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Regio- and stereoselective synthesis of pregnane-fused isoxazolines by nitril-oxide/alkene 1,3-dipolar cycloaddition and an evaluation of their cell-growth inhibitory effect *in vitro*

Gergő Mótyán [a], Ádám Baji [a], István Zupkó [b], Éva Frank [a, *]

[a] Department of Organic Chemistry, University of Szeged, Szeged, H-6720, Hungary
[b] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, H-6720, Hungary

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ABSTRACT

Efficient syntheses of some pregnane-fused isoxazolines from 16-dehydropregnenolone acetate with different arylnitrile oxides were carried out by 1,3-dipolar cycloadditions. The intermolecular ring-closures occurred in a highly regio- and stereoselective manner permitting the formation of a single 16α,17α-condensed diastereomer in which the *O* terminus of the nitrile oxide dipole is attached to C-17 of the sterane core. The conversions were found to be affected significantly by the electronic character of the substituents on the aromatic moiety of the 1,3-dipoles. Deacetylation of the primary products resulted in the corresponding 3β-OH analogs. All of the synthesized compounds were subjected to *in vitro* pharmacological studies for the determination of their antiproliferative effects on four breast cancer cell lines (MCF7, T47D, MDA-MB-231 and MDA-MB-361).

1. Introduction

1,3-Dipolar cycloadditions of nitrile oxides with alkene or alkyne dipolarophiles leading to 2-isoxazoline or isoxazole derivatives [1,2] have been extensively studied and have found general application in organic synthesis owing to the great importance of these heterocycles as structural building blocks of several biologically active molecules [3,4] and versatile intermediates in the synthesis of numerous bifunctional compounds [5].

The propargyl/allenyl-type nitrile oxide 1,3-dipoles, containing a *N* atom in the center can readily undergo cyclization with multiple-bond systems under thermal or catalytic conditions [6]. The reactivity of the dipolarophile as well as the regio- and stereoselectivity of the thermally-induced process depend on certain structural features. Thus, monosubstituted alkenes are generally more reactive than disubstituted olefins or alkynes, and the reactions with nitrile oxides occur with almost complete regiospecificity to furnish exclusively 5-substituted isoxazolines. However, mixtures of regioisomers are usually obtained with 1,2-disubstituted dipolarophiles, although steric effects may have an influence on the regioisomeric ratio, especially in case of polycyclic systems [1,6]. π-Conjugation of the double bond has a strong promoting effect on the reactivity of the alkene and site-selectivity often predominates when different olefinic moieties compete for the dipole. Ring-strain also destabilizes dipolarophiles and enhances their activity toward nitrile oxides. The stereochemical outcome of the cycloadditions is affected by steric and geometric features of the dipolarophile.

Attention to the incorporation of five-membered *N*,*O*-heterocyclic moiety into steroids either connected to or condensed with ring A or D of the sterane core is caused by the fact that some of these modified compounds have been reported to exhibit various biological activities, including anabolic, antibacterial, antiproliferative, hypocholesteremic and anti-inflammatory properties [5,7–10]. Moreover, steroids are attractive chiral models with relatively rigid framework for controlling the regio- and/or stereoselectivity of nitrile oxide/alkene cycloadditions [11]. However, sterane-based dipolarophiles, containing an unactivated and/or sterically less hindered double bond are usually not reactive enough necessitating elevated temperature and/or prolonged reaction time for sufficient conversion and the reactions often suffer from a lack of selectivity [5,12,13]. The introduction of an enone

* Corresponding author.
E-mail address: frank@chem.u-szeged.hu (É. Frank).

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moiety into the vicinity of sterically interfering groups has been found to have a strong driving effect on the reactivity of such alkenes and also on the regio- and stereochemical outcome of the thermally-induced ring-closures [1]. In this regard, we recently reported the regio- and stereoselective synthesis of some novel 5α-androstanes containing an 2-isoxazoline moiety condensed to ring A or D by intermolecular 1,3-dipolar cycloaddition of aryl nitrile oxides to steroidal α,β-unsaturated ketones [14]. We have demonstrated that the cyclic enone moiety of the five-membered ring D is more reactive than that of the six-membered ring A presumably due to ring strain and conformation effects.

