Prenylisoflavonoids from *Erythrina senegalensis* as Novel HIV-1 Protease Inhibitors

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Abstract

Eight compounds were isolated from the CH_2Cl_2 extracts of *Erythrina senegalensis* to assess HIV-1 protease (PR) activity inhibition. The prenylated isoflavone structures, identified by spectroscopic analysis, were 8-prenylluteone (**1**), auriculatin (**2**), erysenegalensein O (**3**), erysenegalensein D (**4**), erysenegalensein N (**5**), derrone (**6**), alpinumisoflavone (**7**), and 6,8-diprenylgenistein (**8**). The constituents showed dose-dependent inhibitory activities on HIV-1 PR with IC_{50} values from 0.5 to 30.0 μM. Compounds **1 – 5** possessing two hydroxy groups in the 2′ and 4′ positions of the B ring, potently inhibited HIV-1 PR activity. In addition, 6,8-diprenylgenistein (**8**) with two prenyl groups in the 6 and 8 positions of the A ring and one hydroxy group in the 4′ position of B-ring was the most potent HIV-1 PR inhibitor.

Key words

Erythrina senegalensis · Leguminosae · prenylated isoflavonoids · HIV-1 protease

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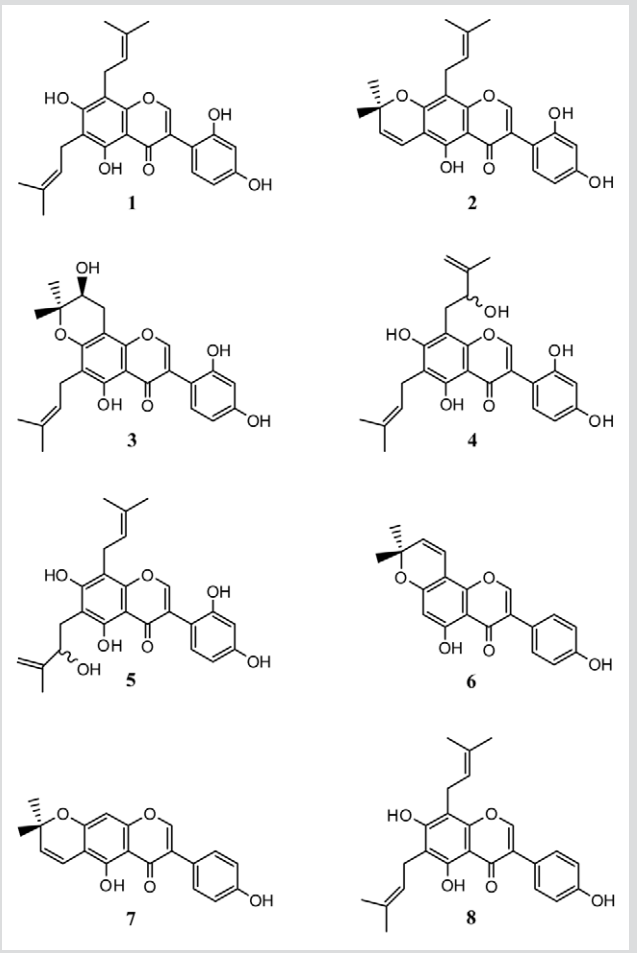

Fig. 1 Structures of compounds **1 – 8** derived from *E. senegalensis*.

Compounds	HIV-1 Protease (IC_{50})[b]
1	4.0 ± 0.1[a]
2	3.5 ± 0.2
3	5.0 ± 0.5
4	2.5 ± 0.1
5	4.5 ± 0.3
6	18.2 ± 0.2
7	30.1 ± 0.1
8	0.5 ± 0.1
Acetyl-pepstatin	0.09 ± 0.01

Table 1 Inhibitory activities against HIV-1 protease *in vitro* by compounds **1 – 8** derived from *E. senegalensis*

[a] Data are expressed as mean ± SEM of three experiments.
[b] Inhibitor concentration required to reduce HIV-1 protease activities on HIV-1 by 50 %.

Human immunodeficiency virus (HIV) is the aetiological agent of acquired immunodeficiency syndrome (AIDS). The viral species HIV-1 encodes a set of proteins that play basic roles in the viral replication cycle. Direct inhibition of viral replication and the inhibition of different steps in its life cycle, e. g., adsorption, fusion, reverse transcription, integration, transcription, maturation and budding, are considered promising approaches in the development of anti-HIV drugs. Thus far, HIV-1 protease (PR) and reverse transcriptase (RT) have been the most effective targets for finding anti-HIV agents. Anti-HIV drugs, used alone or in combination with other drugs, have helped patients live longer. However, these drugs are relatively expensive and require long-term therapy, which is typically accompanied with viral resistance [1]. These problems suggest the need for developing alternate anti-HIV agents that are effective, inexpensive, and less toxic.

