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Research paper

Structure-activity relationship of hybrids of *Cinchona* alkaloids and bile acids with *in vitro* antiplasmodial and antitrypanosomal activities

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ABSTRACT

In this work, a series of hybrid compounds were tested as antiparasitic substances. These hybrids were prepared from bile acids and a series of antiparasitic *Cinchona* alkaloids by the formation of a covalent C–C bond *via* a decarboxylative Barton–Zard reaction between the two entities. The bile acids showed only weak antiparasitic properties, but all the hybrids exhibited high *in vitro* activities (IC_{50}: 0.48–5.39 μM) against *Trypanosoma brucei*. These hybrids were more active than their respective parent alkaloids (up to a 135 fold increase in activity), and displayed good selectivity indices. Additionally, all these compounds inhibited the *in vitro* growth of a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7: IC_{50}: 36.1 nM to 8.72 μM), and the most active hybrids had IC_{50}s comparable to that of artemisinin (IC_{50}: 36 nM). Some structure-activity relationships among the group of 48 hybrids are discussed. The increase in antiparasitic activity may be explained by an improvement in bioavailability, since the more lipophilic derivatives showed the lowest IC_{50}s.

1. Introduction

Infectious diseases, such as malaria and African sleeping sickness, which are caused by the protozoan parasites *Plasmodium sp.* and *Trypanosoma sp.* respectively, are still major global health problems, especially in developing countries. According to the World Health Organisation (WHO), in 2012 malaria caused an estimated 627000 deaths, mostly among African children, and in the same year, 7197 new cases of African trypanosomiasis were reported [1]. Most of the drugs currently in use have the drawbacks of their toxicities, increased resistance, and a variable efficacy against different strains or species, making necessary the search for new antiparasitic compounds [2–5]. Actually, there are only a few efficient treatments for these diseases, and these are often based in a combination of drugs. For example, the World Health Organisation does not recommend the use of arteminisin derivatives as a monotherapy to treat malaria, since this will lead to an increase in drug resistance [1], and instead promotes the use of a combination of drugs with different modes of action. In this regard, the preparation of new bioactive substances by the use of bioconjugation can be considered an alternative approach for the discovery of new drugs. The synthesis of hybrids of bioactive compounds with the combined properties of their individual components has emerged as a fast growing methodology in medicinal chemistry [6–8]. This strategy has been employed in the search for new antimalarial compounds [9,10], and several examples of antiparasitic steroid-based hybrids have been reported [11]. On the other hand, the efficacy of a drug is closely related to its transport properties and bioavailability, which in turn are related to other processes such as intestinal absorption, further biotransformations, and the ease and mechanism of elimination [12,13]. In this sense, bile acids are of great interest in drug discovery because of their efficient transport system and their properties as adsorption enhancers [14]. All these observations led to the preparation of a new series of antiparasitic hybrids of *Cinchona* alkaloids and bile acids [15]. The strategy was to

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combine the antiparasitic properties of the natural *Cinchona* alkaloids, which are currently used to treat severe cases of malaria [16], with the known properties of the bile acids as drug transporters, especially taking into account that some bile acids also have mild antiparasitic activity [17,18]. This strategy involved the formation of a covalent C–C bond between the quinoline core of the alkaloids and the side chain of a bile acid *via* a radical Barton–Zard decarboxylation reaction, constituting the first example of this reaction using natural *Cinchona* alkaloids as substrates. The Barton–Zard reaction can be used for the formation of a new C–C bond between a nucleophilic alkyl radical, obtained by decarboxylation of an *O*-acyl ester (Barton ester), and an electron-deficient position of an aromatic substrate [19,20], such as the C-2′ position of the quinoline core of *Cinchona* alkaloids. In these compounds C-4′of the quinoline core is substituted, leaving C-2′ as the only available position for a nucleophilic attack. From a biological point of view, the incorporation of a norcholane moiety to *Cinchona* alkaloids (quinine, quinidine, cinchonine and cinchonidine), had a positive effect on their *in vitro* antiparasitic activity against *Plasmodium falciparum* and *Trypanosoma brucei* [15]. In the first group of synthesized hybrids, the degree of oxidation of the bile acid moiety had a marked influence on the activity, since the compounds derived from chenodeoxycholic acid were more active than those prepared from lithocholic acid. For this reason, and as a continuation of this work, this promising library of hybrids was expanded by the incorporation of four additional bile acid moieties (cholic, deoxycholic, hyocholic and hyodeoxycholic acids) in order to get a more complete picture of the influence of the different hydroxy groups present on the steroidal core. In the present work, 32 new hybrids were synthesised (Fig. 1) to add to the series of the 16 previous derivatives prepared from lithocholic and chenodeoxycholic acids [15]. The *in vitro* antiparasitic activities of the hybrids **1**–**12a**–**d**, the four parent alkaloids (**QN** = quinine, **QND** = quinidine, **CN** = cinchonine, **CND** = cinchonidine) and the bile acids **1**–**12** were also evaluated against *P. falciparum* and *T. brucei brucei*. The cytotoxicities of all these compounds against the WI-38 cell line (human non cancer fibroblast) were also determined. Based on previous disappointing results [15], the activity of the new hybrids against *Leishmania mexicana mexicana* was not tested. In this work, the antiparasitic activity results of the complete series of 48 hybrids are discussed, and some structure-activity relationships can now be established.

