Steroids 112 (2016) 109–114

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

C(16)-C(22) oxygen-bridged analogues of *ce*DAF-12 and LXR ligands

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ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 17 May 2016
Accepted 24 May 2016
Available online 26 May 2016

Keywords:
Oxysterols
Oxocarbenium reduction
Dafachronic acid
DAF-12 receptor
Liver X Receptor

ABSTRACT

The DAF-12 receptor in nematodes and the Liver X Receptor (LXR) in mammals are structurally related transcription factors that play key roles in determining the life span of the organism. Both types of receptors are activated by oxysterols, cholesterol metabolites with oxidized side chains. Restricting the movement of the oxysterol side chain to certain orientations may have profound effects in the activity profile, however this has not been explored so far. In a first attempt to obtain analogues of natural ligands of DAF-12 and LXR with restricted side chain mobility we introduced a 16,22-oxygen bridge in 26-hydroxycholesterol, a cholestenoic acid and a dafachronic acid (**5**–**7**). Diosgenin was used as starting material, the key step to obtain the 16,22 epoxy functionality was the one pot formation and reduction of a cyclic hemiketal via the oxocarbenium ion using sodium cyanoborohydride. All new compounds were characterized by NMR and mass spectrometry and assayed as *ce*DAF-12 or LXR ligands in transactivation cell-based assays. The dafachronic acid analogue **7** behaved as a *ce*DAF-12 agonist.

1. Introduction

Nuclear hormone receptors are transcription factors that respond to lipophilic hormones such as steroids, to regulate essential processes in living cells [1]. DAF-12 is a nuclear receptor in *Caenorhabditis elegans*, that controls the choice between reproductive growth and arrest at a long-lived, alternate third larval stage formed under harsh environmental conditions [2,3]. The *Ce*DAF-12 ligands, termed dafachronic acids (DAs) are oxidized cholesterol metabolites. It is known that a C-3 keto group, a double bond at C-4 (Δ^4) or C-7 (Δ^7), and an acidic carboxyl group at the end of the cholesterol side chain are required for efficient *Ce*DAF-12 activation (e.g. Δ^4-DA **1** and the synthetic agonist **2**, Fig. 1) [4,5]. Since many of the molecular and cellular pathways occurring in the nematode show analogies to corresponding pathways on higher animals [2,6], a detailed understanding of DAF-12 function may result central to clarify the molecular mechanism involved in human aging. Using sequence similarity searches, the liver X receptor (LXR) has been identified as one of the human nuclear receptors, the protein sequence of which is most similar to *Ce*DAF-12 [7]. The endogenous LXR ligands are also cholesterol metabolites with an oxidized sterol side chain, some of which are closely related to the dafachronic acids, e.g. 26-hydroxycholesterol **3** and 25*R*-cholestenoic acid **4** (Fig. 1) [8–10]. Once activated, LXR isoforms are involved in many physiological functions being regulators of lipid homeostasis, including reverse cholesterol transport. This has lead to propose LXRs as key factors affecting human life span [7]. Although the ligand binding pockets of DAF-12 and LXR accept structurally similar ligands, molecular modeling and X-ray data indicate marked differences in side chain conformation and binding mode [11,12]. As a first approach to evaluating the effect of restricting side chain flexibility of DAF-12 and LXR ligands we prepared the 16,22-epoxysteroids **5**–**7** that are side chain constrained analogues of natural ligands **3**, **4** and **1** respectively.

2. Experimental

2.1. General

Mps were taken on a Fisher-Johns apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance II 500 NMR spectrometer (^{1}H at 500.13 MHz, ^{13}C at 125.77 MHz). Chemical shifts are given in ppm downfield from TMS as internal standard, J values are given in Hz. Multiplicity determinations and 2D spectra (COSY, NOESY, HSQC and HMBC) were obtained

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http://dx.doi.org/10.1016/j.steroids.2016.05.009
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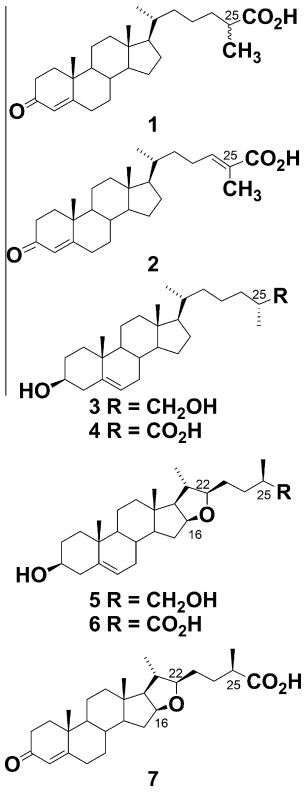

Fig. 1. Structures of DAF-12 and LXR ligands and synthetic analogues.

