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Synthesis and antifungal activity of bile acid-derived oxazoles

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ABSTRACT

Peracetylated bile acids (**1a**–**g**) were used as starting materials for the preparation of fourteen new derivatives bearing an oxazole moiety in their side chain (**6a**–**g**, **8a**–**g**). The key step for the synthetic path was a Dakin–West reaction followed by a Robinson–Gabriel cyclodehydration. A simpler model oxazole (**12**) was also synthesized. The antifungal activity of the new compounds (**6a**–**g**) as well as their starting bile acids (**1a**–**g**) was tested against *Candida albicans*. Compounds **6e** and **6g** showed the highest percentages of inhibition (63.84% and 61.40% at 250 μg/mL respectively). Deacetylation of compounds **6a**–**g**, led to compounds **8a**–**g** which showed lower activities than the acetylated derivatives.

1. Introduction

Bile acids are enantiomerically pure, abundant and inexpensive commercial compounds with a wide range of biological activities [1]. The pharmacological interest in these acids is directly related to the fact that liver cells can specifically recognize such natural ligands. This fact makes them ideal building blocks for the synthesis of novel molecules that can be recognized at the molecular level [2]. The particular conformation, reactivity and amphiphilic properties of bile acids were also exploited in supramolecular chemistry and biotechnological applications [3–4].

Small heterocycles such as imidazole, thiazole and oxazole are present in both marine and terrestrial natural products. In a biosynthetical sense, thiazole and oxazole are masked aminoacids, usually formed by cyclization of serine and cysteine, and are frequent structural fragments in cyclic peptides. Secondary metabolites with these structural fragments have shown diverse biological properties, which include antitumoral, antibacterial and antiviral activities [5]. In medicinal chemistry, an emergent practice is to bind two or more bioactive molecules as a way to improve the biological properties of the starting components [6,7]. In this context, it was expected that a compound combining a bile acid core and an oxazole moiety, may retain intrinsic biological properties belonging to both fragments. For example, synthetic steroidal isoxazoles have already shown a great variety of biological activities [8] and more recently, 6,5 fused cholestane oxazoles have been tested for antifungal activity [9]. In previous work, our group has used the strategy of bioconjugation to prepare compounds which combined bile acids with quinones and *Cinchona* alkaloids [10–12].

In this work, 14 new compounds were synthesized using acetylated bile acids and the 4-methyl ester of aspartic acid as starting materials. The synthetic strategy had as key steps a Dakin-West reaction followed by a Robinson–Gabriel cyclodehydration [13]. The parent acetylated bile acids and their resulting derivatives together with a low-molecular weight compound representing the oxazole fragment itself were evaluated for antifungal activity against *Candida albicans*.

2. Experimental

2.1. General

The bile acids chenodeoxycholic, lithocholic, deoxycholic, hyodeoxycholic, cholic, ursodeoxycholic and hyocholic and all other reagents were obtained from Sigma–Aldrich Co. (St Louis, MO, USA). All chemicals and solvents were of analytical grade.

Silicagel 60 H (Merck) was used for dry column flash chromatography. TLC was carried out on Merck Silicagel 60 F_{254} plates. TLC plates were analyzed by visualization under UV light (254 nm), exposition to iodine vapors or by spraying with either 2% vanillin

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in H_2SO_4 (cc) or 0.2% ninhydrin in ethanol. Among the solvents, CH_2Cl_2 was distilled from phosphorous pentoxide and Me_2CO was distilled from potassium permanganate prior to their use in the syntheses.

UV spectra were obtained on a Hewlett Packard 8453 spectrophotometer and IR spectra were obtained on an FT-IR Nicolet Magna 550 instrument. Optical rotations were measured on a Perkin-Elmer 343 polarimeter, whereas ^1H and ^{13}C NMR data were acquired using Bruker Avance-2 (500 MHz) and AC-200 (200 MHz) spectrometers, in $CDCl_3$ or CD_3OD.

Proton chemical shifts were referenced to the residual signal of protonated $CDCl_3$ at δ 7.26 or δ 3.31 when CD_3OD was used, and ^{13}C NMR were referenced to the central peak of $CDCl_3$ at 77.0 ppm or CD_3OD at 49.0 ppm. Homonuclear ^1H connectivities were determined by COSY experiments. The edited reverse-detected single quantum heteronuclear correlation (DEPT–HSQC) experiment allowed the determination of carbon multiplicities, as well as one-bond proton–carbon connectivities, and the heteronuclear multiple bond correlation (HMBC) experiments allowed the determination of long-range proton–carbon correlations. All 2D NMR experiments were performed using standard pulse sequences. HRESI mass spectra were recorded using a Bruker MicrOTOF QII mass spectrometer. HPLC separations were performed using HPLC-grade solvents, a Thermo Separations Spectra Series P100 pump, a Thermo Separations Refractomonitor IV RI detector, a Thermo Separations SpectraSeries UV 100 UV detector, and a Phenomenex Bondclone 10 C18 (10 μm, 8 mm × 300 mm) column. UV detection was performed at 220 nm.