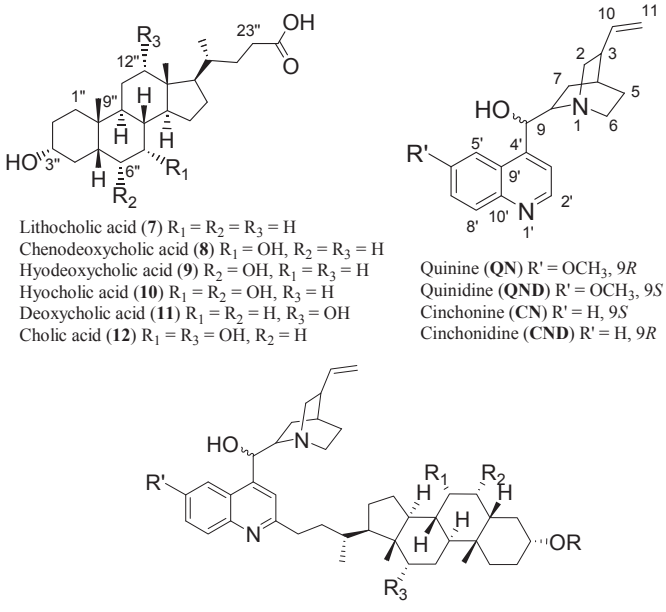

Fig. 1. Generic structure of the hybrids.

2. Chemistry

Hybrids **3**–**6a**–**d** were prepared following the synthetic pathway outlined in Scheme 1, using a Barton–Zard decarboxylation reaction as described previously for the preparation of hybrids **1**–**2a**–**d** [15]. Briefly, the Barton esters of the peracetylated bile acids **3**–**6**, obtained respectively from bile acids **9**–**12**, were prepared by reaction with 2-mercaptopyridine-*N*-oxide and DCC (*N*,*N*′-dicyclohexylcarbodiimide) in dry CH_2Cl_2 at 0 °C. These esters were then subjected to photolytic decarboxylation in the presence of a large excess (10 eq.) of a protonated *Cinchona* alkaloid acting as a radical trap, by irradiation with a 300 W tungsten lamp at 0 °C in CH_2Cl_2 under N_2 atmosphere to yield the corresponding hybrids. The purification of the final products was complicated by the large excess of the free *Cinchona* alkaloids. For this reason, the yields in Table 1 are of the final, purified compounds, and were calculated after the 2 reactions and consecutive purification steps. In general, the purification of the hybrids was achieved by normal-phase VLC, to remove the less polar side-products, such as the thioether which is the main side-product of the reaction [21], followed by reverse-phase VLC to remove most of the large excess of the parent alkaloid, and finally permeation through a Sephadex LH-20 column. The removal of the acetate protecting groups was achieved by treatment with 20% NaOH in MeOH under reflux, yielding quantitatively the corresponding deacetylated hybrids (Scheme 1). Table 1 lists the complete series of 48 hybrids, including those described herein and in the previous work in order to get a full picture of some structure-activity relationships [15].

3. Biological evaluation

The *in vitro* antiparasitic activities of the hybrids **3**–**6a**–**d** and **9**–**12a**–**d** as well as those of the parent free bile acids **7**–**12** and acetylated bile acids **1**–**6**, were evaluated against *T. brucei brucei* bloodstream forms (Tbb), and also against a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7). The cytotoxicities of all these compounds were also evaluated against a human normal fibroblast cell line (WI-38). Suramin, artemisinin and campthotecin were used respectively as positive controls. In the bioassays with *T. brucei brucei* and WI-38 cells, the hybrids were evaluated at a concentration range between 0.009 μg/ml and 20 μg/ml, and the corresponding IC_{50}s (drug concentration resulting in 50% inhibition of parasite or cell growth) were calculated. In the case of *P. falciparum*, the hybrids were first evaluated in duplicate at the same range of concentrations. However, some of the compounds still showed a detectable parasite growth inhibition at concentrations as low as 0.009 μg/ml. In these cases, the evaluation was repeated (in triplicate) at lower concentrations (between 4 μg/ml and 1.8 ng/ml), in order to confirm the IC_{50} values. The selectivity indices were calculated for each parasite according to the formula: IC_{50}(WI38)/IC_{50}(parasite). Table 2 lists the *in vitro* biological activities of the complete library of hybrids, which includes the 32 new compounds described herein, as well as the 16 previously reported derivatives [15] for a better understanding of the structure-activity relationships among the compounds. The antiparasitic activities and cytotoxicities of the parent bile acids **1**–**12** were also evaluated, and those results are displayed in Table 3. In the three bioassays, the bile acids were only evaluated at 2 concentrations: 20 and 100 μg/ml. In the event of a larger than 50% inhibition at 20 μg/ml, additional bioassays were performed at lower concentrations (100–0.5 μg/ml) to determine the IC_{50}s.