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Synthesis, characterization and biological evaluation of some novel 17-isoxazoles in the estrone series

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ABSTRACT

Regioselective 1,3-dipolar cycloadditions of different aryl nitrile oxides to mestranol were carried out to furnish novel steroidal 17α-isoxazoles in good to excellent yields. Copper(I) was found to be an efficient catalyst, accelerating the intermolecular ring-closures and leading exclusively to 3,5-disubstituted isoxazoles. The yields of the cycloadducts, however, were influenced by the substituents on the aromatic moiety of the 1,3-dipoles. Moreover, dehydration of the primary products resulted in the corresponding $\Delta^{16,17}$ *exo*-heterocyclic derivatives. All the synthesized compounds were subjected to *in vitro* pharmacological studies of their antiproliferative effects relative to three human malignant cell lines (HeLa, MCF7 and A2780).

1. Introduction

One of the major aims of steroid chemistry at present is the development of novel derivatives containing a heterocyclic moiety, as a part of, fused to or linked to the sterane framework, in order to extend the already wide range of known biological activities of these compounds. A number of semisynthetic molecules have been described as potent inhibitors of specific enzymes involved in the biosynthesis of endogenous hormones, allowing their potential use in the medication of hormone-dependent diseases. Steroidal *exo*-heterocycles, such as the therapeutically applied abiraterone, can block androgen synthesis at an early stage by inhibiting 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) and can therefore be used in the treatment of prostate cancer [1–4]. Some heterosteroids (*e.g.* finasteride and dutasteride) are known to decrease the circulating levels of dihydrotestosterone by exerting pronounced inhibitory effects on 5α-reductase, and consequently to reduce the excessive prostate growth in benign hyperplasia [5–7].

One of the most frequent modifications of the original steroid molecule is performed at C-17, where the construction of a heteroring can be facilitated by the already existing functional groups. Therefore, the introduction of different heterocyclic moieties (*e.g.* imidazole, pyrazol, oxazole, thiazol or isoxazole) into this position was found to be beneficial from a pharmacological aspect obtaining several compounds as potent inhibitors of $P450_{17\alpha}$ [1,8,9]. Moreover, experimental results during the past few years have revealed that some of these steroidal heterocycles play important roles in complex signal transduction mechanisms in a hormone receptor-independent manner by the inhibition of angiogenesis, tubulin polymerization, and the upregulation of apoptotic pathways [10,11]. Although the detailed mechanisms of action are not very clear in these latter cases, the coordination of 17-heterocycles of steroids to the target biomolecule and thus the better fit of the ligand at the binding site of the receptor is thought to be an important factor in the antiproliferative activity. The cell membrane diffusion may be facilitated at the same time by the hydrophobic steroid unit.

Among the steroidal heterocycles, attention to derivatives of isoxazole is caused by the fact that these compounds have been reported to exhibit various biological activities, including anabolic, myotrophic, hypochloesteremic and tumor-inhibiting properties [12], and moreover they are convenient intermediates in the synthesis of numerous polyfunctional compounds [13]. Danazol, probably the best-known member of this series, was approved as the first drug for the specific treatment of endometriosis. Although its clinical use is limited by its masculinizing side-effects, *in vitro* bioassays have suggested that danazol also exerts a growth-inhibitory effect on human endometrial cancer cells [14].

One of the synthetic tools for the formation of five-membered heterocyclic ring systems include 1,3-dipolar cycloaddition, which has enjoyed a wide range of popularity for decades in view of the large numbers of available dipoles and dipolarophiles [15–17]. In recent years, considerable attention has been focused on

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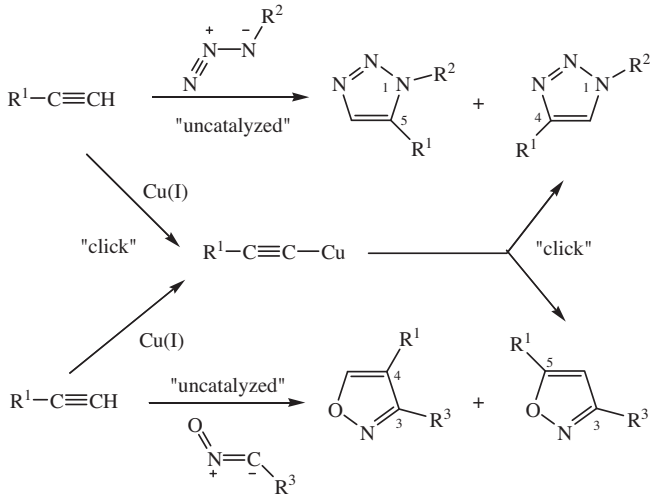


Fig. 1. Synthesis of triazoles and isoxazoles via Huisgen *versus* catalytic 1,3-dipolar cycloadditions.

Cu(I)-catalyzed azide–alkyne cycloaddition [18–20] as it meets all of the criteria for ″click″ chemistry introduced by Sharpless [21] in 2001 (Fig. 1). As compared with the uncatalyzed (Huisgen) version, the presence of the catalyst dramatically improves both the rate and the regioselectivity of the process, leading exclusively to the 1,4-disubstituted 1,2,3-triazole, and eliminating the need for elevated temperature and/or a prolonged reaction time. Although the conventional cycloaddition occurs through a concerted mechanism, the catalytic pathway follows a stepwise route with a copper acetylide as the key intermediate [22,23].

Cycloadditions between alkynes and nitrile oxides also provide convenient possibilities for the construction of isoxazoles (Fig. 1). Nitrile oxides are usually prepared from the corresponding aldoximes directly before application, by oxidative halogenation to imidoyl halides and subsequent base-induced dehydrohalogenation, due to the instability of the precursor and the 1,3-dipole [24]. Although several syntheses of steroidal isoxazoles have been reported, the two possible regioisomers are usually obtained during different reaction times and at different temperatures. A further disadvantage of the concerted reaction is the rapid formation of furoxane-type by-products (especially for reactive nitrile oxide dipoles), which can reduces the yields of the desired cycloadducts [15]. The regiospecific stepwise sequence is not limited to azides, however, but works with nitrile oxide dipoles as well. Thus, a dramatic acceleration was observed experimentally when a Cu(I) catalyst was applied, in good agreement with theoretical predictions; more importantly, only the formation of the 3,5-disubstituted isoxazole occured [25].

As an extension of our program on the synthesis of steroidal heterocycles [26–29] exerting antiproliferative activity, we set out to develop an efficient route for the preparation of novel 17-*exo*-isoxazolyl derivatives from mestranol through use of the ″click″ chemistry approach. Since analogous compounds in the estrone series have been reported to have no estrogenic effect [12], our aim was to investigate wheather the synthetized isoxazoles display any effect on cell-growth. Therefore, all derivatives were screened *in vitro* for their activities against a panel of three human cancer cell lines (HeLa, MCF7 and A2780).

2. Experimental

2.1. General

Melting points (mp) were determined on a Kofler block and are uncorrected. Elementary analysis data were determined with a Perkin Elmer CHN analyzer model 2400. NMR spectra were obtained at room temperature with a Bruker DRX 500 instrument. Chemical shifts are reported in ppm (δ scale), and coupling constants (J) in Hz. 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 micro-well plate autoinjector and a 1946A MSD equipped with an electrospray ion source (ESI) operated in positive ion mode. The ESI parameters were: nebulizing gas N_2, at 35 psi; drying gas N_2, at 350 °C and 12 L/min; capillary voltage (VCap) 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 automated needle wash directly into the solvent flow (0.3 mL/min) 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 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); solvent systems (ss): (A) CH_2Cl_2/EtOAc (98:2 v/v), (B) CH_2Cl_2/EtOAc (95:5 v/v), (C) CH_2Cl_2/*n*-hexane (70:30 v/v), (D) CH_2Cl_2/*n*-hexane (60:40 v/v); (E) CH_2Cl_2. 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. Flash chromatography: Merck silica gel 60, 40–63 μm.

2.2. General procedure for the synthesis of 17β-hydroxy,17α-isoxazolyl derivatives (**6a–k**) in the estrone series

Mestranol (**1**, 310 mg, 1.00 mmol) and the appropriate aromatic imidoyl chloride [25] (**4a–k**, 1.50 mmol) were dissolved in toluene (15 mL), and Ph_3P (52 mg, 0.20 mmol) and CuI (19 mg, 0.10 mmol) were then added to the solution. Finally, *N*,*N*-diidopropyl ethylamine (DIPEA) (0.67 mL, 4.00 mmol) was added dropwise to the gently heated reaction mixture, which was subsequently heated to reflux with stirring for 4 h. During the reaction, the pale-yellow solution became dark-brown. After completion of the reaction (TLC monitoring), the solvent was evaporated off *in vacuo*. The resulting crude products were purified by flash chromatography.