using standard Bruker software. Exact mass spectra were measured on a Bruker micrOTOF-Q II mass spectrometer, equipped with an ESI source operating in positive mode. Medium Pressure Liquid Chromatography (MPLC) was carried out in a Buchi Sepacore purification system C-615 equipped with two pumps of 10 bar maximum pressure; columns (12 × 75 mm or 12 × 150 mm) were filled with silica gel 60, 0.0040–0.0063 mm. Thin layer chromatography (tlc) analysis was performed on silica gel 60 F254 (0.2 mm thick). The homogeneity of all compounds was confirmed by tlc and high field (500 MHz) ^{1}H NMR. Solvents were evaporated at reduced pressure and ca. 45 °C. 3β,16β-diacetoxy-26-hydroxy-5-cholesten-22-one (**8**) was prepared from diosgenin following the procedure described by Fernández-Herrera et al. [13].

2.2. Chemistry

2.2.1. 3β,16β-Diacetoxy-26-(t-butyldimethylsilyloxy)-cholest-5-en-22-one (*9*)

Imidazole (64 mg, 0.940 mmol) and *t*-butyldimethylsilyl chloride (128 mg, 0.849 mmol) were added successively to a solution of alcohol **8** (160 mg, 0.310 mmol) in anhydrous DMF (1.8 mL) and the solution was stirred for 15 min at 25 °C under a nitrogen atmosphere. The reaction mixture was extracted with ether, the organic layer was washed successively with brine and water and dried with sodium sulphate. Evaporation of the solvent followed by MPLC (Flow rate: 20 mL/min; hexane-ethyl acetate 100:0 → 90:10) gave compound **9** as an amorphous solid (186 mg, 95%); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ_H: 5.36 (1H, d, J = 5.0 Hz, H-6); 4.98 (1H, td, J = 8.0 and 4.6 Hz, H-16); 4.60 (1H, tt, J = 11.0 and 5.5 Hz, H-3); 3.44 (1H, dd, J = 10.0 and 6.0 Hz, H-26a); 3.38 (1H, dd, J = 10.0 and 6.5 Hz, H-26b); 2.96 (1H, m, H-20); 2.60 (1H, m, H-23a); 2.42 (1H, m, H-15β); 2.36 (1H, m, H-23b); 2.31 (2H, m, H-4); 2.03 (3H, s, 3-acetate); 1.96 (3H, s, 16-acetate); 1.95 (1H, m, H-7β); 1.94 (1H, m, H-12β); 1.93 (1H, m, H-17); 1.86 (1H, m, H-2α); 1.85 (1H, m, H-1β); 1.66 (1H, m, H-24a); 1.59 (1H, m, H-2β); 1.55 (3H, m, H-25, H-8 and H-7α); 1.51 (2H, m, H-11); 1.31 (1H, m, H-24b); 1.28 (1H, m, H-12α); 1.14 (1H, m, H-1α); 1.13 (3H, d, J = 7.0 Hz, H-21); 1.04 (1H, m, H-15α); 1.03 (1H, m, H-14); 1.02 (1H, s, H-19); 1.00 (1H, m, H-9); 0.89 (9H, s, $(CH_3)_3$C-Si); 0.87 (3H, d, J = 7.0 Hz, H-27); 0.87 (3H, s, H-18); 0.03 (6H, s, $(CH_3)_2$-Si); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ_C: 213.3 (C-22); 170.5 (3-acetate); 169.8 (16-acetate); 139.6 (C-5); 122.3 (C-6); 75.7 (C-16); 73.8 (C-3); 68.2 (C-26); 55.0 (C-17); 53.9 (C-14); 49.7 (C-9); 43.5 (C-20); 41.8 (C-13); 39.6 (C-12); 38.9 (C-23); 38.0 (C-4); 36.9 (C-1); 36.5 (C-10); 35.4 (C-25); 34.8 (C-15); 31.6 (C-7); 31.2 (C-8); 27.7 (C-2); 27.0 (C-24); 25.9 (($\mathbf{C}H_3)_3$C-Si); 21.4 (3-acetate); 21.1 (16-acetate); 20.7 (C-11); 19.3 (C-19); 18.3 (($CH_3)_3\mathbf{C}$-Si); 16.7 (C-27); 16.6 (C-21); 13.2 (C-18); -5.4 (($CH_3)_2$-Si); HRMS-ESI: calculated for $C_{37}H_{62}NaO_6Si$: 653.4208, found 653.4201.

