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Synthesis of conjugated bile acids/azastilbenes as potential antioxidant and photoprotective agents

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ABSTRACT

A series of 14 bile acids/azastilbenes conjugates (**1a–g** and **2a–g**) was prepared through the condensation of bile amides (**1** and **2**) and aromatic aldehydes. The newly synthesized conjugates were evaluated *in vitro* for their antioxidant and photoprotective activities. Six compounds (**1**, **1a**, **1b**, **2**, **2a** and **2b**) showed promising antioxidant activity with IC_{50} values of 19.60–31.83 μg mL^{-1}. The synthesized compounds presented a varied photoprotection profile, with the SPF ranging from 2 to 9. Among the 16 compounds tested for the protection against UVB sunrays, 3 compounds (**2c**, **2e** and **2g**) presented more significant protection than resveratrol and the free azastilbene **3**; while the UVAPF increased from 2 in resveratrol and 5 in **3** to 5–11 in the majority of the conjugates.

1. Introduction

Steroids are natural compounds with several important applications in many fields of research, such as pharmacology, biomimetic and supramolecular chemistry, and nanotechnology [1]. In particular, bile acids such as cholic and deoxycholic acids (Fig. 1) have been considered quite useful in the preparation of new pharmaceutic drugs due to their chemical and biological properties [2,3]. They are end products of cholesterol metabolism in the liver and exhibit a facially amphiphilic nature, due to the polar hydroxyl groups and non-polar hydrocarbon body.

The literature describes a vast amount of pharmacological applications for bile acids derivatives, notably antimicrobial [4–6], antifungal [4,7,8], carbonic anhydrase inhibition [9,10], and antitumor [11]. Yet, one of the most promising applications of bile acid derivatives is as drug carriers, a direct consequence of their amphiphilic character. They have already been reported to improve the permeability of cell membranes, including the bacterial wall [2,4]. The high specificity and capacity of bile acid transport systems during their entero-hepatic circulation allows the use of drug-bile acid conjugates for specific drug targeting to the liver and also improving the intestinal absorption of poorly or non absorbed drugs [2,12].

Within this context, a number of works [13–21] described the conjugation of active substances with bile derivatives through chemical modifications at the 3- and 24-positions of the sterol nucleus. For instance, Pore et al. [17] designed novel fluconazole/bile acid conjugates and the new molecules showed good antifungal activity against *Candida* species. Tolle-Sander et al. [19], in turn, described increased acyclovir oral bioavailability (from 20% to 54%) through a bile acid conjugate: the antiviral drug was conjugated with chenodeoxycholane acid via a valine linker.

Our research group has previously described the conjugation of biliar acid with other bioactive molecules, such as thiopurine [20] and aminoquinoline [21], and the results highlight the importance of steroids as carriers.

In this work, we focused on the conjugation of these steroids with azastilbenes. Stilbenes are a class of plant polyphenols produced in a number of unrelated plant species. They are generally known as phytoalexins, which can be biosynthesized in response to pathogen or herbivore attacks [22]. These compounds are of noticeable interest for medicinal chemistry because of their diverse biological activities [23]; resveratrol, a potent antioxidant agent, is the major stilbene present in grapes and wines. Particularly, azastilbenes comprise a special group of stilbenes derivatives that possess biological properties such as antioxidant [24,25],

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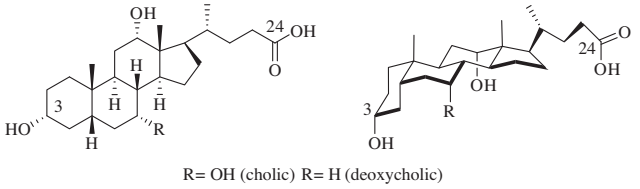

Fig. 1. Chemical structures of cholic and deoxycholic acids.

antibacterial [26,27], antimicrobial [28,29], antifungal [30], and antiproliferative [31]. In a recent effort, our research group has been reporting the antileishmanial [32], depigmenting [33] and photoprotective [34] activities of azastilbenes. In these reports, the azastilbenes are presented as bioisosters of stilbene since may be seen as resulting from the substitution of the central C=C linkage with a C=N double bond (Fig. 2).