As a continuation of our research for the construction of steroidal ring-condensed heterocycles [10,14–18], our aim was to extend the ring closure reactions to the pregnane skeleton to make available some additional ring D-fused isoxazolines. The present work describes the transformations of 16-dehydropregnenolone acetate (16-DPA) containing a reactive dipolarophilic enone moiety, with nitrile oxides via thermal 1,3-dipolar cycloaddition. The regio- and stereoselectivity of the process and the influence of steric and electronic factors on the ring-closure reactions were also investigated. Since several steroidal isoxazoles and their saturated analogs have been reported to possess cell-growth-inhibitory effect on malignant cell lines of diverse origins [7,9–11], our further goal was to investigate our compounds for such activities against four human adherent breast cancer cell lines (MCF7, T47D, MDA-MB-231 and MDA-MB-361).

2. Experimental

2.1. General

Melting points (mp-s) were determined on an SMS Optimelt digital apparatus. Elemental analysis data were obtained with a Perkin Elmer CHN analyzer model 2400 and FT-IR spectra were recorded on a FT/IR-4700 spectrometer (Jasco) using ATR. Infrared absorbance is reported in reciprocal centimeters (cm^{-1}). ^1H NMR spectra were obtained at room temperature in $CDCl_3$ solution at 500 MHz (Brucker DRX 500) and the ^{13}C NMR spectra at 125 MHz with the same instrument. Chemical shifts are reported in ppm (δ scale) relative to TMS; coupling constants (J) are given in Hz. Multiplicity of the ^1H resonance peaks are indicated as singlet (s), doublet (d), triplet (t) and multiplet (m). ^{13}C NMR spectra are ^1H-decoupled. For the determination of multiplicities, the J-MOD pulse sequence was used. Automated flow injection analyses were performed by using an HPLC/MSD system. The system comprised an Agilent 1100 micro vacuum degasser, a quaternary pump, a microwell plate autoinjector and a 1946A MSD equipped with an electrospray ion (ESI) source operated in positive ion mode. The ESI parameters: nebulizing gas N_2, at 35 psi; drying gas N_2, at 350 °C and 12 L min^{-1}; capillary voltage 3000 V; and fragmentor voltage 70 V. The MSD was operated in scan mode with the mass range m/z 60–620. Samples (0.2 μL) were injected with an automated needle wash directly into the solvent flow (0.3 mL min^{-1}) of MeCN/H_2O 70:30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software. All solvents were distilled immediately prior to use. Reagents and materials were obtained from reliable 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 systems (ss): CH_2Cl_2 (A); EtOAc/CH_2Cl_2 (10/80, v/v) (B). Flash chromatography: Merck silica gel 60, 40–63 μm.

2.2. General procedure for the preparation of steroidal isoxazolines (**6a**–**e**)

16-DPA (**1**, 357 mg, 1.00 mmol) and the appropriate aromatic hydroximidoyl chloride (**4a**–**e**, 1.50 mmol) were dissolved in toluene (15 mL), and DIPEA (0.52 mL, 3.00 mmol) was added dropwise to the reaction mixture at room temperature, with subsequent refluxing for 2 h. The solvent was then evaporated off *in vacuo* and the resulting crude product was purified by column chromatography with CH_2Cl_2.

2.2.1. 3β-Acetoxy-3′-phenyl-2′-isoxazolino[4′,5′-d:16α,17α]-pregn-5-en-20-one (**6a**)