2.2.1. Synthesis of 3-methoxy-17α-(3′-phenylisoxazol-5′-yl)estra-1,3,5(10)-trien-17β-ol (**6a**)

According to Section 2.2, *N*-hydroxybenzenecarboximidoyl chloride (**4a**, 233 mg) was used. After purification with CH_2Cl_2/EtOAc (98:2) as eluent, **6a** was obtained as a white solid (399 mg, 93%). Mp 119–122 °C; R_f = 0.24 (ss A). Anal. Calcd for $C_{28}H_{31}NO_3$: C, 78.29; H, 7.27. Found: C, 78.40; H, 7.43. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.93 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.36–1.64 (m, 4H), 1.72–1.79 (m, 2H), 1.91–2.01 (m, 2H), 2.05–2.15 (m, 2H), 2.22 (m, 1H), 2.50 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.52 (s, 1H, 4′-H), 6.62 (d, 1H, J = 2.2 Hz, 4-H), 6.68 (dd, 1H, J = 8.5 Hz, J = 2.2 Hz, 2-H), 7.12 (d, 1H, J = 8.5 Hz, 1-H), 7.47 (overlapping m, 3H, 3″-H, 4″-H and 5″-H), 7.84 (d, 2H, J = 8.2 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 100.1 (C-4′), 111.4 (C-2), 113.8 (C-4), 126.2 (C-1), 126.8 (2C, C-2″ and C-6″), 128.9 (2C, C-3″ and C-5″), 129.0 (C-1″), 130.0 (C-4″), 132.4 (C-10), 137.8 (C-5), 157.4 (C-3), 161.9 (C-3′), 177.6 (C-5′) ppm; ESI–MS: 430 $(M+H)^+$.

*2.2.2. Synthesis of 3-methoxy-17α-[3′-(2″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6b**)*

According to Section 2.2, *N*-hydroxy-2-methylbenzenecarboximidoyl chloride (**4b**, 254 mg) was added to the mixture. After purification with CH_2Cl_2 as eluent, **6b** was obtained as a white solid (435 mg, 98%). Mp 141−143 °C; R_f = 0.40 (ss A). Anal. Calcd for $C_{29}H_{33}NO_3$: C, 78.52; H, 7.50; Found: C, 78.66; H, 7.37. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (m, 1H), 1.07 (s, 3H, 18-H_3), 1.35–1.64 (m, 4H), 1.72–1.80 (m, 2H), 1.91–2.00 (m, 2H), 2.05–2.16 (m, 2H), 2.24 (m, 1H), 2.50 (m, 1H), 2.53 (s, 3H, 2″-Me), 2.87 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.39 (s, 1H, 4′-H), 6.63 (d, 1H, *J* = 2.2 Hz, 4-H), 6.69 (dd, 1H, *J* = 8.5 Hz, *J* = 2.2 Hz, 2-H), 7.13 (d, 1H, *J* = 8.5 Hz, 1-H), 7.27–7.36 (overlapping m, 3H, 3″-H, 4″-H and 5″-H), 7.54 (d, 1H, *J* = 7.9 Hz, 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 21.2 (2″-Me), 23.6 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 102.7 (C-4′), 111.4 (C-2), 113.8 (C-4), 125.9 (C-5″), 126.2 (C-1), 128.7 (C-1″), 129.4, 129.5, 131.1 (3C, C-3″, C-4″ and C-6″), 132.3 (C-10), 136.9 (C-2″), 137.8 (C-5), 157.4 (C-3), 162.5 (C-3′), 176.6 (C-5′) ppm; ESI–MS: 444 $(M+H)^+$.

*2.2.3. Synthesis of 3-methoxy-17α-[3′-(3″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6c**)*

According to Section 2.2, *N*-hydroxy-3-methylbenzenecarboximidoyl chloride (**4c**, 254 mg) was used. After purification with CH_2Cl_2 as eluent, **6c** was obtained as a white solid (426 mg, 96%). Mp 153–155 °C; R_f = 0.46 (ss A). Anal. Calcd for $C_{29}H_{33}NO_3$: C, 78.52; H, 7.50; Found: C, 78.40; H, 7.68. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.92 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.35–1.66 (m, 4H), 1.71–1.78 (m, 2H), 1.91–2.02 (m, 2H), 2.04–2.16 (m, 2H), 2.22 (m, 1H), 2.42 (s, 3H, 3″-Me), 2.50 (m, 1H), 2.87 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.50 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.4 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.4 Hz, 2-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.27 (d, 1H, *J* = 8.2 Hz, 4″-H), 7.35 (t, 1H, *J* = 8.2 Hz, 5″-H), 7.63 (d, 1H, *J* = 8.2 Hz, 6″-H), 7.67 (s, 1H, 2″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 21.4 (3″-Me), 23.6 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 100.2 (C-4′), 111.4 (C-2), 113.8 (C-4), 123.9 (C-6″), 126.2 (C-1), 127.4 (C-2″), 128.8 (CH), 128.9 (C-1″), 130.8 (CH), 132.4 (C-10), 137.8 (C-5), 138.6 (C-3″), 157.4 (C-10), 162.0 (C-3′), 177.5 (C-5′) ppm; ESI–MS: 444 $(M+H)^+$.

*2.2.4. Synthesis of 3-methoxy-17α-[3′-(4″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6d**)*

According to Section 2.2, *N*-hydroxy-4-methylbenzenecarboximidoyl chloride (**4d**, 254 mg) was added to the mixture. After purification with CH_2Cl_2/EtOAc (97:3 v/v) as eluent, **6d** was obtained as a white solid (430 mg, 97%). Mp 139–142 °C; R_f = 0.25 (ss A). Anal. Calcd for $C_{29}H_{33}NO_3$: C, 78.52; H, 7.50; Found: C, 78.71; H, 7.43. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.93 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.36–1.66 (m, 4H), 1.71–1.79 (m, 2H), 1.90–2.00 (m, 2H), 2.04–2.14 (m, 2H), 2.21 (m, 1H), 2.41 (s, 3H, 4″-Me), 2.53 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.49 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.0 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.0 Hz, 2-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.27 (d, 2H, *J* = 8.1 Hz, 3″-H and 5″-H), 7.72 (d, 2H, *J* = 8.1 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0 (C-18), 21.4 (4″-Me), 23.6 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 37.2 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.7 (C-17), 100.1 (C-4′), 111.4 (C-2), 113.8 (C-4), 126.1 (C-1″), 126.2 (C-1), 126.7 (2C, C-2″ and C-6″), 129.6 (2C, C-3″ and C-5″), 132.4 (C-10), 137.8 (C-5), 140.1 (C-4″), 157.4 (C-3), 161.8 (C-3′), 177.4 (C-5′) ppm; ESI–MS: 444 $(M+H)^+$.

*2.2.5. Synthesis of 3-methoxy-17α-[3′-(4″-methoxy)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6e**)*

According to Section 2.2, *N*-hydroxy-4-methoxybenzenecarboximidoyl chloride (**4e**, 279 mg) was added to the mixture. After purification with CH_2Cl_2/EtOAc (98:2 v/v) as eluent, **6e** was obtained as a pale-yellow solid (445 mg, 97%). Mp 155–157 °C; R_f = 0.28 (ss B). Anal. Calcd for $C_{29}H_{33}NO_4$: C, 75.79; H, 7.24; Found: C, 75.89; H, 7.05. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.93 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.35–1.66 (m, 4H), 1.71–1.79 (m, 2H), 1.90–1.99 (m, 2H), 2.04–2.15 (m, 2H), 2.22 (m, 1H), 2.52 (m, 1H), 2.87 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OMe), 3.86 (s, 3H, 4″-OMe), 6.46 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.3 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 6.98 (d, 2H, *J* = 8.4 Hz, 3″-H and 5″-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.76 (d, 2H, *J* = 8.4 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 37.2 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 and 55.3: 3-OMe and 4″-OMe, 83.7 (C-17), 100.0 (C-4′), 111.4 (C-2), 113.8 (C-4), 114.3 (2C, C-3″ and C-5″), 121.5 (C-1″), 126.2 (C-1), 128.2 (2C, C-2″ and C-6″), 132.4 (C-10), 137.8 (C-5), 157.4 (C-3), 161.0 (C-4″), 161.5 (C-3′), 177.4 (C-5′) ppm; ESI–MS: 460 $(M+H)^+$.