In this work we synthesized and characterized new bile acids/azastilbenes conjugates, in which the azastilbenes have been built at carboxylic acid C-24 at cholic and deoxycholic acids through the intermediates amides **1** and **2**.

2. Experimental

2.1. General

Melting points (M.p.) were determined using a Microquímica MQAPF-301 digital apparatus and are uncorrected. Infrared spectra (wave numbers in cm^{-1}) were recorded on a Schimadzu 8400 series FTIR instrument. ^1H NMR spectra were recorded on a Bruker AC-300 and ^{13}C NMR spectra were recorded on a Bruker AC-300 at 75 MHz. The chemical shifts (δ) are given in parts per million relative to tetramethylsilane (TMS). Spectra were acquired in DMSO-d_6 for all compounds. Column chromatography was performed on Merck silica gel (70–230 mesh). All reagents used were of analytical reagent grade. High resolution Mass spectra (HRMS) were recorded in Bruker Daltonics – micrOTOF.

2.2. Chemistry

2.2.1. General procedure for the synthesis of amides 1 and 2

Bile acid (4.89 mmol of cholic or deoxycholic acid) was dissolved in 70 mL of tetrahydrofuran (THF). To this solution 1,4-phenylenediamine (4.89 mmol) and dicyclohexylcarbodiimide (DCC) (5.07 mmol) were added. The mixture was stirred for 24 h, when the formations of the desired bile acid amides were found by thin layer chromatography (TLC). Ethyl acetate was then added in order to promote the precipitation of the byproduct *N,N′*-dicyclohexylurea, which was filtered off. The solvents were evaporated and the solid crude obtained was chromatographed on silica gel using a mixture of dichloromethane/methanol as eluent.

The NMR peaks were assigned according to the numbered general structure (Fig. 3).

*(R)-N-(4-aminophenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[α]phenanthren-17-yl)pentanamide (**1**):* The bile amide **1** was obtained as a gray solid. Yield: 41%. M.p.: 157.8–158.3 °C. I.R. (KBr) *ν*: 3406, 2935, 2866, 1654, 1541, 1515, 1431, 1251 cm^{-1}. ^1H-NMR (300 MHz, DMSO-d_6), δ (ppm), J (Hz): 0.59 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, 21-CH_3, J = 5.4); 1.29–1.77 (m, 22H, CH_2 and CH skeleton); 2.16 (m, 2H, 23-CH_2); 3.17 (br s, 1H, H-3β); 3.61 (br s, 1H, H-7β); 3.79 (br s, 1H, H-12β); 4.01 (br s, 1H, 7-OH); 4.10 (br s, 1H, 12-OH); 4.34 (br s, 1H, 3-OH); 4.82 (br s, 2H, —NH_2); 6.47 (d, 2H, 2H-m, J = 6.0); 7.19 (d, 2H, 2H-o, J = 6.0); 9.42 (s, 1H, —NH). ^{13}C-NMR (75 MHz, DMSO-d_6), δ (ppm): 12.4 (C-18); 17.2 (C-21); 22.6 (C-19); 22.8–46.2 (CH and CH_2 skeleton); 66.2 (C-7); 70.4 (C-3); 71.0 (C-12); 113.8 (2C-m); 120.8 (2C-o); 128.7 (C_{ar}—NH); 144.4 (C-p); 170.8 (C-24). HRMS (LC-MS): calcd. for $[M + H]^+$ ($C_{30}H_{47}N_2O_4$) requires *m/z* 499.3536, found *m/z* 499.3550.

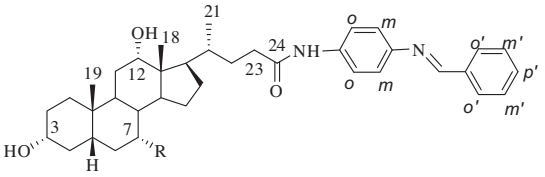

Fig. 3. Structure and numbering of bile acids/azastilbenes analogues conjugates and bile amides.