Peracetylated compounds **1a**–**g** were obtained by standard methods, using Ac_2O/DMAP/Pyr. Compounds **3** and **9** were prepared according to previously reported procedures [14,15].

2.2. Chemistry

2.2.1. Methyl (5-methyl-2-(3α,7α-diacetoxy-24-nor-5β-cholan-23-yl)-1,3-oxazol-4-yl)acetate (**6a**)

To a solution of the peracetylated bile acid **1a** (119 mg, 0.25 mmol) in dry CH_2Cl_2 (5 mL), under inert atmosphere and stirring at 0 °C, a solution of 1.25 mmoles (5 equiv, 0.10 mL) of oxalyl chloride in 2 mL of dry CH_2Cl_2 was added dropwise. After 3 h, the solvent was evaporated at reduced pressure and the acid chloride **2a** was redissolved in dry acetone and used as such in the following step. Sodium carbonate (83.8 mg, 0.88 mmol, 3.5 equiv) was suspended in 2 mL of a 1:1 mixture of pyridine and acetone at 0 °C. Then, the 4-methyl ester of aspartic acid (**3**) (153.5 mg, 0.5 mmol, 2 equiv) was added, followed by a slow addition of the corresponding bile acid chloride (**2a**) in acetone (2 mL), the resulting mixture was stirred for 30 min. After this time, the solution was adjusted to pH = 3 by adding HCl and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic phases were concentrated to yield a yellowish oil (**4a**) that was utilized without further purification. Compound **4a** was dissolved in 0.6 mL of pyridine under stirring with the addition of a catalytic amount of 4-dimethylaminopyridine (DMAP). Slowly, 0.6 mL of acetic anhydride was added and the reaction mixture was kept at 90 °C for one hour. Then, the mixture was acidified to pH = 3 with HCl and extracted with 10 mL of CH_2Cl_2 (3 times). The combined organic layers were successively washed with 2 N HCl and water. A yellow oil (**5a**) was obtained after concentration and used in the following step. Ketoamide **5a** was dissolved in 3 mL of DMF with stirring under inert atmosphere, and then 6.25 μL of phosphoryl chloride ($POCl_3$) were added dropwise. The reaction mixture was heated up to 90 °C for twenty minutes, then diluted with 20 mL of water and finally extracted with diethyl ether (3 × 15 mL). The combined organic phases were washed with water, dried over magnesium sulfate and evaporated under reduced pressure. The crude product was chromatographed on silicagel with cyclohexane:EtOAc (9:1) as mobile phase to give **6a** (7.4 mg, 5.1% from **1a**) as a yellowish oil. ^1H NMR (500 MHz, $CDCl_3$): 4.59 (1H, tt, J = 11.4, 4.4 Hz, H-3), 4.87 (1H, q, J = 2.9 Hz, H-7), 0.63 (3H, s, H-18), 0.93 (3H, s, H-19), 0.96 (3H, d, J = 5.8 Hz, H-21), 2.73 (1H, ddd, J = 15.2, 11.2, 4.3 Hz, H-23a), 2.56 (1H, ddd, J = 14.7, 10.0, 6.0 Hz, H-23b), 2.23 (3H, s, H-6′), 3.45 (2H, s, H-7′), 3.71 (3H, s, 7′-COO$\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 608.3581 (calc. for $C_{34}H_{51}NNaO_7^+$, 608.3558). IR (film, cm^{-1}): 2937, 2871, 1734. α_D ($CHCl_3$, c = 0.39) = +15.8°. UV ($CHCl_3$, 1/Mcm): ε_{241} = 388, ε_{269} = 300.

