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Research paper

Tyrosol and hydroxytyrosol derivatives as antitrypanosomal and antileishmanial agents

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ABSTRACT

Trypanosomiasis and leishmaniasis keep being a real challenge for health and development of African countries. Existing treatments have considerable side effects and increase resistance of the parasites. We have measured antitrypanosomal and antileishmanial activity of natural phenols, tyrosol (TYR) and hydroxytyrosol (HT) and several of their esters and metabolites. We found significant IC_{50} values against *Trypanosoma brucei* for HT decanoate ester and HT dodecanoate ester (0.6 and 0.36 μM, respectively). This represents a large increase in activity with respect to HT (79 and 132 fold, respectively). Moreover, both compounds displayed a high selectivity index against MRC-5, a non-tumoral human cell line (118 and 106, respectively). Then, we synthesized a focused library of compounds to explore structure-activity. We found the ether and thiourea analogs of HT decanoate ester and HT dodecanoate ester also showed IC_{50} values against *T. brucei* in the low micromolar range. In conclusion, the di-ortho phenolic ring and medium size alkyl chain are essential for activity whereas the nature of the chemical bond among them seems less important.

1. Introduction

Infectious diseases caused by protozoan parasites affect millions of people around the world, especially in tropical and subtropical areas. Among them, leishmaniasis (caused by different species of *Leishmania*) is a major global health problem with around 12 million people infected and causing 1–2 million new cases every year. *Trypanosoma brucei*, transmitted by the tsetse fly, is responsible for sleeping sickness in humans and preclude the development of productive livestock and agricultural activity based on domesticated animals where it causes Nagana [1,2]. For these reasons, this parasite is considered to be one of the major root causes of hunger and poverty in sub-Sahara Africa. Current treatments for both diseases present important drawbacks such as high toxicity, increased resistance and variability on their efficacy depending on the different strain of the parasite. Thus, the development of new antiparasitic therapies continues to be necessary.

Natural products have been tested for decades to find new antitrypanosomal and antileishmanial agents. Among them, several phenolics and polyphenols have shown relevant activity. For example, flavonoids such as 7,8-dihydroxyflavone, 3-hydroxyflavone and rhamnetin showed low micromolar IC_{50} values for *T. brucei rhodesiense* and others such as luteolin and quercetin also displayed low micromolar IC_{50}s for *Leishmania donovani* [3]. In fact, quercetin glycosides present in the aqueous extract of *Kalanchoe pinnata* were active by oral administration in experimental cutaneous and visceral leishmaniasis infections produced *in vivo* [4,5].

Tyrosol (TYR, **1**) and hydroxytyrosol (HT, **2**) (Fig. 1) are natural phenolic antioxidants present in olives and olive oil. They have shown a diverse biological activity such as antibacterial [6,7], antiviral [8], anti-inflammatory [9–12], neuroprotective [13] and anticancer activity [14], inhibition of human LDL oxidation [15] and

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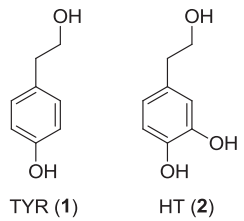

Fig. 1. Chemical structures of tyrosol (TYR, **1**) and hydroxytyrosol (HT, **2**).

prevention of platelet aggregation [16]. Recently, Moradi-Afrapoli et al. reported medium activity of TYR against *T. brucei rhodesiense* (IC_{50} > 15 μM) [17]. To the best of our knowledge, HT has not been examined for activity against *T. brucei*. Though, moderate antileishmanial activity was reported for HT against both, promastigotes of *Leishmania infantum*, *L. donovani*, and *Leishmania major*, and against *L. donovani* amastigotes that parasitize J774A.1 macrophages [18].

The preparation of chemically-modified natural products to improve their antiparasitic activity is still a very active source of potential new drugs. This is the case of chalcone [19] or caracasine acid derivatives [20], or the preparation of hybrids such as *Cinchona* alkaloids with bile acids [21] or caffeine-based chalcones [22]. Tyrosol and hydroxytyrosol derivatives have also been synthesized in order to improve the antioxidant and biological properties of the parent compounds. Hydroxytyrosol fatty acid esters increased the protection of proteins and lipids against oxidation caused by peroxyl radicals in a brain homogenate as an ex vivo model [23]. Similarly, hydroxytyrosol acetate was able to reduce the metabolic imbalance induced by a high-cholesterol diet in rats to a higher extent than HT [24]. Finally, HT alkyl ether derivatives, more stable under biological conditions than HT, have been reported to exert antiproliferative [25], neuroprotective [26,27], antiplatelet and anti-inflammatory effects [28] that are greater than those of HT.

Due to the improved biological activity found for several HT derivatives with respect to HT itself, we decided to investigate the antitrypanosomal and antileishmanial activity of a series of TYR and HT derivatives. We prepared a series of tyrosol and hydroxytyrosol fatty acid esters together with three metabolites of HT and TYR, tyrosol sulfate, tyrosol glucuronate and hydroxytyrosol glucuronate for a first *in vitro* screening. Later, we synthesized a focused library of compounds to explore structure-activity relevance varying the number of phenolic OH groups, the type of chemical bond between the phenolic ring and the alkyl chain, and the length of the alkyl chain.