*(R)-N-(4-aminophenyl)-4-((3R,5R,8R,9S,10S,12S,13R,14S,17R)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[α]phenanthren-17-yl)pentanamide (**2**):* The bile amide **2** was obtained as a lilac solid. Yield: 44%. M.p.: 134.4–136.0 °C. I.R. (KBr) *ν*: 3433, 3398, 2933, 2864, 1650, 1515, 1512 cm^{-1}. ^1H-NMR (300 MHz, DMSO-d_6), δ (ppm), J (Hz): 0.59 (s, 3H, 18-CH_3); 0.84 (s, 3H, 19-C_3); 0.96 (d, 3H, 21-CH_3, J = 5.4); 1.19–1.79 (m, 23H, CH_2 and CH skeleton); 2.18 (m, 2H, 23-CH_2); 3.80 (s, 1H, H-12β); 4.22 (s, 1H, 12-OH); 4.50 (s,1H, 3-OH); 4.84 (br s, 2H, —NH_2); 6.47 (d, 2H, J = 6.0, 2H-m); 7.19 (d, 2H, J = 6.0, 2H-o); 9,42 (s, 1H, —NH). ^{13}C-NMR (75 MHz, DMSO-d_6), δ (ppm): 12.0 (C-18); 17.0 (C-21); 23.0 (C-19); 26.9–46.2 (CH and CH_2 skeleton); 47.4 (C-7); 69.9 (C-3); 71.0 (C-12); 113.8 (2C-m); 120.8 (2C-o); 128.7 (C_{ar}—NH); 144.4 (C-p); 170.7 (C-24). HRMS (LC-MS): calcd. for $[M + H]^+$ ($C_{30}H_{47}N_2O_3$) requires *m/z* 483.3587, found *m/z* 483.3618.

2.2.2. General procedure for the synthesis of bile acids/azastilbenes conjugates (1a–g and 2a–g)

Bile amide (0.100 g; 0.20 mmol of amide **1** or 0.21 mmol of amide **2**) was dissolved in 3 mL of methanol and then, under constant stirring, 1 M equivalent of an aromatic aldehyde was added. The mixture was kept at room temperature and after a short time interval (5 min–10 h) the formation of a precipitate was noticed. The reaction medium was then filtered and the precipitate was washed with methanol and dried in an oven. All compounds of this class were obtained as colored solid.

*2-((E)-(4-((R)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[α]phenanthren-17-yl)pentanamido)phenylimino)methyl)benzoic acid (**1a**):* The compound **1a** was obtained as a beige solid. Yield: 100%. M.p.: 175.5–177.0 °C. I.R. (KBr) *ν*: 3348, 2931, 2860, 1747, 1660, 1523, 1465 cm^{-1}. ^1H-NMR (300 MHz, DMSO-d_6), δ (ppm), J (Hz): 0.59 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.97 (d, 3H, 21-CH_3, J = 5.4); 1.33–2.00 (m, 22H, CH_2 and CH skeleton); 2.17 (m, 2H, 23-CH_2); 3.17 (br s, 2H, H-3β); 3.61 (br s, 1H, H-7β); 3.81(br s, 1H, H-12β); 4.04 (br s, 1H, 7-OH); 4.14 (br s, 1H, 12-OH); 4.37 (br s, 1H, 3-OH); 6.88 (br s, 2H, 2H-m); 7.14 (br s, 2H, 2H-o); 7.44 (br s, 2H, H-o′ and H-m′); 7.70 (m, 2H, H-m′ and H-p′); 7.88 (br s, 2H, HC=N and —COOH); 9.68 (s, 1H, —NH). ^{13}C-NMR (75 MHz, DMSO-d_6), δ (ppm):13.0 (C-18); 17.9 (C-21); 23.3 (C-19); 23.9–56.5 (CH and CH_2 skeleton); 66.8 (C-7); 70.9 (C-3); 71.5 (C-12); 115.0 (2C-o); 120.9 (2C-m); 124.5 (C-m′); 125.1 (C-o′); 130.9 (C-p′); 132.3 (C-o′—COOH); 134.7 (C-m′); 141.2 (C_{ar}—CH=N); 145.1 (C_{ar}—NH); 146.3 (C_{ar}—N=CH); 169.5 (HC=N); 171.7 (C-24).

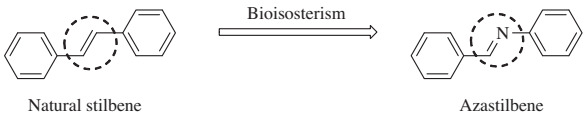

Fig. 2. Comparison of the basic structures of stilbene and azastilbene skeletons.