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Synthesis, stereochemistry and cytotoxic activity of novel steroidal 16-spiro-1,3,2-dioxaphosphorinanes

János Wölfling [a], Piroska Kovács-Pénzes [a,b], István Zupkó [c], Gyula Schneider [a], Éva Frank [a,*]

[a] Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary
[b] Glycom A/S, Anker Engelundsvej 201, DK-2800 Kgs. Lyngby, Denmark
[c] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary

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ABSTRACT

The epimeric pairs **a** and **b** of novel steroidal 16-spiro-dioxaphosphorinanes **4–8** were synthetized via the phosphorylation of 16,16-*bis*(hydroxymethyl)androst-4-ene-3,17-dione (**2**) and their stereostructures were investigated by NMR methods. The dioxaphosphorinane moiety exists mainly as one of the possible chair conformers or as a chair–twist equilibrium in solution as a consequence of the rigidity of the sterane framework. The contributions of the conformers depend strongly on the configuration of the P atom and the stereoelectronic properties of the substituents on it. The antiproliferative activities of the structurally related products were determined *in vitro* with the MTT assay on three malignant human cell lines (HeLa, MCF7 and A431).

1. Introduction

During the past few decades, P-containing six-membered heterocycles, and especially 1,3,2-dioxaphosphorinanes, have been thoroughly studied from both pharmacological and stereochemical aspects [1–3]. Besides the natural cyclic nucleotides (e.g. cAMP), which play important regulatory functions as second messengers in many endogenous processes [4], a number of synthetic 1,3,2-dioxaphosphorinanes have been reported to exhibit diverse biological activities [5–8]. The main driving force behind the earlier stereostructural studies was the desire to determine the unique conformational behavior of variously substituted hetero rings [9–11]. The stereostructures of *O*, *P*, *O* heterocycles are known to be significantly different from those of cyclohexanes as a consequence of the electron lone pairs on the ring heteroatoms and the differences in bond angles and lengths [12,13]. Especially in structurally biased bulky molecules, one of the two alternative chair forms or intermediate conformations can predominate in solution [14–16]. The conformational preference is strongly influenced by the steric and electronic features of the substituents on the P and by its configuration [9].

The construction of a dioxaphosphorinane ring on a relatively rigid sterane skeleton furnishes an excellent possibility via which to restrict the conformational flexibility of the hetero ring, as was demonstrated earlier on other steroid-type model compounds [17,18]. As a continuation of our research on steroidal P heterocycles, our present aim was to prepare novel 16-spiro-dioxaphosphorinanes in the androst-4-ene series and to investigate the stereochemistry of the hetero ring in the cases of different P substituents. Since certain diheterophosphorinanes have been reported to exert considerable antiproliferative activity [19,20], and the joining of a hetero ring to a sterane framework can often be useful [21,22], the synthetized compounds were subjected to a high-throughput screen in order to determine their *in vitro* cell-growth inhibitory effects against a panel of human cancer cell lines.

2. Experimental

2.1. General

All solvents were distilled prior to use. Reagents and other materials were obtained from commercial suppliers and were used without purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm; solvent system: EtOAc/CH_2Cl_2 = 30:70. Flash chromatography: Merck silica gel 60, 40–63 μm. Melting points (mp) were determined on a Kofler block

* Corresponding author. Fax: +36 62 544200.
E-mail addresses: zupko@pharm.u-szeged.hu (I. Zupkó), frank@chem.u-szeged.hu (É. Frank).

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and are uncorrected. Specific rotation was measured in $CHCl_3$ ($c = 1$) at 25 °C with a Polamat-A polarimeter. Elementary analysis data were determined with a PerkinElmer CHN analyzer model 2400. NMR spectra were recorded on a Bruker DRX 400 spectrometer in $CDCl_3$, with TMS (^1H and ^{13}C NMR) as an internal and 85% H_3PO_4 (^{31}P NMR) as an external standard. ^{13}C and ^{31}P NMR spectra were measured at 75 and 121 MHz with Varian Mercury Vx-300 and Varian Unity 300 instruments, respectively. Chemical shifts are reported in ppm (δ scale), and coupling constants (J) in Hz. For the determination of multiplicities, the APT pulse sequence was used.

