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ScienceDirect
Phytomedicine 16 (2009) 659–664
Phytomedicine
www.elsevier.de/phymed

SHORT COMMUNICATION

Antitrypanosomal activity of some pregnane glycosides isolated from *Caralluma* species

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Abstract

Pregnane glycosides previously isolated from genus *Caralluma* (*C. Penicillata, C. tuberculata* and *C. russelliana*) were tested for their antitrypanosomal activity. Penicilloside E showed the highest antitrypanosomal activity (IC_{50} 1.01 μg/ml) followed by caratuberside C (IC_{50} 1.85 μg/ml), which exhibited the highest selectivity index (SI 12.04). It was noticed that acylation is required for the antitrypanosomal activity while glycosylation at C-20 has no significant effect on the activity.

Keywords: *Caralluma penicillata*; *Caralluma russelliana*; *Caralluma tuberculata*; Asclepiadaceae; Penicillosides; Russeliosides; Caratubersides; Antitrypanosomal activity; Cytotoxicity

Introduction

Genus *Caralluma* belongs to family Asclepiadaceae, which comprises about 200 genera and 2500 species (Evans 2002). Several members of the genus *Caralluma* have found medicinal uses in the treatment of rheumatism, diabetes, and leprosy and as antiseptics and disinfectants (Neuwinger 1994). Previous studies on the plants of the genus *Caralluma* have reported the isolation of several pregnane glycosides or their esters (Ahmed et al. 1988; Tanaka et al. 1990; Lin et al. 1994; Qiu et al. 1999), with antitumor activity (Deepak et al. 1989, 1997) and recently showed antitrypanosomal activity (Abdel-Sattar et al. 2008).

African trypanosomes are protozoan parasites which responsible for human African trypanosomiasis disease or (HAT) 'sleeping sickness' and nagana in cattle. HAT are transmitted by the bite of an infected tsetse fly, *Trypanosoma brucei brucei*. The causative agent of nagana, is closely related to *Trypanosoma brucei rhodesiense* (which present in East to South Africa) and *Trypanosoma brucei gambiense* (which present in West and Central Africa).

Sleeping sickness currently affects about half a million people in sub-Saharan Africa and an estimated 60 million people are at risk of attaching this disease, which is fatal if untreated (WHO 1998; Barrett 1999). However, the currently available treatments are far from being ideal.

Research for development of new chemotherapy of HAT over the past century has yielded only four clinically approved drugs (Fig. 1), three of which were introduced more than 50 years ago. Parasitic diseases in general, and trypanosomiasis in particular, showed extremely badly research development. After merging

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doi:10.1016/j.phymed.2009.02.009