2.2.2. (22R)-16β,22-Epoxycholest-5-ene-3β,26-diol (*5*)

Method A: A solution of KOH 8% in methanol (0.92 mL, 1.3 mmol) was added to a solution of compound **9** (138 mg, 0.219 mmol) in dichloromethane (0.2 mL) and methanol (4 mL). After stirring for 24 h at 25 °C, water was added to the mixture and a precipitate was formed. The solid was filtered, washed with water and purified by MPLC (Flow rate: 10 mL/min; hexane-ethyl acetate 100:0 → 60:40) to give hemiketal **10** as an amorphous solid (106 mg, 92%): ^{1}H NMR (500.13 MHz, DMSO-d_6) δ_H: 5.27 (1H, d, J = 5.0 Hz, H-6); 4.60 (1H, m, 3-OH); 4.44 (1H, td, J = 7.0 and 6.8 Hz, H-16); 3.26 (1H, m, H-3); 3.39 (2H, dd, J = 5.5.8 and 2.0 Hz, H-26); 2.14 (1H, m, H-4β); 2.09 (1H, m, H-4α); 1.95 (1H, m, H-20); 1.92 (1H, m, H-7β); 1.86 (1H, m, H-15β); 1.77 (1H, m, H-1β); 1.70 (1H, m, H-12β); 1.68 (1H, m, H-2α); 1.65 (1H, m, H-17); 1.61 (1H, m, H-24a);1.53 (1H, m, H-8); 1.52 (1H, m, H-23a); 1.51 (2H, m, H-7α and H-25); 1.49 (2H, m, H-11α and H-24b); 1.40 (1H, m, H-11β); 1.35 (1H, m, H-2β); 1.13 (1H, m, H-12α); 1.12 (2H, m, H-15α and H-23b); 1.09 (1H, m,H-14); 0.98 (1H, m, H-1α); 0.96 (1H, s, H-19); 0.92 (3H, d, J = 7.0 Hz, H-21); 0.90 (1H, m, H-9); 0.88 (9H, s, $(CH_3)_3$C-Si); 0.83 (3H, d, J = 6.5 Hz, H-27); 0.75 (3H, s, H-18); 0.03 (6H, s, $(CH_3)_2$-Si); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ_C: 141.2 (C-5); 120.2 (C-6); 109.5 (C-22); 79.5 (C-16); 69.9 (C-3); 67.3 (C-26); 62.4 (C-17); 55.6 (C-14); 49.5 (C-9); 42.1 (C-4); 40.0 (C-13); 39.1 (C-12); 38.6 (C-20); 36.8 (C-1); 36.1 (C-10); 35.7 (C-24); 35.4 (C-25); 31.5 (C-15); 31.4 (C-7); 31.3 (C-2); 30.9 (C-8); 26.8 (C-23); 25.7 (($\mathbf{C}H_3)_3$C-Si); 20.3 (C-11); 19.0 (C-19); 17.8 (($CH_3)_3\mathbf{C}$-Si); 16.5 (C-27); 15.9 (C-18); 15.7 (C-21); −5.52,−5.54 (($CH_3)_2$-Si); HRMS-ESI: calculated for $C_{33}H_{58}NaO_4Si$: 569.3997, found 569.3981.

Sodium cyanoborohydride (50 mg, 0.796 mmol) was added to a solution of the solid obtained above in dichloromethane (1.6 mL) and MeOH (3 mL) containing a trace of methyl orange. The reaction mixture was acidified with 1 M HCl until the solution turned orange (pH 3) and stirred for 30 min at 25 °C, the orange color was maintained by periodic additions of 1 M HCl (ca. 2 mL) during the reaction. The mixture was diluted with water, concentrated to a third of its volume and extracted with dichloromethane. The organic layer was washed with saturated sodium bicarbonate solution and water and dried with sodium sulphate. Evaporation of the solvent followed by MPLC (Flow rate: 20 mL/min; hexane-ethyl acetate 100:0 → 50:50) gave compound **5** as a white solid (65 mg, 82%), mp 160–162 °C (from hexane-ethyl acetate; lit [14]. 164–166 °C); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ_H: 5.35 (1H, dt, J = 5.3 and 1.8 Hz, H-6); 4.31 (1H, td, J = 7.5 and 5.0 Hz, H-16); 3.51 (1H, m, H-3); 3.50 (1H, dd, J = 10.7 and 6.0 Hz, H-26a); 3.45 (1H, dd, J = 10.5 and 6.0 Hz, H-26b); 3.33 (1H, td, J = 8.0 and 3.5 Hz, H-22); 2.29 (1H, m, H-4β); 2.23 (1H, m, H-4α); 2.01 (1H, m, H-15β); 2.00 (1H, m, H-7β); 1.85 (1H, m, H-1β); 1.84 (1H, m, H-2α); 1.75 (1H, m, H-20); 1.72 (1H, m, H-12β); 1.67 (1H, m, H-25), 1.63 (1H, m, H-8); 1.61 (1H, m, H-17); 1.60 (2H, m, H-23);