2.2.2. Methyl (5-methyl-2-(3α-acetoxy-24-nor-5β-cholan-23-yl)-1,3-oxazol-4-yl)acetate (**6b**)

The title compound was prepared in 6.2% yield (8 mg) as an oil from **1b** (105 mg, 0.25 mmol) via the procedure used to prepare **6a**. ^1H NMR (500 MHz, $CDCl_3$): 4.71 (1H, tt, J = 11.4, 4.7 Hz, H-3), 0.63 (3H, s, H-18), 0.92 (3H, s, H-19), 0.95 (3H, d, J = 6.2 Hz, H-21), 2.73 (1H, ddd, J = 15.3, 11.3, 4.4 Hz, H-23a), 2.56 (1H, ddd, J = 14.8, 10.1, 5.9 Hz, H-23b), 2.23 (3H, s, H-6′), 3.45 (2H, s, H-7′), 3.71 (3H, s, 7′-COO$\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 550.3522 (calc. for $C_{32}H_{49}NNaO_5^+$, 550.3503). IR (film, cm^{-1}): 2939, 2867, 1735. α_D ($CHCl_3$, c = 0.75) = +23.3°. UV ($CHCl_3$, 1/Mcm): ε_{242} = 995, ε_{268} = 828.

2.2.3. Methyl (5-methyl-2-(3α,12α-diacetoxy-24-nor-5β-cholan-23-yl)-1,3-oxazol-4-yl)acetate (**6c**)

The compound was prepared in 24.2% yield (35.4 mg) as an oil from **1c** (119 mg, 0.25 mmol) via the procedure used to prepare **6a**. ^1H NMR (500 MHz, $CDCl_3$): 4.69 (1H, m, H-3), 5.07 (1H, brs, H-12), 0.71 (3H, s, H-18), 0.90 (3H, s, H-19), 0.84 (3H, d, J = 6.1 Hz, H-21), 2.71 (1H, ddd, J = 15.3, 10.8, 3.8 Hz, H-23a), 2.54 (1H, ddd, J = 15.3, 10.2, 5.8 Hz, H-23b), 2.22 (3H, s, H-6′), 3.44 (2H, s, H-7′), 3.70 (3H, s, 7′-COO$\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 608.3611 (calc. for $C_{34}H_{51}NNaO_7^+$, 608.3563). IR (film, cm^{-1}): 2925, 2869, 1733, 1623. α_D ($CHCl_3$, c = 0.56) = +79.4°. UV ($CHCl_3$, 1/Mcm): ε_{241} = 304, ε_{268} = 178.

2.2.4. Methyl (5-methyl-2-(3α,6α-diacetoxy-24-nor-5β-cholan-23-yl)-1,3-oxazol-4-yl)acetate (**6d**)

The compound was prepared in 9.5% yield (13.9 mg) as an oil from **1d** (119 mg, 0.25 mmol) applying the procedure used to prepare **6a**. ^1H NMR (500 MHz, $CDCl_3$): 4.69 (1H, m, H-3), 5.14 (1H, dt, J = 12.3, 4.8 Hz, H-6), 0.63 (3H, s, H-18), 0.96 (3H, s, H-19), 0.95 (3H, d, J = 6.1 Hz, H-21), 2.72 (1H, m, H-23a), 2.57 (1H, ddd, J = 15.3, 10.2, 5.9 Hz, H-23b), 2.22 (3H, s, H-6′), 3.42 (2H, s, H-7′), 3.70 (3H, s, 7′-COO$\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 608.3589 (calc. for $C_{34}H_{51}NNaO_7^+$, 608.3563). IR (film, cm^{-1}): 2925, 2869, 1736. α_D ($CHCl_3$, c = 0.23) = +22.2°. UV ($CHCl_3$, 1/Mcm): ε_{242} = 726, ε_{270} = 562.

2.2.5. Methyl (5-methyl-2-(3α,7α,12α-triacetoxy-24-nor-5β-cholan-23-yl)-1,3-oxazol-4-yl)acetate (**6e**)

The compound was obtained in 16% yield (25.7 mg) as an oil from **1e** (133 mg, 0.25 mmol) by the same procedure used for **6a**. ^1H NMR (500 MHz, $CDCl_3$): 4.57 (1H, tt, J = 11.3, 4.3 Hz, H-3), 4.90 (1H, q, J = 3.2 Hz, H-7), 5.08 (1H, t, J = 2.9 Hz, H-12), 0.72 (3H, s, H-18), 0.91 (3H, s, H-19), 0.85 (3H, d, J = 5.8 Hz, H-21), 2.72 (1H, m, H-23a), 2.55 (1H, ddd, J = 15.4, 10.0, 6.0 Hz, H-23b), 2.22 (3H, s, H-6′), 3.44 (2H, s, H-7′), 3.70 (3H, s, 7′-COO$\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 666.3662 (calc. for $C_{36}H_{53}NNaO_9^+$, 666.3613). IR (film, cm^{-1}) 3448, 2950, 2872, 1735, 1654, 1648. α_D ($CHCl_3$, c = 0.36) = +66.4°. UV ($CHCl_3$, 1/Mcm): ε_{242} = 631, ε_{272} = 183.