2. Results and discussion

2.1. Chemistry

Tyrosol and hydroxytyrosol fatty acid esters (**3**–**7** and **11**–**16**, respectively) were synthesized by enzymatic acylation using Novozym 435 and the corresponding vinyl acyl donor in t-butyl methyl ether as reported previously [29–31] (Scheme 1). Tyrosol and hydroxytyrosol glucuronates **8** and **17, 18** were prepared by glycosylation of the acetyl protected TYR or HT derivatives (**3** and **10,** respectively). We used the acetyl protected trichloroacetimidate glucuronosyl derivative **19** as glycosyl donor and boron trifluoride etherate as the catalyst in the glycosylation step. Final acetyl deprotection yielded glucuronate derivatives **8** and **17,18** as described previously [32]. TYR sulfate **9** was prepared following a similar strategy to the one used for the glucuronate derivative. Sulfation of tyrosol acetate **3** was carried out with $SO_3{\bullet}NMe_3$ as sulfating reagent, NEt_3 as base, and acetonitrile as solvent at 100 °C under microwave radiation. The reaction afforded the sulfated TYR derivative in good yield. Final acyl deprotection and reverse phase purification gave TYR sulfate **9**.

We identified hydroxytyrosol decanoate ester **13** and hydroxytyrosol dodecanoate ester **14** as relevant hit compounds against *T. brucei* (see below, Table 2) after carrying out the first *in vitro* screening on *T. brucei* and *L. donovani*. We decided then to perform structure-activity studies on **13** and **14** by changing the phenolic hydroxyl groups, the type of chemical bond between the phenolic ring and the alkyl chain, and the length of the alkyl chain (Fig. 2) in order to improve its biological activity.

We synthesized a hydroxyl protected version of compound **14** by formation of the acetal derivative **22** and also a shorter version with a decanoate alkyl chain, compound **21** (Scheme 2). The cyclic acetal intermediate **20** was obtained by reaction of HT with CH_2Cl_2 in basic conditions and then acylated using the enzymatic conditions used previously for HT yielding the desired compounds. The ether analog of **14**, compound **29**, was prepared as reported previously [33] by benzyl protection of the phenolic groups, alkylation with 1-iodododecane under basic conditions and final hydrogenation. The HT ether decanoate derivative **28** was prepared following the same procedure. We also prepared the p-methoxybenzyl (PMB) protected HT derivative **24** trying to improve the yields of the route. However, deprotection with trifluoroacetic acid of the ether intermediate **27** was unsuccessful. The synthesis of the thioether analog of **14** was attempted next. Tosylation of derivatives **23** and **24** was followed by nucleophilic displacement with 1-dodecanethiol producing the protected thioether derivatives **32** and **33** with moderate yields. Neither hydrogenation nor reaction under strong acidic conditions yielded the final thioether deprotected products.

Dopamine hydrochloride was the starting material for the synthesis of the amide and thiourea analogs of **13** and **14** (compounds **37**–**40**) (Scheme 3). Dopamine was also synthesized to compare its activity with HT. The thiourea derivatives were synthesized by reaction of the isothiocyanate intermediate **36** with the corresponding primary alkylamine to obtain the products in moderate yields. The amide analogs of **13** and **14** were prepared by reaction of dopamine hydrochloride with the corresponding fatty acids using HATU as the coupling reagent.

2.2. Biological evaluation

The *in vitro* antiparasitic activities of TYR, HT and their derivatives **3**–**18** were evaluated against *T. brucei brucei* and against axenic amastigotes of *L. donovani*. Those compounds exhibiting over 40% inhibition of axenic amastigotes at 20 μM were also evaluated on intracellular amastigotes of *L. donovani*. The cytotoxicity of these compounds was also evaluated against a human non-tumoral lung cell line (MRC-5). Suramin and amphotericin B were used as positive drug controls for *T. brucei* and *L. donovani*, respectively. Selectivity indices (SI) were calculated according to the formula: IC_{50} (MRC-5)/IC_{50} parasite. The activity gain (AG) of each compound with respect to the reference compound (TYR or HT) was calculated according to the formulas: IC_{50} (TYR)/IC_{50} compound and IC_{50} (HT)/IC_{50} compound.

Table 1 presents the antiparasitic and cytotoxic data for TYR and its derivatives **3**–**9**. IC_{50} values against *T. brucei* ranged from 10 to 62.7 μM and selectivity indices from 1.5 to 5. None of the TYR derivatives improved the activity of TYR itself as can be observed from the AG values. In the case of *L. donovani*, compounds **4** and **5** showed inhibition over 40% of axenic amastigotes at 20 μM concentration. These two derivatives were evaluated on intracellular amastigotes and showed IC_{50} values > 10 μM. Aissa et al. [34]