Traditional medicines have been investigated as potential sources of anti-HIV agents. New HIV-1 protease inhibitors from natural plant sources were previously reported by the authors [2], [3]. In their screening of natural plants for HIV-1 PR inhibitors, the authors found that the methanolic extracts of *Erythrina senegalensis* DC (Leguminosae), a Cameroonian medicinal plant, showed inhibitory activity on this enzyme. Extracts of leaves, barks, and roots of the genus *Erythrina* have a history of medicinal use, including treatment of female infertility, stomach pain, and gonorrhea [4], [5]. The aqueous extract of *E. senegalensis* showed antiplasmodial, analgesic, and anti-inflammatory activities [6], and prenylated isoflavonoids were isolated from *E. senegalensis* as phospholipase Cγ1 inhibitors [7], [8], [9]. However, components with anti-HIV activity have not been reported.

Eight compounds were isolated from the CH_2Cl_2 extracts of *E. senegalensis* for HIV-1 PR inhibitors. The structures of these compounds were determined based on UV, IR, ^1H, ^{13}C-NMR and mass spectral data using authentic samples. The spectral data were in agreement with literature values [7], [8], [10], [11], [12], [13], [14], [15] for the following prenylated isoflavones: 8-prenylluteone (**1**), auriculatin (**2**), erysenegalensein O (**3**), erysenegalensein D (**4**), erysenegalensein N (**5**), derrone (**6**), alpinumisoflavone (**7**), and 6, 8-diprenylgenistein (**8**).

In addition to prenylated isoflavonoids reported from *E. senegalensis* and *E.* species [7], [8], [10], [11], [12], [17], [18], [19], [20], [21], [22], [23], [24], prenylated isoflavonoids have been isolated from *Bolusanthus speciosus*, *Moghania philippinensis*, *Lespedeza bicolor*, *Cudrania tricuspidata, Macular pomifera* and *Derris scandens* in the Moraceae family [16], [25], [26], [27], [28], [29], [30], [31].

Although prenylated isoflavonoids have been isolated from some plants, only few studies into their biological activities have been reported. Prenylated isoflavonoids from *E. senegalensis* inhibited phopholipase Cγ1 [7], [9], while osajin and pomiferin, prenylated isoflavones from the fruits of *M. pomifera*, showed antioxidative and mouse hepatic microsomal 7-ethoxyresorufin-*O*-deethylase (EROD) inhibitory activities [16]. The prenylated isoflavonoids of wighteone, erysenegalensein E, alpinumisoflavone, and 8-prenylerythrinin C from *E. indica* showed cytotoxic effects against KB cells [24]. Warangalone and robustic acid from the insecticidal plant, *D. scandens*, selectively and potently inhibited the activities of cyclic AMP-dependent protein kinase [29], and gancaonin, 4′-*O*-methylalpinumisoflavone, and alpinumisoflavone from *C. tricuspidata* fruits exhibited inhibitory activities against monoamine oxidase (MAO) [30]. In addition, prenylated flavonoids from the roots of *M. philippinensis* showed estrogenic and antiestrogenic activities [31].

However, the HIV-1 PR activities of these groups have not been reported. Prenylated isoflavones isolated from *E. senegalensis* were evaluated for HIV-1 PR inhibitory activity. These compounds showed dose-dependent inhibitory activities on HIV-1 PR with IC_{50} values ranging from 0.5 to 30.0 μM. Their inhibitory potencies were similar to or more potent than those of previously reported inhibitors. Of the *E. senegalensis* prenylated isoflavones, compounds **1 – 5**, possessing two hydroxy groups at the 2′ and 4′ positions in the B ring, were found to have potent HIV-1 inhibitory activities with IC_{50} values of 2.5 – 5.0 μM. The structural activity relationship of **1 – 5** showed that the structural A ring differences may not influence the inhibitory activities of 2′,4′-dihydroxy isoflavonoids. In addition, 6,8-diprenylgenistein (**8**), which has one hydroxy group at the 4′ position of the B-ring and two prenyl groups at the 6 and 8 positions of the A ring, showed the greatest inhibitory potency among 4′-hydroxy isoflavonoids on HIV-1 PR, with an IC_{50} value of 0.5 μM. Derrone (**6**), and alpinumisoflavone (**7**), which do not have two prenyl groups at the 6 and 8 positions of the A ring, showed markedly lower (IC_{50} values, 18.2 and 30.1 μM) inhibitory activities compared to that of 6,8-diprenylgenistein (**8**). These results suggest that the 6,8-diprenyl groups play an important role in the potency of HIV-1 PR inhibition in 4′-hydroxy isoflavonoids.