*2.2.6. Synthesis of 3-methoxy-17α-[3′-(3″,4″-dimethoxy)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6f**)*

According to Section 2.2, *N*-hydroxy-3,4-dimethoxybenzenecarboximidoyl chloride (**4f**, 323 mg) was used. After purification with CH_2Cl_2/EtOAc (98:2 v/v) as eluent, **6f** was obtained as a white solid (465 mg, 95%). Mp 108–111 °C; R_f = 0.28 (ss B). Anal. Calcd for $C_{30}H_{35}NO_5$: C, 73.59; H, 7.21; Found: C, 73.70; H, 7.34. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.35–1.64 (m, 4H), 1.70–1.80 (m, 2H), 1.90–1.99 (m, 2H), 2.02–2.15 (m, 2H), 2.23 (m, 1H), 2.50 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 3.93 (s, 3H) and 3.96 (s, 3H, 3″-OMe and 4″-OMe), 6.47 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.3 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 6.93 (d, 1H, *J* = 8.4 Hz, 5″-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.33 (dd, 1H, *J* = 8.4 Hz, *J* = 1.6 Hz, 6″-H), 7.44 (d, 1H, *J* = 1.6 Hz, 2″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 55.9 and 56.0: 3″-OMe and 4″-OMe, 83.8 (C-17), 100.0 (C-4′), 109.3 and 111.1: C-2″ and C-5″, 111.4 (C-2), 113.8 (C-4), 119.9 (C-6″), 121.7 (C-1″), 126.2 (C-1), 132.3 (C-10), 137.8 (C-5), 149.3 and 150.6: C-3″ and C-4″, 157.4 (C-3), 161.6 (C-3′), 177.5 (C-5′) ppm; ESI–MS: 490 $(M+H)^+$.

*2.2.7. Synthesis of 3-methoxy-17α-[3′-(3″,5″-dichloro-2″,4″,6″-trimethoxy)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6g**)*

According to Section 2.2, *N*-hydroxy-3,5-dichloro-2,4,6-trimethoxybenzenecarboximidoyl chloride (**4g**, 472 mg) was added to the mixture. After purification with CH_2Cl_2 as eluent, **6g** was obtained as a white solid (577 mg, 98%). Mp 131–134 °C; R_f = 0.52 (ss B). Anal. Calcd for $C_{31}H_{35}Cl_2NO_6$: C, 63.27; H, 5.99; Found: C, 63.15; H, 6.08. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.84 (m, 1H), 1.07 (s, 3H, 18-H_3), 1.35–1.69 (m, 4H), 1.76–1.82 (m, 2H), 1.90–1.97 (m, 2H), 2.00–2.06 (m, 1H), 2.11–2.17 (m, 1H), 2.23 (m, 1H), 2.49 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.74 (s, 6H, 2 × OMe), 3.77 (s, 3H, 3-OMe), 3.96 (s, 3H, OMe), 6.37 (s, 1H, 4′-H), 6.63 (d, 1H, *J* = 2.6 Hz, 4-H), 6.69 (dd, 1H, *J* = 8.5 Hz, *J* = 2.6 Hz, 2-H), 7.14 (d, 1H, *J* = 8.5 Hz, 1-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0 (C-18), 23.5 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 33.2 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.5 (CH), 48.4 (C-13), 48.9 (CH), 55.2 (3-OMe), 60.9 (OMe), 62.1 (2C, 2 × OMe), 83.7 (C-17), 104.2 (C-4′), 111.5 (C-2), 113.8 (C-4), 116.9 (2C, C-3″ and C-5″), 120.2 (C-1″), 126.2 (C-1), 132.1 (C-10), 137.8 (C-5), 154.3 (2C, C-2″ and C-6″), 154.9 (C-3′), 155.6 (C-4″), 157.5 (C-3), 176.5 (C-5′) ppm; ESI–MS: 589 $(M+H)^+$.

*2.2.8. Synthesis of 3-methoxy-17α-[3′-(4″-fluoro)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6h**)*

According to Section 2.2, *N*-hydroxy-4-fluorobenzenecarboximidoyl chloride (**4h**, 260 mg) was used. After purification with CH_2Cl_2 as eluent, **6h** was obtained as a white solid (363 mg, 81%). Mp 140–142 °C; R_f = 0.49 (ss A). Anal. Calcd for $C_{28}H_{30}FNO_3$: C, 75.14; H, 6.76; Found: C, 75.29; H, 6.94. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.36–1.64 (m, 4H), 1.71–1.78 (m, 2H), 1.90–2.00 (m, 2H), 2.05–2.14 (m, 2H), 2.22 (m, 1H), 2.52 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.48 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.3 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 7.11–7.18 (overlapping m, 3H, 1-H, 3″-H and 5″-H), 7.82 (dd, 2H, *J* = 8.2 Hz, *J* = 4.9 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 100.0 (C-4′), 111.4 (C-2), 113.8 (C-4), 116.0 (2C, *J* = 21.8 Hz, C-3″ and C-5″), 125.2 (C-1″), 126.2 (C-1), 128.7 (2C, *J* = 8.4 Hz, C-2″ and C-6″), 132.3 (C-10), 137.8 (C-5), 157.4 (C-3), 161.0 (C-3′), 163.8 (C-4″, *J* = 248.1 Hz), 177.8 (C-5′) ppm; ESI–MS: 448 $(M+H)^+$.

*2.2.9. Synthesis of 3-methoxy-17α-[3′-(4″-chloro)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6i**)*

According to Section 2.2, *N*-hydroxy-4-chlorobenzenecarboximidoyl chloride (**4i**, 285 mg) was used. After purification with CH_2Cl_2 as eluent, **6i** was obtained as a white solid (376 mg, 81%). Mp 144–147 °C; R_f = 0.43 (ss A). Anal. Calcd for $C_{28}H_{30}ClNO_3$: C, 72.48; H, 6.52; Found: C, 72.60; H, 6.67. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.36–1.65 (m, 4H), 1.71–1.79 (m, 2H), 1.91–2.00 (m, 2H), 2.05–2.15 (m, 2H), 2.22 (m, 1H), 2.52 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.49 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.5 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.5 Hz, 2-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.44 (d, 2H, *J* = 8.4 Hz, 3″-H and 5″-H), 7.77 (d, 2H, *J* = 8.4 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 29.7 (CH_2), 33.1 (CH_2), 37.3 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 100.0 (C-4′), 111.4 (C-2), 113.8 (C-4), 126.2 (C-1), 127.5 (C-1″), 128.1 (2C, C-2″ and C-6″), 129.2 (2C, C-3″ and C-5″), 132.3 (C-10), 136.0 (C-4″), 137.8 (C-5), 157.4 (C-3), 160.9 (C-3′), 178.0 (C-5′) ppm; ESI–MS: 465 $(M+H)^+$.

*2.2.10. Synthesis of 3-methoxy-17α-[3′-(4″-bromo)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6j**)*

According to Section 2.2, *N*-hydroxy-4-bromobenzenecarboximidoyl chloride (**4j**, 352 mg) was added to the mixture. After purification with CH_2Cl_2 as eluent, **6j** was obtained as a white solid (397 mg, 78%). Mp 98–100 °C; R_f = 0.53 (ss A). Anal. Calcd for $C_{28}H_{30}BrNO_3$: C, 66.14; H, 5.95; Found: C, 66.01; H, 5.77. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.90 (m, 1H), 1.06 (s, 3H, 18-H_3), 1.35–1.65 (m, 4H), 1.71–1.78 (m, 2H), 1.91–2.00 (m, 2H), 2.04–2.15 (m, 2H), 2.22 (m, 1H), 2.48 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 6.49 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.2 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.2 Hz, 2-H), 7.12 (d, 1H, *J* = 8.5 Hz, 1-H), 7.59 (d, 2H, *J* = 8.3 Hz, 2″-H and 6″-H), 7.70 (d, 2H, *J* = 8.3 Hz, 3″-H and 5″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 33.1 (CH_2), 37.4 (CH_2), 39.4 (CH), 43.2 (CH), 48.3 (C-13), 48.8 (CH), 55.2 (3-OMe), 83.8 (C-17), 100.0 (C-4′), 111.4 (C-2), 113.8 (C-4), 124.3 (C-4″), 126.2 (C-1), 127.9 (C-1″), 128.3 (2C, C-2″ and C-6″), 132.1 (2C, C-3″ and C-5″), 132.3 (C-10), 137.8 (C-5), 157.5 (C-3), 161.0 (C-3′), 178.1 (C-5′) ppm; ESI–MS: 509 $(M+H)^+$.

