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Two new tryptophan derivatives from the seed kernels of *Entada rheedei*: Effects on cell viability and HIV infectivity

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ABSTRACT

Two new tryptophan derivatives, N-sulfonyl-L-tryptophan (tryptorheedei A) (**1**) and 3-(N-sulfonylindolyl)-D-lactic acid (tryptorheedei B) (**2**) together with the known 5-*O*-β-D-glucopyranosyl-2-hydroxyphenylacetic acid (**3**), 1-*O*-methylglucopyranoside, entadamide A, homogentisic acid and 3-*O*-β-D-glucopyranosyl-β-sitosterol, were isolated from the seed kernels of *Entada rheedei* (Mimosaceae). Their structures were established using 1D and 2D NMR spectroscopy, mass spectrometry and by comparison with spectroscopic data reported in the literature. Compounds **1** and **2** showed no toxicity to TZM and Human PBMC cells. Both compounds **1** and **2** were found to promote early infection events in HIV, likely by inhibiting the enzyme indolamine 2,3-dioxygenase (IDO) and preventing tryptophan depletion. Inhibition of IDO acutely in HIV infection inhibits viral replication, but chronic activation of IDO leads to immune impairment in AIDS. IDO is also the gatekeeper enzyme for kynurenine metabolism, a pathway involved in serotonin and melatonin biosynthesis and the regulation of glutamate and dopamine levels in the brain. Therefore inhibition of IDO might explain both the reported medicinal and neuropsychiatric effects of *E. rheedei*.

1. Introduction

As part of our continuous investigation on Cameroonian medicinal plants [1], we have conducted a phytochemical study of the seed kernels of *Entada rheedei* Spreng (Mimosaceae), also known as *E. rheedii* [2], a large woody liana or climber growing naturally throughout tropical Africa and Southeastern Asia. Tobacco made from the seeds of this plant has been reported to cause vivid dreaming and, for this reason, the plant is commonly known as African dream herb or snuff box sea bean (entheology.org). *E. rheedei* has various medicinal uses, including treatment of jaundice, diarrhea [3], musculo-skeletal problems[4] and mumps [5]. Previous phytochemical studies on the genus *Entada* revealed the presence of saponins [6,7], thioamides [8–10] and phenylacetic acid derivatives [11,12]. More recently, investigation of the seed kernels of *E. rheedei* led to the isolation of oleanane-type saponins, thioamide glycosides [13], and phenylpropanoid glycosides [14]. In a recent paper some of us reported the isolation and characterization of two antiproliferative and antioxidant saponins from the n-butanol extract of the seed kernels of this plant [15]. In the present study, we describe the isolation and structure elucidation of two new tryptophan derivatives, N-sulfonyl-L-tryptophan (tryptorheedei A) (**1**) and 3-(N-sulfonylindolyl)-D-lactic acid (tryptorheedei B) (**2**), together with the known 5-*O*-β-D-glucopyranosyl-2-hydroxyphenylacetic acid (**3**) (Fig. 1), 1-*O*-methylglucopyranoside, entadamide A ((E)-N-(2-hydroxyethyl)-3-(methylthio)acrylamide), homogentisic acid (2-(2,5-dihydroxyphenyl)acetic acid) and 3-*O*-β-D-glucopyranosyl-β-sitosterol. Given the important role of tryptophan metabolism via the enzyme indolamine 2,3-dioxygenase 1

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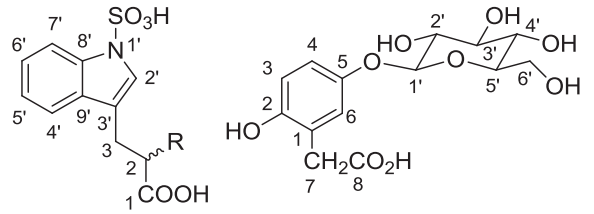

1 R = α-NH_2 **3**

2 R = β-OH

Fig. 1.

and 2 (IDO) in both HIV-1 pathogenesis and neurological diseases [16,17] and their structural similarities to an existing IDO inhibitor, 1-methyltryptophan (1-MT), compounds **1** and **2** were assayed on HIV infection and for their toxicity to TZM and Human PBMC (peripheral blood mononuclear) cells. Both compounds significantly enhanced HIV infection at concentrations above 40 μM. No significant toxicity of the compounds was observed on cells at concentrations of 80 μM and below. Since IDO inhibits HIV-1 replication, the loss of inhibition suggests that these compounds may be competitive inhibitors of IDO and have an effect at 5 fold less concentration of existing inhibitors of IDO (200 μM for 1-MT [18]).

