Diacylglycerol Acyltransferase-Inhibitory Compounds from *Erythrina senegalensis*

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Inhibition of acyl CoA:diacylglycerol acyltransferase (DGAT) is proposed to be a drug target for the treatment of obesity and type 2 diabetes. Bioassay-guided fractionation of the CH\(_2\)Cl\(_2\)-soluble extract of the stem bark of *Erythrina senegalensis*, using an *in vitro* DGAT enzyme assay, resulted in the isolation of eight known prenylflavonoids, 8-prenylleutone (\(^1\)), auriculatin (\(^2\)), erysenegalensein O (\(^3\)), erysenegalensein D (\(^4\)), erysenegalensein N (\(^5\)), derrone (\(^6\)), alpinumisoflavone (\(^7\)), and 6,8-diprenylgenistein (\(^8\)). Compounds \(^1\), \(^2\)-\(^4\), \(^6\), and \(^8\) inhibited DGAT activity, with IC\(_{50}\) values ranging from 1.1 ± 0.3 to 15.1 ± 1.1 µg/mL. On the basis of the data obtained, we propose isoflavonoids with isoprenyl groups as a novel class of DGAT inhibitors.

**Key words:** *Erythrina senegalensis*, Prenylflavonoid, Diacylglycerol acyltransferase

**INTRODUCTION**

Triglycerides (TG) represent the major form of energy stored in eukaryotes. Disorders or imbalances in TG metabolism are implicated in the pathogenesis and increased risk of obesity, insulin resistance syndrome and type II diabetes, as well as coronary heart disease (Lewis et al., 2002). A key enzyme in the synthesis of triglycerides is acyl CoA:diacylglycerol acyltransferase (DGAT). DGAT is a microsomal enzyme widely expressed in mammalian tissue; it catalyzes the joining of 1, 2-diacylglycerol (DAG) and fatty acyl CoA to form TG at the endoplasmic reticulum (Coleman et al., 2004). The molecular understanding of the function of DGAT was initiated by recent identification of two DGAT genes: *DGAT 1* and *DGAT 2* (Cases et al., 1998, 2001). *DGAT1* belongs to the acyl-CoA:cholesterol acyltransferase (ACAT) gene family while *DGAT2* is a member of a distinct and independent gene family.

Previous studies reported that DGAT1 played a major role in modulating signals of energy homeostasis and a minor role in bulk TG synthesis. Mice lacking *DGAT1* (*Dgat1\(^{-/-}\)* mice) were viable and had reduced TG levels in tissues, including white adipose tissue. *Dgat1\(^{-/-}\)* mice were resistant to diet-induced obesity through a mechanism involving increased energy expenditure; these mice exhibited increased sensitivity to leptin and insulin (Smith et al., 2000; Chen et al., 2002). On the other hand, *DGAT2* was shown to play a comparatively greater role in basal TG synthesis and storage, and *DGAT2* deficiency is known to be incompatible with survival (Stone et al., 2004). These findings suggested that inhibiting specific components of *DGAT1* might be more feasible as a therapeutic strategy in combating human obesity and type 2 diabetes (Chen et al., 2005).

During the screening process we found that a CH\(_2\)Cl\(_2\)-soluble extract of the stem bark of *Erythrina senegalensis* DC inhibited DGAT1 activity (> 50% inhibition at 50 µg/mL). The genus *Erythrina* of the family Leguminosae comprises over 110 species of trees and shrubs that are widely distributed in tropical and subtropical regions, and representative species have been used in indigenous medicine (Oliver-Bever, 1981). Alkaloids, pterocarpans,
and other flavonoids have been reported as constituents of this genus, and possess a wide range of biological activities that include anti-HIV, antioxidant, antimicrobial and anti-inflammatory activities (McKee et al., 1997; Njamen et al., 2004; Chacha et al., 2005). Bioassay-guided fractionation of a CH$_2$Cl$_2$-soluble extract of the stem bark of this plant led to the isolation of a series of prenylflavonoids as the active principles affecting DGAT activity.

**MATERIALS AND METHODS**

**General experimental procedures**

Melting points were determined using a Yanaco micro melting point apparatus and were uncorrected. UV-vis spectra were taken in MeOH using a Beckmann Du-64 instrument. Nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 500 MHz spectrometer using as solvents CDCl$_3$, DMSO-$d_6$ (Aldrich) with TMS as the internal standard. $^{13}$C DEPT, $^1$H-$^1$H COSY, NOESY, HMQC, and HMBC NMR spectra were obtained using standard Varian pulse sequences. All accurate mass experiments were performed on a VG Autospec Ultima mass spectrometer. Column chromatography was conducted using silica gel 60 (40-63 and 63-200 μm particle size) for semi-preparative runs.