*2.2.11. Synthesis of 3-methoxy-17α-[3′-(4″-nitro)phenylisoxazol-5′-yl]estra-1,3,5(10)-trien-17β-ol (**6k**)*

According to Section 2.2, *N*-hydroxy-4-nitrobenzenecarboximidoyl chloride (**4k**, 300 mg) was added to the mixture. After purification with CH_2Cl_2 as eluent, **6k** was obtained as a yellow solid (299 mg, 63%). Mp 92–95 °C; R_f = 0.38 (ss A). Anal. Calcd for $C_{28}H_{30}N_2O_5$: C, 70.87; H, 6.37; Found: C, 71.06; H, 6.20. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (m, 1H), 1.07 (s, 3H, 18-H_3), 1.36–1.67 (m, 4H), 1.72–1.79 (m, 2H), 1.92–2.02 (m, 2H), 2.04–2.16 (m, 2H), 2.22 (m, 1H), 2.51 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OMe), 6.60 (s, 1H, 4′-H), 6.62 (d, 1H, *J* = 2.1 Hz, 4-H), 6.68 (dd, 1H, *J* = 8.5 Hz, *J* = 2.1 Hz, 2-H), 7.11 (d, 1H, *J* = 8.5 Hz, 1-H), 8.02 (d, 2H, *J* = 8.4 Hz, 2″-H and 6″-H), 8.33 (d, 2H, *J* = 8.4 Hz, 3″-H and 5″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-18), 23.6 (CH_2), 26.2 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 33.2 (CH_2), 37.4 (CH_2), 39.4 (CH), 43.2 (CH), 48.4 (C-13), 48.9 (CH), 55.2 (3-OMe), 83.9 (C-17), 100.2 (C-4′), 111.5 (C-2), 113.8 (C-4), 124.2 (2C, C-3″ and C-5″), 126.2 (C-1), 127.6 (2C, C-2″ and C-6″), 132.2 (C-10), 135.2 (C-1″), 137.8 (C-5), 148.7 (C-4″), 157.5 (C-3), 160.1 (C-3′), 178.9 (C-5′) ppm; ESI–MS: 475 $(M+H)^+$.

*2.3. Synthesis of 3-methoxy-17α-(3′-phenylisoxazol-5′-yl)estra-1,3,5(10)-trien-17β-ol (**6a**)*

Mestranol (**1**, 310 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL), and a solution of $CuSO_4{\cdot}5H_2O$ (12.5 mg, 0.05 mmol) and sodium ascorbate (30 mg, 0.15 mmol) in water (10 mL) was poured into the organic phase, and **4a** (233 mg, 1.5 mmol) was then added to the mixture under vigorous stirring. Finally, DIPEA (0.67 mL, 4.00 mmol) was added dropwise to the two-phase system, and the reaction mixture was stirred for 4 h at ambient temperature. After completion of the reaction (TLC monitoring), the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was washed with water (10 mL), dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography with CH_2Cl_2/EtOAc (98:2) to give **6a** as a white solid (395 mg, 92%).

*2.4. General procedure for the synthesis of $\Delta^{16,17}$-17-isoxazolyl derivatives (**7a–k**) in the estrone series*

The appropriate 17β-hydroxy-17α-isozaxole derivative (**6a–k**, 0.50 mmol) was dissolved in pyridine (10 mL), and $POCl_3$ (0.8 mL, 12 mmol) was added dropwise at 0 °C under vigorous stirring. The reaction mixture was allowed to warm to room temperature and after 24 h it was poured into a mixture of ice and concentrated HCl (20 mL), and extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with water (10 mL), followed by saturated $NaHCO_3$ solution (10 mL), then dried over Na_2SO_4 and evaporated *in vacuo*. The resulting crude product was purified by flash chromatography.

*2.4.1. Synthesis of 3-methoxy-17α-(3′-phenylisoxazol-5′-yl)estra-1,3,5(10),16-tetraene (**7a**)*

Compound **6a** (215 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (25:75 v/v) as eluent, **7a** was obtained as a yellow solid (183 mg, 89%). Mp 167−170 °C; R_f = 0.52 (ss C). Anal. Calcd for $C_{28}H_{29}NO_2$: C, 81.72; H, 7.10; Found: C, 81.93; H, 6.96. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.65–1.85 (m, 4H), 1.96 (m, 1H), 2.21 (m, 1H), 2.32–2.40 (m, 2H), 2.42–2.49 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.80 (s, 3H, 3-OMe), 6.52 (overlapping m, 2H, 4′-H and 16-H), 6.67 (d, 1H, *J* = 2.0 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.5 Hz, *J* = 2.0 Hz, 2-H), 7.23 (d, 1H, *J* = 8.5 Hz, 1-H), 7.47 (overlapping m, 3H, 3″-H, 4″-H and 5″-H), 7.84 (d, 2H, *J* = 7.2 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.7 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.9 (CH), 97.4 (C-4′), 111.4 (C-2), 113.8 (C-4), 126.0 (C-1), 126.7 (2C, C-2″ and C-6″), 128.8 (2C, C-3″ and C-5″), 129.3 (C-1″), 129.8

(C-4″) 132.5 (C-10), 133.0 (C-16), 137.8 (C-5), 141.9 (C-17), 157.5 (C-3), 162.4 (C-3′), 167.4 (C-5′) ppm; ESI–MS: 412 $(M+H)^+$.

*2.4.2. Synthesis of 3-methoxy-17α-[3′-(2″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7b**)*

Compound **6b** (222 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7b** was obtained as a white solid (196 mg, 92%). Mp 145–147 °C; R_f = 0.51 (ss C). Anal. Calcd for $C_{29}H_{31}NO_2$: C, 81.85; H, 7.34; Found: C, 81.98; H, 7.51. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.06 (s, 3H, 18-H_3), 1.52 (m, 1H), 1.64–1.86 (m, 4H), 1.97 (m, 1H), 2.22 (m, 1H), 2.32–2.38 (m, 2H), 2.41–2.48 (m, 2H), 2.52 (s, 3H, 2″-Me), 2.92 (m, 2H, 6-H_2), 3.80 (s, 3H, 3-OMe), 6.40 and 6.53 (bs, 2H, 4′-H and 16-H), 6.67 (d, 1H, *J* = 2.1 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.5 Hz, *J* = 2.1 Hz, 2-H), 7.23 (d, 1H, *J* = 8.5 Hz, 1-H), 7.27–7.36 (overlapping m, 3H, 3″-H, 4″-H and 5″-H), 7.54 (d, 1H, *J* = 7.2 Hz, 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 21.1 (2″-Me), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 56.0 (CH), 100.2 (C-4′), 111.4 (C-2), 113.8 (C-4), 125.9 (C-5″), 126.0 (C-1), 129.0 (C-1″), 129.3, 129.4, 131.0 (3C, C-3″, C-4″ and C-6″), 132.5 (C-10), 132.9 (C-16), 136.8 (C-2″), 137.8 (C-5), 141.9 (C-17), 157.5 (C-3), 163.0 (C-3′), 166.5 (C-5′) ppm; ESI–MS: 426 $(M+H)^+$.