Thus, prenylated isoflavonoids are suggested as a novel class of HIV-1 PR inhibitors. This is particularly feasible for 6,8-diprenylgenistein, the most potent inhibitor in the present study. These prenylated isoflavones should be considered for use in combination with other chemotherapeutic agents in the treatment of AIDS.

Materials and Methods

The stem bark of *Erythrina senegalensis* DC (Leguminosae) was collected at Foumban, in western Cameroon, in April 1988 and was identified by Dr. Z. Tanee Fomum, from the University of Yaounde in Yaounde, Cameroon. Herbarium specimens (CNPH-0013) documenting the collection have been deposited at the National Herbarium of Cameroon, in Yaounde, Cameroon. The stem barks (5.0 kg) were extracted with 80% MeOH in an ultrasonic apparatus for 3 hours to yield an 80% MeOH extract (230.2 g) upon removal of the solvent under a vacuum. The CH_2Cl_2 extract was obtained through CH_2Cl_2 fractionation with an aqueous suspension of the 80% MeOH extract. Eight compounds were isolated from the CH_2Cl_2 extract of *E. senegalensis* as HIV-1 PR inhibitors using previously reported methods [6], [7], [8]. In brief, a portion of sample (7.0 g) was chromatographed on silica gel (Kieselgel 60, 230 – 400 mesh; Merck) with $CHCl_3$/MeOH 50/1 and Sephadex LH-20 columns with $CHCl_3$/MeOH/*n*-hexane 2/1/3 to result in fractions I and II. For fraction I, an oily residue of 280.3 mg was applied to rechromatography on ODS RP_{18} with MeOH/H_2O (6/4 → 8/2, stepwise) to produce compounds **1** (5.0 mg) and **2** (13.1 mg). The other fraction (40 mg) was then re-applied to Sephadex LH-20 with $CHCl_3$/MeOH/*n*-hexane (2/1/3) and was further purified by semipreparative HPLC using YMC-Pack ODS (ψ 6.0 mm × 250 mm) to afford three flavonoids: pure compounds **3** (1.3 mg), **4** (13.7 mg), and **5** (25.0 mg) as a pale yellow oil.

Fraction II (150 mg) was subjected to ODS RP-18 column chromatography using MeOH/H_2O (8/2) to give compound **6** (5.2 mg) as white needles. The other fraction was then purified repeatedly by applying it to preparative HPLC. Elution with 85% MeOH resulted in compounds **7** (5.3 mg) and **8** (3.2 mg) as a yellow oil.

HIV-1 PR activity assay was performed as described previously by Lee et al. [2], [3]. The inhibitory activity of the test samples against HIV-1 PR was determined with a HPLC method using a synthetic heptapeptide [His-Lys-Ala-Arg-Val-Leu-(*p*NO_2-Phe)-Glu-Ala-Nle-Ser-NH_2; Bachem Feinchemikalien AG] as a substrate. The fused recombinant HIV-1 PR was also obtained from Bachem Feinchemikalien AG.

Recombinant HIV-1 PR (2.5 μL, 0.175 μg protein) was added to a reaction mixture (25 μL) that contained 50 μM acetate buffer (pH 5.0), 2.5 μg of the substrate and 2.5 μL of a DMSO solution of the test compound (over 99% purity). It was then incubated at 37 °C for 15 min.

The reaction was stopped by addition of 2.5 μL of 10% trifluoroacetic acid. The hydrolysate and remaining substrate were quantitatively analyzed by HPLC. The Shimadzu HPLC system used here was composed of an LC9A liquid chromatograph, an SPD-6A UV spectrophotometric detector, an SLC-6B autoinjector and a C-R6A Chromatopac integrator (Shimadzu Corporation). Five μL of the reaction mixture were injected into an RP-18 column (4.6 × 150 mm; YMC Co. Ltd) and were eluted with a gradient of acetonitrile (15 – 40%) in 0.1% trifluoroacetic acid at a flow rate of 1.0 mL/min. The elution profile was monitored at 280 nm. The substrate and *p*-NO_2-Phe-bearing hydrolysate were eluted at 11.9 and 7.3 min, respectively. Acetyl-pepstatin (over 99% purity; Bachem Feinchemikalien AG), used as a positive control, showed an IC_{50} of 0.09 μM under these conditions.

Three experiments were conducted with each compound and a control. All values are expressed as mean ± SEM. Differences between the drug-treated and the control groups were evaluated

by independent sample *t*-test using SPSS statistical software (SPSS Inc.). $P < 0.05$ was considered as significant.

Supporting information

The isolation scheme for compounds **1 – 8** and the inhibitory activities against HIV-1 protease *in vitro* of compounds **1 – 8** are available as Supporting Information.

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