Bovine serum albumin and Trizma-base were obtained from Sigma Chemical Co (St. Louis, MO, USA). [1-14C] oleoyl CoA and [1-14C] oleate were purchased from Amersham. For the cell culture, Dulbecco’s modified eagle medium (DMEM), L-glutamine, kanamycin sulfate, and fetal bovine serum (FBS) were purchased from GIBCO-BRL (Gaithersburg, MD, USA).

**Plant material**

The stem bark of *E. senegalensis* DC was collected at Foumban, West Cameroon, in April, 1988. The botanical sample was identified by Prof. Fomum at the Cameroon National Herbarium (Yaounde, Cameroon) where a voucher specimen was deposited.

**Extraction and isolation**

The dried and ground stem bark (17 kg) was extracted with MeOH at room temperature for two weeks. The evaporated MeOH extract was then re-extracted with CH$_2$Cl$_2$ to give 580 g of extract. Successive column chromatography with followed by thin layer chromatography (TLC) analysis gave several fractions consisting of A-G series. Series G (20 g) was separated by silica gel column chromatography (10×30 cm; 63-200 μm particle size) using a gradient of hexane-EtOAc (from 8:1 to 0:1), then EtOAc-MeOH (from 20:1 to 1:1), to yield six fractions (Fr. 1-Fr. 6). The DGAT inhibitory activity was concentrated in Fr. 3 (IC$_{50}$ = 10.1 μg/mL, 120 mg) eluted with hexane-EtOAc (from 2:1 to 1:1). Further purification of Fr. 3 by semi-preparative HPLC [YMC Pak$^\text{®}$ ODS-AM column (6.0×250 mm, 10 μm particle size); mobile phase MeOH-H$_2$O (80:20); flow rate 3 mL/min; UV detection at 254 nm] resulted in the isolation of compounds 3 (4.7 mg), 4 (4.2 mg), and 5 (67 mg), respectively. Fr. 4 [eluted with hexane-EtOAc (4:1), 912 mg] was separated by reversed-phase C$_{18}$ (RP-18) column chromatography using a stepwise gradient of MeOH-H$_2$O (from 60:40 to 80:20; 1 L for each step), to afford two compounds 1 (137 mg) and 2 (230 mg). Fr. 2 [eluted with hexane-EtOAc (from 8:1 to 5:1), 150 mg] was subjected to RP-18 column chromatography eluting with a gradient of MeOH-H$_2$O (from 85:25 to 100:0) to obtain compound 6 (25 mg), 7 (13 mg), and 8 (8 mg).

**8-Prenyleuteone (1)**

Yellow oil (137 mg). HREI-MS m/z 422.1969 (calcd. for C$_{25}$H$_{26}$O$_6$ 422.1787) EIMS; m/z 422 [M]$^+$ (100), 379 (69), 367 (14), 351 (36), 323 (37), 311 (86), 217 (11).

**Auriculatin (2)**

Yellow powder (230 mg). HREI-MS m/z 420.0306 (calcd. for C$_{25}$H$_{24}$O$_6$ 420.0306); EIMS m/z 420 [M]$^+$ (55), 405 (100), 377 (18), 365 (20).

**Erysenegalensein O (3)**

Pale yellow oil (4.7 mg). UV (MeOH) $\lambda_{\text{max}}$ 268 nm.

**Erysenegalensein D (4)**

Yellow oil (4.2 mg). UV (MeOH) $\lambda_{\text{max}}$ 268 nm. IR $\nu_{\text{max}}$ 3400, 1652, 1558, 1097 cm$^{-1}$. HREI-MS m/z 438.1619 (calcd. for C$_{25}$H$_{26}$O$_7$ 438.1678), EIMS m/z 438 [M]$^+$ (10), 367 (48), 311 (100), 293 (14), 177 (27), 167 (22), 149 (73), 134 (8).

**Erysenegalensein N (5)**

Pale yellow oil (63 mg), [α]$_D^{20}$ = -3.3 (c = 0.8, MeOH). UV (MeOH) $\lambda_{\text{max}}$ 268 nm. IR $\nu_{\text{max}}$ 3325, 1620, 1431, 1079 cm$^{-1}$. HREI-MS m/z 438.1661 (calcd. for C$_{25}$H$_{26}$O$_7$ 438.1678), EIMS m/z 438 [M]$^+$ (10), 376 (63), 311 (100), 293 (13), 177 (19), 134 (6).