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# HPLC-DAD-SPE-NMR isolation of tetracyclic spiro-alkaloids with antiplasmodial activity from the seeds of *Erythrina latissima*

Yancho Zarev[a,d], Kenn Foubert[a], Paul Cos[b], Louis Maes[b], Esameldin Elgorashi[c], Sandra Apers[a,†], Iliana Ionkova[d] and Luc Pieters[a,*]

[a] Natural Products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610, Antwerp, Belgium

[b] Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610, Antwerp, Belgium

[c] Toxicology and Ethnoveterinary Medicine; Food, Feed and Veterinary Public Health; ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South-Africa

[d] Department of Pharmacognosy; Faculty of Pharmacy, Medical University - Sofia, Str. Dunav 2, 1000 Sofia, Bulgaria

*Corresponding author at: Natural Products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610, Antwerp, Belgium.

E-mail address: luc.pieters@uantwerpen.be (L. Pieters)

† We dedicate this paper to our colleague, Prof Sandra Apers, who passed away much too early on February 5th, 2017.

## ABSTRACT

Seven tetracyclic spiro-alkaloids, i.e. glucoerysodine (**1**), erysodine (**2**), *epi*-erythratidine (**3**), erysovine (**4**), erythratidine (**5**), erysotrine (**6**) and erythraline (**7**) were isolated from the seeds of *Erythrina latissima* by means of conventional separation methods and HPLC-DAD-SPE-NMR. Their structures were elucidated by spectroscopic means. This is the first report on the isolation of compounds **3**, **5** and **6** from this plant. Antiplasmodial activity against the chloroquine-resistant strain *Plasmodium falciparum* K1 and cytotoxicity against MRC-5 cells (human fetal lung fibroblast cells) was assessed *in vitro*. Erysodine (**2**) and erysovine (**4**) showed moderate activity ($IC_{50}$ 6.53 µM and 4.05 µM, respectively), compared with the standard chloroquine ($IC_{50}$ = 0.14 µM). No cytotoxicity was observed in a concentration up to 64.0 µM.

**KEYWORDS**: *Erythrina latissima*, antiplasmodial acivity, cytotoxicity, HPLC-DAD-SPE-NMR, *Erythrina* alkaloids

## 1. Introduction

Malaria is an infectious disease caused by protozoan parasites from the genus *Plasmodium*. Different species of *Plasmodium* can cause human malaria, *P. falciparum* being the most dangerous one. According to the WHO (World Health Organization 2017), 216 million cases of malaria from 91 countries worldwide were reported in 2016, an increase of 5 million cases over the previous year. Natural products always have played a prominent role in the search for new antimalarial drugs, quinine and artemisinin being notable examples (Xu et al. 2013).

The genus *Erythrina* (Leguminosae) consist of 110 species, mainly trees and scrubs that are widely distributed throughout the tropical regions of the world. The extracts of the roots *E. abyssinica*, *E. burttii* and *E. sacleuxii* showed significant antimalarial activities, which justified the wide traditional use of *E. abyssinica* for treatment of malaria in East Africa. Chukwujekwu et al. (2016) demonstrated antiplasmodial activity of stem bark of *E. caffra*. The activities of these plants are associated mostly with flavonoids and isoflavonoids (Yenesew et al. 2004).

Various flavonoids, isoflavonoids and pterocarpans have been obtained from different parts of *E. latissima* (Wanjala and Majinda 2001; Chacha et al. 2005), while the seeds are well recognized as bioactive alkaloid-rich sources (Wanjala et al. 2002; Wanjala and Majinda, 2000a). Wanjala and Majinda (2000b) have reported two isoflavonoids, six flavanones and a feruloyl ester from the stem bark. In our laboratory five prenylated flavonoids were identified as new compounds from the stem bark of *E. latissima*, ten were reported for the first time from *E. latissima* and glycyrrhizoflavone was new for the genus *Erythrina*. Prenylated flavonoids such as sigmoidin A and B, 4-*O*-methylsigmoidin B, abyssinin I, II and III present in *E. latissima* stem bark were described as highly active antigenotoxic compounds (Zarev et al. 2017). In the present work the isolation, structure elucidation and evaluation of antiplasmodial activity and cytotoxicity of a series

of *Erythrina* alkaloids obtained from seeds of *E. latissima* is reported, as a continuation of our investigations on potentially antimalarial species and their constituents from Central, Eastern and Southern Africa (Mesia et al. 2008; Mbwambo et al. 2004).