*2.4.3. Synthesis of 3-methoxy-17α-[3′-(3″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7c**)*

Compound **6c** (222 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7c** was obtained as a white solid (192 mg, 90%). Mp 165–167 °C; R_f = 0.33 (ss D). Anal. Calcd for $C_{29}H_{31}NO_2$: C, 81.85; H, 7.34; Found: C, 81.70; H, 7.45. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 3H, 18-H_3), 1.51 (m, 1H), 1.65–1.85 (m, 4H), 1.97 (m, 1H), 2.21 (m, 1H), 2.32–2.39 (m, 2H), 2.42 (s, 3H, 3″-Me), 2.43–2.50 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.80 (s, 3H, 3-OMe), 6.51 (overlapping m, 2H, 4′-H and 16-H), 6.67 (d, 1H, *J* = 2.4 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.5 Hz, *J* = 2.4 Hz, 2-H), 7.23 (d, 1H, *J* = 8.5 Hz, 1-H), 7.27 (d, 1H, *J* = 7.8 Hz, 4″-H), 7.36 (t, 1H, *J* = 7.8 Hz, 5″-H), 7.63 (d, 1H, *J* = 7.8 Hz, 6″-H), 7.67 (s, 1H, 2″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 21.4 (3″-Me), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.9 (CH), 97.5 (C-4′), 111.4 (C-2), 113.8 (C-4), 123.9 (C-6″), 126.0 (C-1), 127.4 (C-2″), 128.7 (CH), 129.1 (C-1″), 130.6 (CH), 132.5 (C-10), 132.9 (C-16), 137.8 (C-5), 138.6 (C-3″), 141.9 (C-17), 157.5 (C-3), 162.5 (C-3′), 167.3 (C-5′) ppm; ESI–MS: 426 $(M+H)^+$.

*2.4.4. Synthesis of 3-methoxy-17α-[3′-(4″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7d**)*

Compound **6d** (222 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7d** was obtained as a yellow solid (187 mg, 88%). Mp 197–199 °C; R_f = 0.30 (ss D). Anal. Calcd for $C_{29}H_{31}NO_2$: C, 81.85; H, 7.34; Found: C, 82.01; H, 7.17. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.03 (s, 3H, 18-H_3), 1.49 (m, 1H), 1.62–1.84 (m, 4H), 1.95 (m, 1H), 2.18 (m, 1H), 2.31–2.38 (m, 2H), 2.39 (s, 3H, 4″-Me), 2.40–2.48 (m, 2H), 2.90–2.91 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OMe), 6.46–6.51 (overlapping m, 2H, 4′-H and 16-H), 6.66 (d, 1H, *J* = 2.0 Hz, 4-H), 6.73 (dd, 1H, *J* = 8.5 Hz, *J* = 2.0 Hz, 2-H), 7.21 (d, 1H, *J* = 8.5 Hz, 1-H), 7.26 (d, 2H, *J* = 8.0 Hz, 3″-H and 5″-H), 7.72 (d, 2H, *J* = 8.0 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 21.4 (4″-Me), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.9 (CH), 97.4 (C-4′), 111.5 (C-2), 113.8 (C-4), 126.0 (C-1), 126.4 (C-1″), 126.6 (2C, C-2″ and C-6″), 129.5 (2C, C-3″ and C-5″), 132.5 (C-10), 132.8 (C-16), 137.8 (C-5), 139.9 (C-4″), 141.9 (C-17), 157.5 (C-3), 162.3 (C-3′), 167.2 (C-5′) ppm; ESI–MS: 426 $(M+H)^+$.

*2.4.5. Synthesis of 3-methoxy-17α-[3′-(4″-methoxy)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7e**)*

Compound **6e** (230 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (20:80 v/v) as eluent, **7e** was obtained as a yellow solid (194 mg, 88%). Mp 171–174 °C; R_f = 0.26 (ss C). Anal. Calcd for $C_{29}H_{31}NO_3$: C, 78.88; H, 7.08; Found: C, 79.02; H, 7.29. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.63–1.84 (m, 4H), 1.96 (m, 1H), 2.20 (m, 1H), 2.31–2.39 (m, 2H), 2.40–2.48 (m, 2H), 2.91 (m, 2H, 6-H_2), 3.79 (s, 3H, 3-OMe), 3.86 (s, 3H, 4″-OMe), 6.46–6.50 (overlapping m, 2H, 4′-H and 16-H), 6.67 (d, 1H, *J* = 2.1 Hz, 4-H), 6.73 (dd, 1H, *J* = 8.5 Hz, *J* = 2.1 Hz, 2-H), 6.99 (d, 2H, *J* = 8.2 Hz, 3″-H and 5″-H), 7.22 (d, 1H, *J* = 8.5 Hz, 1-H), 7.78 (d, 2H, *J* = 8.2 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.7 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 and 55.3: 3-OMe and 4″-OMe, 55.9 (CH), 97.2 (C-4′), 111.4 (C-2), 113.8 (C-4), 114.2 (2C, C-3″ and C-5″), 121.8 (C-1″), 126.0 (C-1), 128.1 (2C, C-2″ and C-6″), 132.5 (C-10), 132.8 (C-16), 137.8 (C-5), 142.0 (C-17), 157.5 (C-3), 160.8 (C-4″), 162.0 (C-3′), 167.2 (C-5′) ppm; ESI–MS: 442 $(M+H)^+$.

*2.4.6. Synthesis of 3-methoxy-17α-[3′-(3″,4″-dimethoxy)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7f**)*

Compound **6f** (245 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with CH_2Cl_2/EtOAc (98:2 v/v) as eluent, **7f** was obtained as a white solid (207 mg, 87%). Mp 188–191 °C; R_f = 0.31 (ss C). Anal. Calcd for $C_{30}H_{33}NO_4$: C, 76.41; H, 7.05; Found: C, 76.27; H, 6.86. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.65–1.84 (m, 4H), 1.97 (m, 1H), 2.22 (m, 1H), 2.31–2.39 (m, 2H), 2.41–2.48 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.79 (s, 3H, 3-OMe), 3.94 (s, 3H) and 3.97 (s, 3H): 3″-OMe and 4″-OMe, 6.47 and 6.51 (bs, 2H, 4′-H and 16-H), 6.66 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 6.94 (d, 1H, *J* = 8.4 Hz, 5″-H), 7.22 (d, 1H, *J* = 8.5 Hz, 1-H), 7.33 (dd, 1H, *J* = 8.4 Hz, *J* = 1.6 Hz, 6″-H), 7.45 (d, 1H, *J* = 1.6 Hz, 2″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.8 and 55.9: 2 × OMe, 56.0 (CH), 97.3 (C-4′), 109.3 and 111.0 (2C, C-2″ and C-5″), 111.5 (C-2), 113.8 (C-4), 119.8 (C-6″), 122.0 (C-1″), 126.0 (C-1), 132.4 (C-10), 132.9 (C-16), 137.8 (C-5), 141.9 (C-17), 149.3 and 150.5 (2C, C-3″ and C-4″), 157.5 (C-3), 162.1 (C-3′), 167.3 (C-5′) ppm; ESI–MS: 472 $(M+H)^+$.

*2.4.7. Synthesis of 3-methoxy-17α-[3′-(3″,5″-dichloro-2″,4″,6″-trimethoxy)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7g**)*

Compound **6g** (294 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7g** was obtained as a white solid (260 mg, 91%). Mp 65–68 °C; R_f = 0.33 (ss C). Anal. Calcd for $C_{31}H_{33}Cl_2NO_5$: C, 65.26; H, 5.83; Found: C, 65.38; H, 5.95. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.51 (m, 1H), 1.64–1.86 (m, 4H), 1.97 (m, 1H), 2.21 (m, 1H), 2.28–2.37 (m, 2H), 2.40–2.50 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.76 (s, 6H, 2 × OMe), 3.79 (s, 3H, OMe), 3.97 (s, 3H, OMe), 6.42 and 6.54 (bs, 2H, 4′-H and 16-H), 6.66 (d, 1H, *J* = 2.6 Hz, 4-H), 6.73 (dd, 1H, *J* = 8.5 Hz, *J* = 2.6 Hz, 2-H), 7.22 (d, 1H, *J* = 8.5 Hz, 1-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.1 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 56.0 (CH), 60.9 (OMe), 62.1 (2 × OMe), 101.5 (C-4′), 111.5 (C-2), 113.9 (C-4), 117.2 (2C, C-3″ and C-5″), 120.2 (C-1″), 126.0 (C-1), 132.5 (C-10), 133.0 (C-16) 137.8 (C-5), 141.7

(C-17), 154.3 (2C, C-2″ and C-6″), 154.8 (C-3′), 156.1 (C-4″), 157.5 (C-3), 166.5 (C-5′) ppm; ESI–MS: 571 $(M+H)^+$.