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a PerkinElmer 241 MC Polarimeter. IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. ESIMS was carried out on a Hewlett-Packard HP-1100 series LC–MSD system while FABMS was recorded on a Joel-JMS-700 mass spectrometer using glycerol as matrix. NMR spectra were recorded in deuterated solvents (CD_3OD, C_5D_5N) on a Bruker AM-500 and a Varian Mercury Plus 400 spectrometer at 500 or 400 MHz (^1H) and 125 or 100 MHz (^{13}C), respectively. All chemicals shifts (δ) are given in ppm units with reference to the residual solvent signal and the coupling constant (J) are in Hz. Column chromatography was performed using silica gel 60 Merck (63–200 μm and 32–63 μm). TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates developed with EtOAc-MeOH-H_2O (9:1:0.5 or 8:2:1). TLC plates were visualized by spraying with 50% H_2SO_4 and heating for 10 min at 110 C.

2.2. Plant material

The seeds of *E. rheedei* were collected in Konda village, Momo Division, North-West region of Cameroon, in August 2005, and authenticated by Dr. Gaston Achoundong, head of the National Herbarium of Cameroon. A voucher specimen (No. 19966/SRI/CAM) was deposited at the National Herbarium of Cameroon, Yaoundé.

2.3. Extraction and isolation

The dried and powdered seeds kernel (2.5 kg) of *E. rheedei* was extracted three times by maceration with 95% EtOH at room temperature for 24 h. The filtrate obtained was evaporated under reduced pressure to yield a brown residue (315 g). Part of this extract (300 g) was suspended in water (500 ml) and successively partitioned between EtOAc and *n*-BuOH, yielding 19.3 and 105.2 g of extracts after evaporation to dryness, respectively. Part of the EtOAc extract (17.5 g) was subjected to silica gel (63–200 μm) column chromatography, using a gradient of MeOH in EtOAc to give six main fractions (A–F). Fraction E (EtOAc-MeOH (7–3)) was rechromatographed using silica gel (32–63 μm) with EtOAc-MeOH-H_2O (8–2–1) as eluent to afford compounds **1** (50 mg) and **2** (10 mg). Fraction D (EtOAc-MeOH (8–2)) was purified by column chromatography over silica gel (32–63 μm) using EtOAc-MeOH-H_2O (10:2:1) as eluent to yield compound **3** (11 mg) and 1-*O*-methylglucopyranoside (50 mg). Fraction C (EtOAc-MeOH (9–1)) was purified by column chromatography over silica gel (32–63 μm) using EtOAc-MeOH-H_2O (95–5–2) as eluent to yield entadamide A (25 mg) and 3-*O*-β-D-glucopyranosyl-β-sitosterol (150 mg) whereas fraction B (EtOAc) was rechromatographed using silica gel (32–63 μm) with hexane-EtOAc (3:7) as eluent to afforded homogentisic acid (17 mg).

N-sulfonyl-L-tryptophan (*tryptorheedei A*) (**1**): brown oil; $[\alpha]^{20}_D$-12.0 (*c* 0.7, CH_3OH); IR (KBr) υ_{max} 3419, 3208, 1731, 1593 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 283 $[M–H]^-$, 224$[M–CO_2–NH_2]^-$.

3-(N-sulfonylindolyl)-D-lactic acid (*tryptorheedei B*) (**2**): brown oil; $[\alpha]^{20}_D$ +22 (*c* 1.7, CH_3OH); ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 284 $[M–H]^-$, 286 $[M + H]^+$, 224 $[M + H–CO_2–H_2O]^+$, 142 $[M–CO_2–H_2O–SO_3H]^-$.

2.4. Acid hydrolysis of compound **3**

A solution of compound **3** (3 mg) in water (1 mL) and 2 N aqueous CF_3COOH (10 mL) was heated at reflux at 100 °C for 2 h. The mixture was then diluted in water (10 mL) and extracted with EtOAc (3 × 3 mL). The aqueous residue was concentrated to dryness by adding repeatedly MeOH to remove acid and analysed by TLC (silica gel) in comparison with standard sugars by using a mixture of $CHCl_3$–MeOH–AcOH–H_2O (60:32:12:8) as the eluant. The absolute configuration of the sugar residue was determined by GC analysis of its chiral derivative [19].

2.5. Bioassay procedure

TZM-bl cell line was obtained from The NIH AIDS Reagent Program, USA; LuSIV cells were obtained from J. Clements, and were maintained as previously described [20]; PE Annexin V Apoptosis Detection Kit was purchased from BD Biosciences; Vybrant MTT Cell Proliferation Assay Kit from Invitrogen; and One-Glo Luciferase Assay System from Promega.

2.5.1. HIV.RF virus preparation

The chronically infected H9 cell line (H9/HIV.RF) was maintained in cRPMI medium and used to produce infectious viral particles. H9/HIV.RF cells were cultured in a T-75 flask to