2.2. General procedure for the synthesis of steroidal 16-spiro-dioxaphosphorinanes (**4–8**)

To a stirred solution of Et_3N (0.43 ml, 4.0 mmol) and **2** [23] (347 mg, 1.0 mmol) in dichloromethane (20 ml), phenylphosphonic dichloride (**3a**), phenyldichlorophosphate (**3b**) or substituted phenyldichlorophosphate (**3c–e**) (1.2 mmol) was added drop-wise at room temperature under a nitrogen atmosphere. The reaction mixture was refluxed for 3 h then was poured into water, and extracted with dichloromethane (3 × 10 ml), and the combined organic phases were dried over Na_2SO_4, and evaporated in *vacuo*. The crude product was separated by column chromatography (CC).

2.2.1. Synthesis of 16-spiro(2′-oxo-2′-phenyl[1′,3′,2′]dioxaphosphorino)-4-androsten-3,17-dion (**4a** and **4b**)

According to the general procedure, **3a** (0.17 ml) was used. After purification of the crude product (EtOAc/CH_2Cl_2 = 40:60), compound **4b** (226 mg, 48%) as the fast-eluting diastereomer was obtained. The less mobile epimer was identified as isomer **4a** (135 mg, 29%). Compound **4a**: Mp. 261–264 °C; $[\alpha]_D$ = +122.5; R_f = 0.15; ^{31}P NMR (121 MHz, $CDCl_3$): δ = 14.3; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.97 (s, 3H, 18-H_3), 1.05 (m, 1H), 1.17 (m, 1H), 1.23 (s, 3H, 19-H_3), 1.27 (m, 1H), 1.39 (m, 1H), 1.48 (m, 1H), 1.67–1.90 (m, 5H), 2.03 (m, 2H), 2.33–2.51 (m, 4H), 2.61 (dd, 1H, J = 13.3 Hz, J = 5.8 Hz), 4.11 (ddd, 1H, 16b-H^b), 4.20 (dd, 1H, 16b-H^a), 4.26 (ddd, 1H, 16a-H^a), 4.36 (dd, 1H, 16a-H^b), 5.78 (s, 1H, 4-H), 7.54 (m, 2H, 3′-H and 5′-H), 7.62 (m, 1H, 4′-H), 7.82 (m, 2H, 2′-H and 6′-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.8 (C-18), 17.2 (C-19), 20.0 (CH_2), 30.6 (CH_2), 30.9 (CH_2), 31.4 (CH_2), 32.3 (CH_2), 33.8 (CH_2), 34.5 (CH), 35.6 (CH_2), 38.5 (C-10), 47.8 (CH), 49.1 (C-13), 51.8 (d, J = 7.1 Hz, C-16), 53.7 (CH), 71.3 (d, J = 6.5 Hz, C-16b), 74.2 (d, J = 6.5 Hz, C-16a), 124.2 (C-4), 125.5 (d, J = 182.4 Hz, C-1′), 129.1 (d, 2C, J = 15.3 Hz, C-2′ and C-6′), 131.3 (d, 2C, J = 10.5 Hz, C-3′ and C-5′), 133.0 (d, J = 3.1 Hz, C-4′), 169.5 (C-5), 198.9 (C-3), 215.6 (C-17). Anal. Calcd for $C_{27}H_{33}O_5P$ C, 69.22; H, 7.10; Found: C, 68.97; H, 7.32.Compound **4b**: Mp. 163–166 °C; $[\alpha]_D$ = +14.5; R_f = 0.24; ^{31}P NMR (122 MHz, $CDCl_3$): δ = 18.1; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.99 (s, 3H, 18-H_3), 1.07 (m, 1H), 1.19 (m, 1H), 1.24 (s, 3H, 19-H_3), 1.31 (m, 1H), 1.43 (m, 1H), 1.51 (m, 1H), 1.73 (m, 2H), 1.83 (m, 1H), 1.93 (m, 2H), 2.06 (m, 2H), 2.34–2.52 (m, 4H), 2.60 (dd, 1H, J = 12.8 Hz, J = 5.8 Hz), 3.91 (ddd, 1H, 16b-H^b), 4.09 (ddd, 1H, 16a-H^b), 4.77 (d, 1H, 16b-H^a), 4.97 (dd, 1H, 16a-H^a), 5.78 (s, 1H, 4-H), 7.50 (m, 2H, 3′-H and 5′-H), 7.63 (m, 1H, 4′-H), 7.88 (m, 2H, 2′-H and 6′-H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 13.7 (C-18), 17.3 (C-19), 20.0 (CH_2), 30.7 (2C, 2 × CH_2), 31.3 (CH_2), 32.3 (CH_2), 33.8 (CH_2), 34.6 (CH), 35.6 (CH_2), 38.5 (C-10), 47.8 (CH), 48.9 (C-13), 52.6 (d, J = 5.3 Hz, C-16), 53.7 (CH), 69.0 (d, J = 6.2 Hz, C-16b), 72.6 (d, J = 6.2 Hz, C-16a), 124.3 (C-4), 126.9 (C-1′), 128.5 (d, 2C, J = 15.7 Hz, C-2′ and C-6′), 132.2 (d, 2C, J = 10.4 Hz, C-3′ and C-5′), 133.6 (d, J = 3.0 Hz, C- 4′), 169.4 (C-5), 199.0 (C-3), 215.3 (C-17); Anal. Calcd for $C_{27}H_{33}O_5P$ C, 69.22; H, 7.10; Found: C, 69.05; H, 7.27.