*2.4.8. Synthesis of 3-methoxy-17α-[3′-(4″-fluoro)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7h**)*

Compound **6h** (224 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7h** was obtained as a white solid (191 mg, 89%). Mp 157−160 °C; R_f = 0.34 (ss D). Anal. Calcd for $C_{28}H_{28}FNO_2$: C, 78.30; H, 6.57; Found: C, 78.17; H, 6.73. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.51 (m, 1H), 1.65–1.85 (m, 4H), 1.97 (m, 1H), 2.21 (m, 1H), 2.32–2.40 (m, 2H), 2.42–2.49 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.80 (s, 3H, 3-OMe), 6.48 and 6.53 (bs, 2H, 4′-H and 16-H), 6.67 (d, 1H, J = 1.8 Hz, 4-H), 6.74 (dd, 1H, J = 8.5 Hz, J = 1.8 Hz, 2-H), 7.16 (t, 2H, J = 8.1 Hz, 3″-H and 5″-H) ppm, 7.23 (d, 1H, J = 8.5 Hz, 1-H), 7.83 (dd, 2H, J = 8.1 Hz, J = 5.5 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.9 (CH), 97.3 (C-4′), 111.4 (C-2), 113.8 (C-4), 116.0 (2C, J = 21.8 Hz, C-3″ and C-5″), 125.5 (C-1″, J = 3.1 Hz,), 126.1 (C-1), 128.7 (2C, J = 8.3 Hz, C-2″ and C-6″), 132.4 (C-10), 133.2 (C-16), 137.8 (C-5), 141.8 (C-17), 157.5 (C-3), 161.5 (C-3′), 163.7 (C-4″, J = 248.1 Hz), 167.6 (C-5′) ppm; ESI–MS: 430 $(M+H)^+$.

*2.4.9. Synthesis of 3-methoxy-17α-[3′-(4″-chloro)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7i**)*

Compound **6i** (232 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent, **7i** was obtained as a white solid (190 mg, 85%). Mp 179–182 °C; R_f = 0.37 (ss D Anal. Calcd for $C_{28}H_{28}ClNO_2$: C, 75.41; H, 6.33; Found: C, 75.64; H, 6.24. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.65−1.85 (m, 4H), 1.97 (m, 1H), 2.21 (m, 1H), 2.31−2.38 (m, 2H), 2.40–2.48 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.79 (s, 3H, 3-OMe), 6.48–6.53 (overlapping m, 2H, 4′-H and 16-H), 6.67 (d, 1H, J = 2.3 Hz, 4-H), 6.74 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz, 2-H), 7.22 (d, 1H, J = 8.5 Hz, 1-H), 7.44 (d, 2H, J = 8.4 Hz, 3″-H and 5″-H), 7.78 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 55.9 (CH), 97.3 (C-4′), 111.5 (C-2), 113.8 (C-4), 126.0 (C-1), 127.8 (C-1″), 128.0 (2C, C-2″ and C-6″), 129.1 (2C, C-3″ and C-5″), 132.4 (C-10), 133.3 (C-16), 135.8 (C-4″), 137.8 (C-5), 141.8 (C-17), 157.5 (C-3), 161.4 (C-3′), 167.7 (C-5′) ppm; ESI–MS: 447 $(M+H)^+$.

*2.4.10. Synthesis of 3-methoxy-17α-[3′-(4″-bromo)phenylisoxazol-5′-yl]-estra-1,3,5(10),16-tetraene (**7j**)*

Compound **6j** (254 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with *n*-hexane/CH_2Cl_2 (20:80 v/v) as eluent, **7j** was obtained as a white solid (221 mg, 90%). Mp 187–190 °C; R_f = 0.56 (ss C). Anal. Calcd for $C_{28}H_{28}BrNO_2$: C, 68.57; H, 5.75; Found: C, 68.75; H, 5.93. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.64–1.84 (m, 4H), 1.96 (m, 1H), 2.21 (m, 1H), 2.31–2.38 (m, 2H), 2.41–2.48 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.79 (s, 3H, 3-OMe), 6.48 and 6.53 (bs, 2H, 4′-H and 16-H), 6.66 (d, 1H, J = 2.2 Hz, 4-H), 6.74 (dd, 1H, J = 8.5 Hz, J = 2.2 Hz, 2-H), 7.22 (d, 1H, J = 8.5 Hz, 1-H), 7.60 (d, 2H, J = 8.3 Hz, 2″-H and 6″-H), 7.71 (d, 2H, J = 8.3 Hz, 3″-H and 5″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 56.0 (CH), 97.3 (C-4′), 111.5 (C-2), 113.8 (C-4), 124.1 (C-4″), 126.0 (C-1), 128.3 (2C, C-2″ and C-6″), 132.1 (2C, C-3″ and C-5″), 132.5 (C-10), 133.4 (C-16), 137.8 (C-5), 141.8 (C-17), 157.5 (C-3), 161.5 (C-3′), 167.7 (C-5′) ppm; ESI–MS: 491 $(M+H)^+$.

*2.4.11. Synthesis of 3-methoxy-17α-[3′-(4″-nitro)phenylisoxazol-5′-yl]-estra-1,3,5(10),16-tetraene (**7k**)*

Compound **6k** (237 mg) was used for the synthesis as described in Section 2.4. After purification by column chromatography with CH_2Cl_2 as eluent, **7j** was obtained as a pale-yellow solid (199 mg, 87%). Mp 196−198 °C; R_f = 0.52 (ss C). Anal. Calcd for $C_{28}H_{28}N_2O_4$: C, 73.66; H, 6.18; Found: C, 73.82; H, 5.98. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 3H, 18-H_3), 1.50 (m, 1H), 1.66–1.85 (m, 4H), 1.97 (m, 1H), 2.22 (m, 1H), 2.32–2.40 (m, 2H), 2.42–2.50 (m, 2H), 2.92 (m, 2H, 6-H_2), 3.79 (s, 3H, 3-OMe), 6.58 (overlapping m, 2H, 4′-H and 16-H), 6.66 (d, 1H, J = 2.6 Hz, 4-H), 6.74 (dd, 1H, J = 8.5 Hz, J = 2.6 Hz, 2-H), 7.22 (d, 1H, J = 8.5 Hz, 1-H), 8.02 (d, 2H, J = 8.7 Hz, 2″-H and 6″-H), 8.33 (d, 2H, J = 8.7 Hz, 3″-H and 5″-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.5 (C-18), 26.5 (CH_2), 27.7 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 35.2 (CH_2), 37.1 (CH), 44.1 (CH), 47.1 (C-13), 55.2 (3-OMe), 56.0 (CH), 97.4 (C-4′), 111.5 (C-2), 113.8 (C-4), 124.1 (2C, C-3″ and C-5″), 126.0 (C-1), 127.6 (2C, C-2″ and C-6″), 132.3 (C-10), 134.1 (C-16), 135.4 (C-1″), 137.8 (C-5), 141.5 (C-17), 148.6 (C-4″), 157.5 (C-3), 160.6 (C-3′), 168.4 (C-5′) ppm; ESI–MS: 457 $(M+H)^+$.

*2.5. Synthesis of 3-methoxy-17-ethynylestra-1,3,5(10),16-tetraene (**8**)*

Compound **1** (310 mg, 1.00 mmol) was dissolved in pyridine (20 mL), and $POCl_3$ (1.6 mL, 24 mmol) was added dropwise at 0 °C under vigorous stirring. The reaction mixture was allowed to warm to room temperature and after 24 h it was poured into a mixture of ice and HCl, and extracted with EtOAc (2 × 15 mL). The combined organic phases were washed with water (15 mL), followed by saturated $NaHCO_3$ solution (15 mL), then dried over Na_2SO_4 and evaporated *in vacuo*. The resulting crude product was purified by flash chromatography with CH_2Cl_2 as eluent to give **8** as a pale-yellow solid (257 mg, 88%).