In accordance with the general procedure, *N*-hydroxybenzenecarboximidoyl chloride (**4a**, 233 mg) was used for the synthesis. The product **6a** (452 mg) was obtained as a white precipitate. Mp 201–204 °C (Mp 196–198 °C [22]); $R_f = 0.25$ (ss A); IR: 1730, 1708, 1593, 1442, 1193 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz): δ 0.79 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.04 (m, 1H, 9α-H), 1.14 (m, 1H, 1α-H), 1.45–1.61 (overlapping m, 5H, 2α-H, 8β-H, 11β-H, 14α-H, 15α-H), 1.69–1.77 (overlapping m, 4H, 7β-H, 15β-H, 11α-H, 12β-H), 1.87 (m, 3H, 1β-H, 2β-H, 7α-H), 2.02 (s, 3H Ac-CH_3), 2.08 (m, 1H, 12α-H), 2.28 (m, 2H, 4-H_2), 2.31 (s, 3H, 21-H_3), 4.42 (t-like m, 1H, 16-H), 4.58 (m, 1H, 3-H), 5.30 (m, 1H, 6-H), 7.39 (m, 3H, 3″-H, 4″-H and 5″-H), 7.66 (m, 2H, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.2 (C-18), 19.2 (C-19), 20.5 (C-11), 21.4 (Ac-CH_3), 27.4 (C-21), 27.6 (C-2), 31.2 (C-8), 31.6 (2C, C-7 and C-12), 31.8 (C-15), 35.8 (C-10), 36.9 (C-1), 38.0 (C-4), 48.3 (C-13), 49.1 (C-9), 50.4 (C-14), 51.0 (C-16), 73.7 (C-3), 106.1 (C-17), 121.8 (C-6), 127.1 (2C, C-2″ and C-3″), 128.6 (C-1″), 128.7 (2C, C-3″ and C-5″), 130.1 (C-4″), 139.6 (C-5), 159.9 (C-3′), 170.4 (Ac-CO), 206.4 (C-20); ESI-MS: 476 $[M+H]^+$; Anal. Calcd for $C_{30}H_{37}NO_4$ C, 75.76; H, 7.84; Found: C, 75.92; H, 7.69.

2.2.2. 3β-Acetoxy-3′-4″-tolyl-2′-isoxazolino[4′,5′-d:16α,17α]-pregn-5-en-20-one (**6b**)

In accordance with the general procedure, *N*-hydroxy-4-methylbenzenecarboximidoyl chloride (**4b**, 255 mg) was used for the synthesis. The product **6b** (480 mg) was obtained as a white precipitate. Mp 164–166 °C (Mp 121–123 °C [22]); $R_f = 0.26$ (ss A); IR: 1727, 1712, 1593, 1456, 1197 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.78 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 1.04 (m, 1H, 9α-H), 1.14 (m, 1H, 1α-H), 1.44–1.61 (overlapping m, 5H), 1.68–1.75 (overlapping m, 4H), 1.87 (m, 3H), 2.02 (s, 3H Ac-CH_3), 2.07 (m, 1H), 2.28 (m, 2H, 4-H_2), 2.30 (s, 3H, 21-H_3), 2.36 (s, 3H, 4″-CH_3), 4.40 (t-like m, 1H, 16-H), 4.58 (m, 1H, 3-H), 5.30 (m, 1H, 6-H), 7.19 (d, 2H, $J = 7.8$ Hz, 3″-H and 5″-H), 7.55 (d, 2H, $J = 7.8$ Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.2 (C-18), 19.2 (C-19), 20.5 (C-11), 21.4 (2C, Ac-CH_3 and 4″-CH_3), 27.4 (C-21), 27.6 (C-2), 31.2 (C-8), 31.6 (2C, C-7 and C-12), 31.8 (C-15), 36.5 (C-10), 36.9 (C-1), 38.0 (C-4), 48.3 (C-13), 49.1 (C-9), 50.4 (C-14), 51.1 (C-16), 73.7 (C-3), 105.9 (C-17), 121.9 (C-6), 125.7 (C-1″), 127.0 (2C, C-2″ and C-6″), 129.4 (2C, C-3″ and C-5″), 139.6 (C-5), 140.4 (C-4″), 159.9 (C-3′), 170.4 (Ac-CO), 206.6 (C-20); ESI-MS: 490 $[M+H]^+$; Anal. Calcd for $C_{31}H_{39}NO_4$ C, 76.04; H, 8.03; Found: C, 76.16; H, 7.86.

2.2.3. 3β-Acetoxy-3′-4″-methoxyphenyl-2′-isoxazolino[4′,5′-d:16α,17α]-pregn-5-en-20-one (**6c**)

In accordance with the general procedure, N-hydroxy-4-methoxybenzenecarboximidoyl chloride (**4c**, 279 mg) was used for the synthesis. The product **6c** (490 mg) was obtained as a white precipitate. Mp 187–189 °C (Mp 119–121 °C [22]); $R_f = 0.20$ (ss A); IR: 1722, 1712, 1609, 1465, 1190 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.78 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.04 (m, 1H, 9α-H), 1.13 (m, 1H, 1α-H), 1.44–1.61 (overlapping m, 5H), 1.67–1.75 (overlapping m, 4H), 1.86 (m, 3H), 2.01 (s, 3H Ac-CH_3), 2.07 (m, 1H), 2.28 (m, 2H,