2.2.2. Synthesis of 16-spiro(2′-oxo-2′-phenoxy[1′,3′,2′]dioxaphosphorino)-4-androsten-3,17-dion (**5a** and **5b**)

According to the general procedure, **3b** (0.17 ml) was used. After purification of the crude product (EtOAc/CH_2Cl_2 = 20:80), compound **5a** (148 mg, 31%) as the fast-eluting diastereomer was obtained. The less mobile epimer was identified as isomer **5b** (244 mg, 50%). Compound **5a**: Mp. 295–297 °C; $[\alpha]_D$ = +92.4; R_f = 0.44; ^{31}P NMR (121 MHz, $CDCl_3$): δ = −14.1; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.98 (s, 3H, 18-H_3), 1.05 (m, 1H), 1.17 (m, 1H), 1.23 (s, 3H, 19-H_3), 1.29 (m, 1H), 1.41 (m, 1H), 1.51 (m, 1H), 1.73 (m, 2H), 1.81–1.92 (m, 3H), 2.03 (m, 2H), 2.33–2.50 (m, 4H), 2.63 (dd, 1H, J = 13.2 Hz, J = 5.8 Hz), 4.04 (ddd, 1H, 16b-H^b), 4.20 (ddd, 1H, 16a-H^b), 4.52 (d, 1H, 16b-H^a), 4.72 (dd, 1H, 16a-H^a), 5.78 (s, 1H, 4-H), 7.20 (t, 1H, J = 7.3 Hz, 4′-H), 7.27 (m, 2H, 2′-H and 6′-H), 7.36 (m, 2H, 3′-H and 5′-H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 13.7 (C-18), 17.2 (C-19), 20.0 (CH_2), 30.6 (2C, 2 × CH_2), 31.3 (CH_2), 32.2 (CH_2), 33.8 (CH_2), 34.5 (CH), 35.6 (CH_2), 38.5 (C-10), 47.8 (CH), 49.1 (C-13), 52.1 (d, J = 6.4 Hz, C-16), 53.7 (CH), 72.8 (d, J = 7.7 Hz, C-16b), 76.3 (d, J = 7.7 Hz, C-16a), 119.4 (d, 2C, J = 5.2 Hz, C-2′ and C-6′), 124.3 (C-4), 125.3 (C-4′), 129.9 (2C, C-3′ and C-5′), 150.0 (d, J = 6.6 Hz, C-1′), 169.4 (C-5), 199.0 (C-3), 215.0 (C-17); Anal. Calcd for $C_{27}H_{33}O_6P$ C, 66.93; H, 6.86; Found: C, 67.08; H, 6.94. Compound **5b**: Mp. 218–220 °C; $[\alpha]_D$ = +63.8; R_f = 0.29; ^{31}P NMR (121 MHz, $CDCl_3$): δ = −11.4; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.93 (s, 3H, 18-H_3), 1.00 (m, 1H), 1.09 (m, 1H), 1.20 (s, 3H, 19-H_3), 1.28 (m, 2H), 1.43 (m, 1H), 1.56 (m, 1H), 1.66–1.81 (m, 4H), 1.88 (m, 1H), 2.01 (m, 2H), 2.31–2.47 (m, 4H), 4.01 (ddd, 1H, 16b-H^b), 4.18 (ddd, 1H, 16a-H^b), 4.57 (dd, 1H, 16b-H^a), 4.75 (dd, 1H, 16a-H^a), 5.76 (s, 1H, 4-H), 7.21 (m, 3H, 2′-H, 4′-H and 6′-H), 7.36 (m, 2H, 3′-H and 5′-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.8 (C-18), 17.2 (C-19), 20.0 (CH_2), 30.2 (CH_2), 30.4 (CH_2), 31.3 (CH_2), 32.2 (CH_2), 33.8 (CH_2), 34.5 (CH), 35.6 (CH_2), 38.5 (C-10), 47.6 (CH), 48.9 (C-13), 51.2 (d, J = 6.3 Hz, C-16), 53.6 (CH), 72.2 (d, J = 6.1 Hz, C-16a), 75.2 (d, J = 6.1 Hz, C-16b), 120.4 (d, 2C, J = 4.6 Hz, C-2′ and C-6′), 124.3 (C-4), 125.6 (d, J = 1.3 Hz, C-4′), 129.6 (d, 2C, J = 0.94 Hz, C-3′ and C-5′), 150.3 (d, J = 8.2 Hz, C-1′), 169.3 (C-5), 199.0 (C-3), 214.6 (C-17); Anal. Calcd for $C_{27}H_{33}O_6P$ C, 66.93; H, 6.86; Found: C, 67.17; H, 6.95.