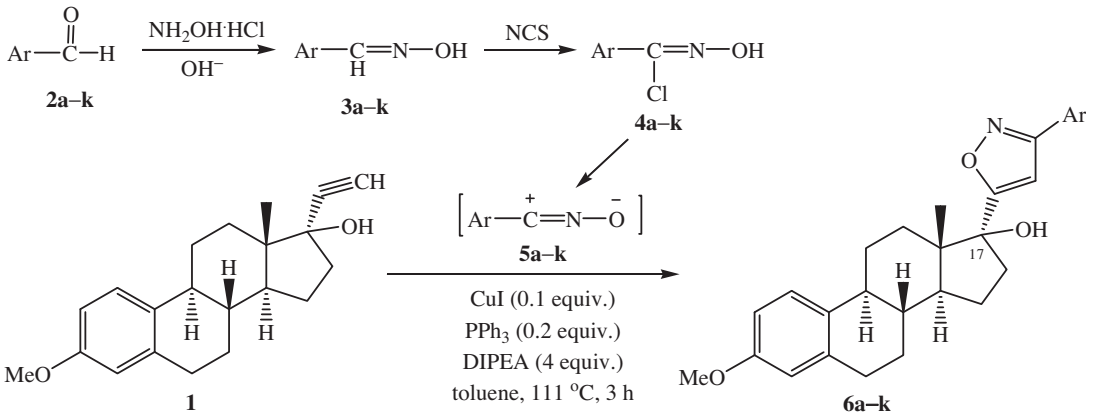
*2.6. Synthesis of 3-methoxy-17α-[3′-(2″-methyl)phenylisoxazol-5′-yl]estra-1,3,5(10),16-tetraene (**7b**) from 3-methoxy-17-ethynylestra-1,3,5(10),16-tetraene (**8**)*

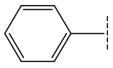
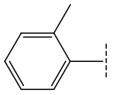
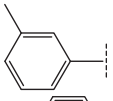
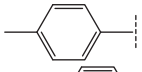
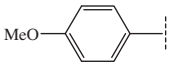
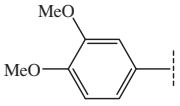
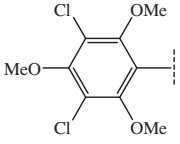
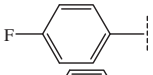
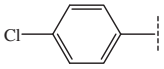
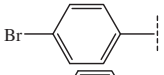
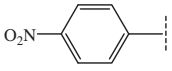
3-Methoxy-17-ethynylestra-1,3,5(10),16-tetraene (**8**, 250 mg, 0.85 mmol) and *N*-hydroxy-2-methylbenzenecarboximidoyl chloride (**4b**, 288 mg, 1.70 mmol) were dissolved in toluene (10 mL), and Ph_3P (44 mg, 0.17 mmol) and CuI (17 mg, 0.09 mmol) were then added to the solution. DIPEA (0.57 ml, 3.40 mmol) was added dropwise to the gently heated reaction mixture, which was subsequently heated to reflux under stirring for 4 h. After the disappearance of the starting material (TLC monitoring), the solvent was evaporated off *in vacuo*. The resulting crude product was purified by flash chromatography with *n*-hexane/CH_2Cl_2 (50:50 v/v) as eluent to give **7b** as a white solid (137 mg, 38%).

2.7. Determination of antiproliferative activities

Antiproliferative effects were measured *in vitro* on three human cancer cell lines (ECACC; Salisbury, UK): HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A2780 (ovarian carcinoma). The cells were cultivated in minimal essential medium (Sigma–Aldrich, Budapest, Hungary) supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. Near-confluent cells were seeded into a 96-well plate (5000 cells/well) and, after overnight standing, the medium (200 μL) containing the tested compound (at 10 or 30 μM) was added. Following a 72-h incubation in a humidified atmosphere of 5% CO_2 at 37 °C, the living cells were assayed by the addition of 20 μL of 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution [30]. During a 4-h contact period, the MTT was converted by intact mitochondrial reductase and

Table 1
Synthesis of steroidal isoxazoles by Cu(I)-catalyzed cycloaddition.



Entry	Imidoyl chloride/nitrile oxide	Ar	Product	Yield[a] (%)
1	**4a**/**5a**		**6a**	93
2	**4b**/**5b**		**6b**	98
3	**4c**/**5c**		**6c**	96
4	**4d**/**5d**		**6d**	97
5	**4e**/**5e**		**6e**	97
6	**4f**/**5f**		**6f**	95
7	**4g**[b]/**5g**		**6g**	98
8	**4h**/**5h**		**6h**	81
9	**5i**/**5i**		**6i**	81
10	**5j**/**5j**		**6j**	78
11	**5k**/**5k**		**6k**	63

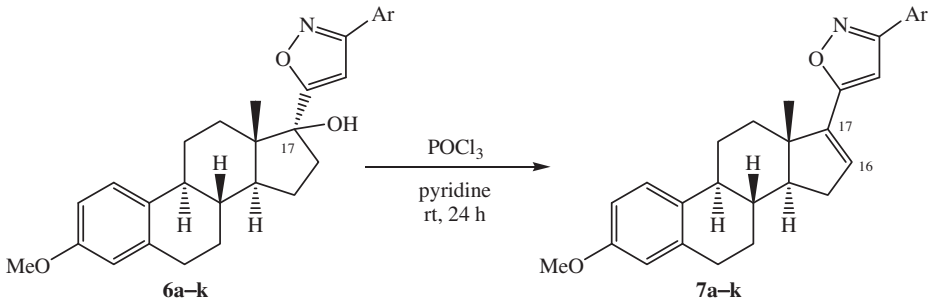
[a] After purification by column chromatography.
[b] During treatment of 2,4,6-trimethoxybenzaldehyde oxime (**3g**) with NCS, *bis*-chlorination of the aromatic ring also occurred.

precipitated as blue crystals. The medium was then removed, the precipitated formazan crystals were solubilized in DMSO (100 μL) during a 60-min period of shaking at 25 °C, and the absorbance was read at 545 nm with a microplate reader. Wells with untreated cells were utilized as controls. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Stock solutions of the tested substances (10 mM) were prepared with DMSO. The DMSO concentration (0.3%) of the medium did not have any significant effect on cell proliferation. Cisplatin was used as reference compound.

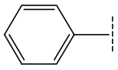
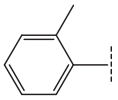
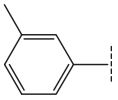
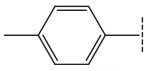
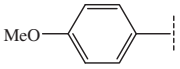
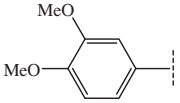
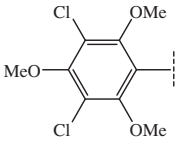
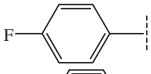
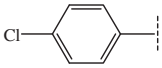
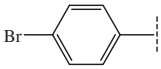
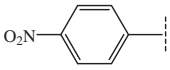
3. Results and discussion

For the preparation of novel steroidal isoxazoles, mestranol **1**, containing an α-ethynyl group on C-17 of the sterane skeleton, was used as starting material. Preliminary Cu(I)-catalyzed ring-closure experiments on **1** with benzonitrile oxide **5a** were first carried out in order to optimize the reaction conditions (Table 1, entry 1). The stable precursor **4a** of the nitrile oxide dipole (**5a**) was synthetized in a two-step pathway by condensation of benzaldehyde **2a** with hydroxylamine hydrochloride in alkaline medium and

Table 2
Synthesis of $\Delta^{16,17}$ 17-*exo*-isoxazolyl derivatives.



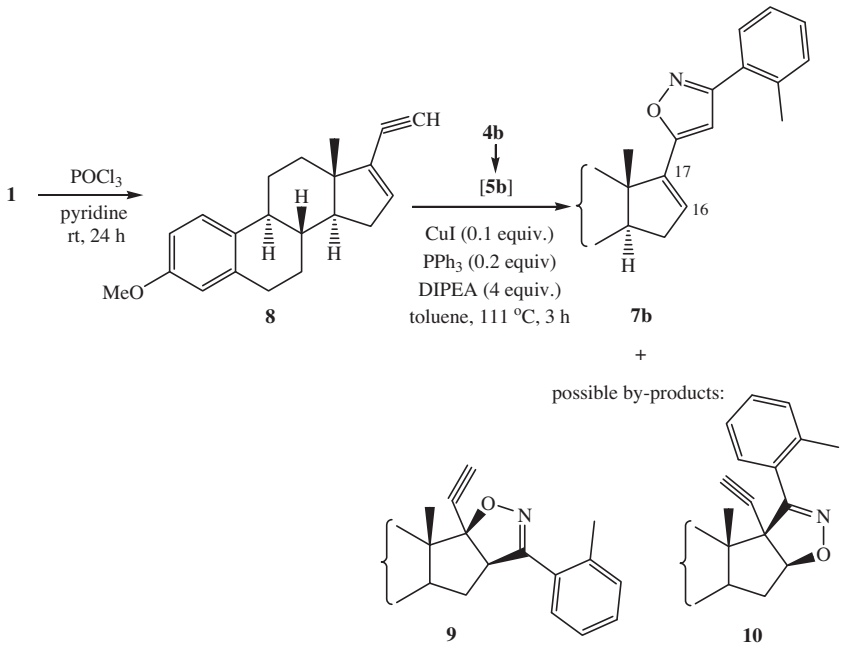
6a–k **7a–k**

Entry	Substrate	Ar	Product	Yield[a] (%)
1	**6a**		**7a**	89
2	**6b**		**7b**	92
3	**6c**		**7c**	90
4	**6d**		**7d**	88
5	**6e**		**7e**	88
6	**6f**		**7f**	87
7	**6g**		**7g**	91
8	**6h**		**7h**	89
9	**6i**		**7i**	85
10	**6j**		**7j**	90
11	**6k**		**7k**	87