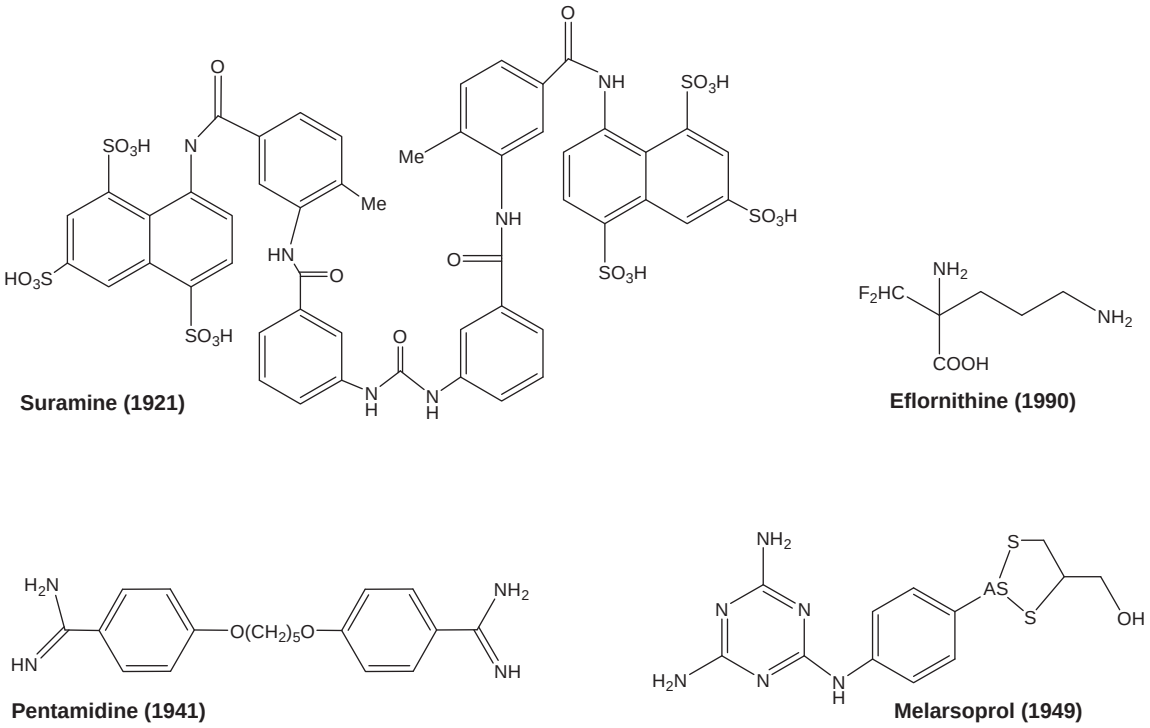

Fig. 1. Registered anti-trypanosomal drugs.

of large pharmaceutical companies, their investment has been declined in drug development for tropical diseases and other less profitable 'orphan diseases'. With the exception of antimalarials, there are currently only four drugs (suramine developed in 1921; pentamidine developed in 1941; melarsoprol developed in 1949 and eflornithine developed in 1990) approved to treat HAT (Mackey et al. 2006). However, eflornithine and pentamidine are ineffective against sleeping sickness caused by *T. b. rhodesiense*. Treatment with melarsoprol, the only generally effective first-line drug, required lengthy parentral adminstration and can result in up to 10% mortality.

The toxicity and the upsurge in the number of patients failing to respond to melarsoprol because of drug resistance (Pepin and Milord 1994) reflects the need for discovery of new chemotherapeutic agents against HAT.

Results of biological screening of plants derived products showed that many of them possess antiparasitic properties with high efficacy and good selectivity (Camacho et al. 2000; Tagboto and Townson 2001; Kayser et al. 2003; Abdel-Sattar et al. 2008).

Materials and methods

Antitrypanosomal activity

Samples were tested by *in vitro* antitrypanosomal assay system by a dose response curve using Alamar Blue sensitivity assay according to the method reported by Räz et al. (1997). Melarsoprol and pentamidine were used as positive controls (Table 1).

In vitro antitrypanosomal assay against *T. brucei brucei*

T. brucei brucei GUTat 3.1 bloodstream-form of trypomastigotes were maintained in Isocove's modified Dulbecco's medium (IMDM) with various supplements containing 10% heat-inactivated fetal bovine serum (FBS) at 37 °C, under 5% CO_2–95% air according to the method of Yabu et al. (1998).

In vitro antitrypanosomal activity of test sample has been estimated by a dose response curve using Alamar Blue sensitivity assay according to the method of Räz et al. (1997): bloodstream forms of *T. brucei brucei* were inoculated into 96-well microtiter plates (Costar, USA). The trypanosomes were incubated with the serial sample dilutions for 70 h at 37 °C in 5% CO_2. Serial dilutions of the standard drugs (Table 1) were prepared and tested in the same manner. Control experiment was performed as previously mentioned, containing medium only. Then 10 µl Alamar Blue were added and after 2 h of incubation the fluorescence was determined at an excitation wavelength of 530 nm and an emission wavelength of 590 nm using a Gemini Plate Reader (Molecular Devices). Fluorescence development was expressed as the percentage of that of the control, and