymnemagenin and deacyl gymnemic acid DAGA (Liu et al., 1992), supported that a tigloyl, a benzoyl and a glucose unit are attached to the genin portion besides two methoxy units. The protons of two methoxy groups at δ 3.85 and 3.76 (both s, 3H) in ^{1}H NMR spectrum were directly attached to the carbons at δ 52.8 and 51.8 in HMQC spectrum. Thus, the genin was established to be 3β,16β,21β,22α,23,28-hexahydroxyolean-12-ene (gymnemagenin). For determination of the position of attachment of acyl/aryl and glucose residues, ^{1}H-^{1}H and ^{1}H-^{13}C chemical shift correlation spectra were examined in detail (Fig. S5–S7, see Supplemental material). In the ^{13}C NMR spectrum, a glycosylation shift of +6.0 ppm (from δ 64.4 for

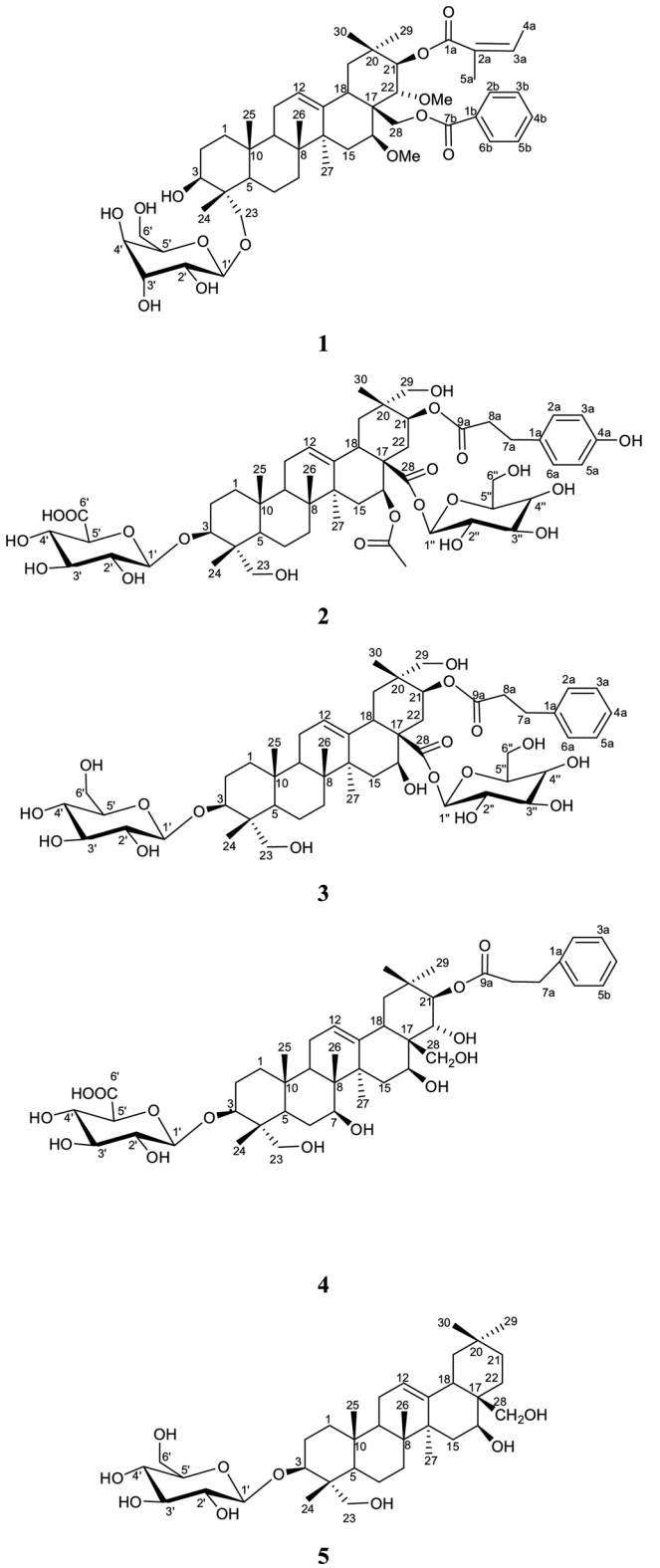

Fig. 1. Structures of compounds **1–5** isolated from *Gymnema sylvestre*.