## 2. Results and discussion

In this study the 80% methanolic extract of *Erythrina latissima* seeds was subjected to targeted pH controlled fractionation. The isolation of single compounds was performed with HPLC-DAD-SPE-NMR and in this way seven spirocyclic *Erythrina* alkaloids were obtained: glucoerysodine (**1**) (Wandji et al. 1994), erysodine (**2**) , *epi*-erythratidine (**3**), erysovine (**4**), erythratidine (**5**), erysotrine (**6**) and erythraline (**7**) (Chawla et al. 1983). Their structures were elucidated by 1D ($^{1}$H, $^{13}$C) and 2D NMR experiments (COSY, HSQC, HMBC) and confirmed by HRESIMS (Figure 1). Epimers **3** and **5** could be distinguished based on the difference in specific optical rotation. The signals for H-2 and H-3 of compounds **3** and **5** are multiplets and the coupling constants cannot be easily described; however, the chemical shifts of C2 and C3 for both isomers show a different dispersion (García-Beltrán et al. 2012). The stereoisomers *epi*-erythratidine and erythratidine, as well as erysotrine were isolated for the first time form *E. latissima*; *epi*-erythratidine has been reported only in *E. fusca* and *E. variegata*.

The antiplasmodial activity against *Plasmodium falciparum* strain K1 and cytotoxicity against MRC-5 cells (human fetal lung fibroblast cells) were evaluated for compounds **1**-**7** (Table 1) according to published procedures (Tuenter et al. 2016). Only the structural isomers erysodine (**2**) and erysovine (**4**) as well as erythraline (**7**) showed antiplasmodial activity with $IC_{50}$ values <10 µM, ranging between 4.1 and 7.3 µM. Erythraline (**7**) was the only compound that showed

cytotoxicity with an $IC_{50}$ value of 37.8 ± 2.3 µM; for the other compounds no cytotoxicity was observed in a concentration up to 64.0 µM ($IC_{50}$ > 64.0 µM).

The antiplasmodial activity of the genus *Erythrina* is mostly attributed to flavonoids and related compounds. This is the first report on the antiplasmodial activity of spirocyclic *Erythrina* alkaloids. (+)-10,11-Dioxoerythratine and (+)-10,11-dioxoepierythratidine had been evaluated for antiplasmodial, antimycobacterial and cytotoxic activities, but were inactive (Rukachaisirikul et al. 2008). Callejon et al. (2014) have evaluated the leishmanicidal activity of tetrahydroprotoberberine and spirocyclic *Erythrina* alkaloids, but compounds belonging to the latter group showed a low activity and low selectivity. Although the alkaloids evaluated in this work may be not sufficiently active to be developed as such as antimalarial medicines, at least erysodine (**2**) and erysovine (**4**) showed a good selectivity. Their spirocyclic skeleton may serve as a promising lead structure for further investigations on semisynthetic derivatives or synthetic analogues with improved activity.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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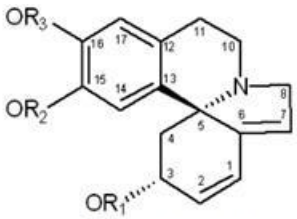
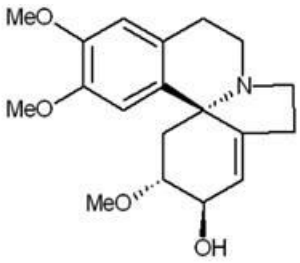
**Tables**

Table 1. Antiplasmodial activity against *P. falciparum* strain K1 and cytotoxicity against MRC5-cells ($IC_{50}$ µM ± SD) for compounds **1**-**7**.

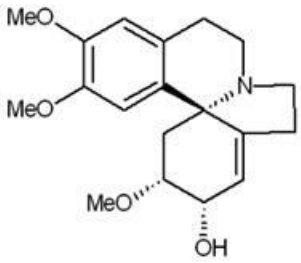
| Compound | *P. Falciparum K1* | MRC-5 |
|---|---|---|
| **1** | >64.00 | >64.00 |
| **2** | 6.5 ± 4.7 | >64.00 |
| **3** | >64.00 | >64.00 |
| **4** | 4.1 ± 0.6 | >64.00 |
| **5** | >64.00 | >64.00 |
| **6** | 20.6 ± 8.6 | >64.00 |
| **7** | 7.3 ± 4.9 | 37.8 ± 2.3 |
| chloroquine | 0.1 | - |
| tamoxifen | - | 9.7 |




| | | | |
|---|---|---|---|
| **1** | $R_1 = CH_3$ | $R_2 = CH_3$ | $R_3 = Glu$ |
| **2** | $R_1 = CH_3$ | $R_2 = CH_3$ | $R_3 = H$ |
| **4** | $R_1 = CH_3$ | $R_2 = H$ | $R_3 = CH_3$ |
| **6** | $R_1 = CH_3$ | $R_2 = CH_3$ | R3 = $CH_3$ |
| **7** | $R_1 = CH_3$ | $R_2^+ = R_3 = -CH_2^-$ | |




**3**




**5**