2.2.3. Synthesis of 16-spiro[(2′-oxo-2′-(4″-chlorophenoxy)-[1′,3′,2′]dioxaphosphorino)]-4-androsten-3,17-dion (**6a** and **6b**)

According to the general procedure, **3c** (0.19 ml) was used. After purification of the crude product (EtOAc/CH_2Cl_2 = 20:80), compound **6a** (165 mg, 32%) as the fast-eluting diastereomer was obtained. The less mobile epimer was identified as isomer **6b** (219 mg, 42%). Compound **6a**: Mp. 249–252 °C; $[\alpha]_D$ = +61.0; R_f = 0.50; ^{31}P NMR (121 MHz, $CDCl_3$): δ = −14.2; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.98 (s, 3H, 18-H_3), 1.06 (m, 1H), 1.17 (m, 1H), 1.23 (s, 3H, 19-H_3), 1.29 (m, 1H), 1.41 (m, 1H), 1.52 (m, 1H), 1.73 (m, 2H), 1.87 (m, 3H), 2.03 (m, 2H), 2.34–2.50 (m, 4H), 2.61 (dd, 1H, J = 13.2 Hz, J = 5.7 Hz), 4.05 (ddd, 1H, 16b-H^b), 4.21 (ddd, 1H, 16a-H^b), 4.49 (d, 1H, 16b-H^a), 4.70 (d, 1H, 16a-H^a), 5.78 (s, 1H, 4-H), 7.22 (d, 2H, J = 8.7 Hz, 2′-H and 6′-H), 7.33 (d, 2H, J = 8.7 Hz, 3′-H and 5′-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.7 (C-18), 17.3 (C-19), 20.0 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 31.3 (CH_2), 32.2 (CH_2), 33.8 (CH_2), 34.5 (CH), 35.6 (CH_2), 38.5 (C-10), 47.9 (CH), 49.1 (C-13), 52.0 (d, J = 6.4 Hz, C-16), 53.7 (CH), 72.9 (d, J = 7.7 Hz, C-16b), 76.5 (d, J = 7.7 Hz, C-16a), 120.9 (d, 2C, J = 5.2 Hz, C-2′ and C-6′), 124.3 (C-4), 129.9 (2C, C-3′ and C-5′), 130.7 (C-4′), 148.6 (d, J = 6.3 Hz, C-1′), 169.3 (C-5), 198.9 (C-3), 214.9 (C-17); Anal. Calcd for $C_{27}H_{32}ClO_6P$ C, 62.49; H, 6.22; Found: C, 62.58; H, 6.33. Compound **6b**: Mp. 240–243 °C; $[\alpha]_D$ = +54.5; R_f = 0.38; ^{31}P NMR (121 MHz, $CDCl_3$): δ = −11.9; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.93 (s, 3H, 18-H_3), 1.00 (m, 1H), 1.09 (m, 1H), 1.21 (s, 3H, 19-H_3), 1.28 (m, 2H), 1.47 (m, 2H), 1.69–1.81 (m, 4H), 1.88 (m, 2H), 2.03 (m, 1H), 2.34–2.47 (m, 4H), 4.01 (dd,