[a] After purification by column chromatography.

subsequent chlorination of the aldoxime **3a** with *N*-chlorosuccinimide (NCS) [31]. Benzonitrile oxide **5a** can be generated *in situ* from **4a** by dehydrochlorination with a base in the presence of the dipolarophile **1**. A similar intermolecular ring-closure reaction of 17α-ethynylestradiol (the 3-demethylated derivative of mestranol) with 4-methoxybenzonitrile oxide **5e** in a 1:1 (v/v) mixture of H_2O/*t*BuOH, using $CuSO_4{\cdot}5H_2O$/Na-ascorbate as Cu(I) source, was reported previously by Sharpless et al. [25]. A single isoxazole regioisomer was obtained in 98% yield after 1 h at ambient temperature on the application of $KHCO_3$ as base to release the 1,3-dipole (**5e**) from its precursor (**4e**). However, mestranol **1** contains a methoxy instead of a hydroxy group at C-3 and is therefore less polar and cannot be dissolved in the above solvent system. Accordingly, the reaction conditions needed modification in order to avoid the solubility problems.

During optimization of the Cu(I)-catalyzed cycloaddition of **1** with benzonitrile oxide (**5a**), the best conversion was found to occur on the use of a catalytic amount of CuI as Cu(I) source, PPh_3 as stabilizing ligand, and excess DIPEA as base in refluxing toluene. The presence of Ph_3P is important to stabilize the catalytically active Cu(I) by complexation so as to prevent its oxidation to Cu(II) [32,33]. Furthermore, the solubility of Cu(I) in the organic medium is also improved. The dual function of DIPEA is to facilitate the formation of Cu(I)-acetylide in sufficient amount and the *in situ* generation of the 1,3-dipole (**5a**) from its relatively stable precursor (**4a**). Since dimerization of unhindered nitrile oxides to furoxanes can reduce their proportion available for cycloaddition [34], the amine base was added to the reaction mixture terminally in order to ensure a low stationary concentration of the dipole and to minimize the undesired side-reaction. Under the applied



Scheme 1. Dehydration of mestranol (**1**) and the following Cu(I)-catalyzed cycloaddition with 2-methylbenzonitrile oxide (**5b**). The possible by-products (**9** and **10**) were not isolated.

Table 3
Antiproliferative effects of the synthetized compounds.

Isoxazole	Growth inhibition % ± (SEM)					
	HeLa		MCF7		A2780	
	10 μM	30 μM	10 μM	30 μM	10 μM	30 μM
6a	<25[a]	98 (0.1)	28 (1.7)	95 (0.2)	29 (2.1)	98 (0.2)
6b	<25	96 (0.7)	<25	92 (1.2)	<25	94 (0.3)
6c	<25	90 (0.5)	<25	94 (0.4)	29 (2.0)	91 (0.4)
6d	<25	91 (0.3)	33 (2.4)	91 (0.4)	28 (1.9)	81 (2.5)
6e	<25	92 (0.2)	54 (1.1)	91 (0.5)	32 (1.9)	78 (0.3)
6f	<25	37 (2.4)	35 (2.6)	92 (0.3)	43 (2.7)	71 (0.4)
6g	<25	70 (1.8)	<25	72 (1.0)	49 (1.4)	78 (0.7)
6h	<25	96 (0.4)	48 (1.3)	96 (0.8)	40 (2.3)	98 (0.4)
6i	<25	98 (0.1)	35 (1.9)	95 (0.5)	29 (1.5)	97 (0.2)
6j	<25	94 (0.5)	25 (1.9)	92 (0.2)	28 (2.7)	84 (0.3)
6k	28 (1.1)	95 (0.4)	43 (1.0)	91 (0.6)	41 (1.5)	96 (0.2)
7a	32 (2.1)	59 (2.0)	<25	48 (1.8)	29 (1.7)	45 (2.3)
7b	<25	35 (2.4)	<25	<25	<25	38 (1.8)
7c	<25	59 (0.7)	<25	26 (1.2)	<25	38 (1.3)
7d	35 (0.6)	59 (0.6)	27 (1.3)	50 (0.3)	<25	45 (1.5)
7f	58 (0.8)	58 (0.9)	36 (2.2)	57 (2.9)	43 (2.9)	65 (1.2)
7g	31 (1.8)	43 (2.4)	<25	<25	36 (2.5)	39 (1.4)
7h	25 (0.8)	58 (0.9)	<25	30 (2.9)	<25	33 (2.5)
7i	32 (1.2)	57 (1.2)	<25	34 (1.7)	<25	40 (2.0)
7j	37 (1.4)	65 (1.3)	<25	41 (2.7)	<25	43 (1.7)
7k	68 (0.7)	78 (0.3)	63 (1.8)	68 (1.1)	46 (2.5)	79 (0.9)
Cisplatin	43 (2.3)	100 (0.3)	53 (2.3)	87 (1.2)	84 (1.2)	95 (0.3)

[a] Compounds eliciting less than 25% inhibition of proliferation were considered ineffective and the exact results are not given, for simplicity.

conditions, the reaction was complete within 3 h and the corresponding isoxazole **6a** was obtained in a yield of 93% after chromatographic purification. The conversion, however, was observed to be significantly lower (by *ca.* 20%) when DIPEA was added to the reaction mixture in an early stage, due to the greater extent of dipole dimerization.

After determination of the optimal reaction parameters, similar cycloadditions of **1** with different substituted benzonitrile oxides (**5b–k**), synthetized from the corresponding aryl aldehydes (**2b–k**) by the well-known sequence mentioned above, were performed to furnish novel 17α-isoxazoles (**6b–k**) in good to excellent yields (Table 1, entries 2–11). During the reactions of oximes (**3b–k**) with NCS, *bis*-chlorination of the aromatic ring was observed for the 2,4,6-trimethoxybenzaldehyde oxime **3g**, which is not surprising in view of the additive *ortho*-directing effects of the electron-donating methoxy-substituents. Although NMR characterizations of the imidoyl chlorides (**4a–k**) were not carried out, and their formation was monitored only by TLC, the 3′,5′-dichloro substitution of product **6g** was confirmed by NMR and MS methods. Otherwise, both the oximes (**3a–k**) and their halogenated derivatives (**4a–k**) appeared as double spots on the TLC plates, which indicated their formation as mixtures of *E* and *Z* isomers. However, the imidoyl chlorides (**4a–k**) were used directly after preparation without further purification. The yields of the cycloadducts **6b–k** were found to depend on the electronic and steric features of the substituents on the aromatic ring of the dipole **5b–k**. The electron-donating substituents in **5b–g** (Table 1, entries 2–7) favored cycloaddition to **1**, in consequence of the lower tendency of these dipoles to undergo dimerization to furoxanes, while the yields of the desired products (**6h–k**) were decreased in the case of the electron-withdrawing groups on the aromatic moiety in **5h–k** (entries 8–11). The lowest conversion was achieved for the reaction of **1** with *p*-nitrobenzonitrile oxide **5k**, owing to its shortest lifetime as a monomer.

Although novel isoxazoles (**6a–k**) were prepared in high yields in the presence of CuI as catalyst under the optimized conditions above, the reactions can also be performed at room temperature by using $CuSO_4{\cdot}5H_2O$/Na-ascorbate as Cu(I) source. The reaction of **1** with **5a** in a mixture of CH_2Cl_2 as solvent and water as co-solvent resulted in the corresponding product **6a** in 92% yield after purification. Consequently, the two kinds of methods were found to work similarly.

E2-type dehydration of **6a–k** was carried out with $POCl_3$ in pyridine, to furnish $\Delta^{16,17}$ 17-isoxazolyl derivatives **7a–k** (Table 2). The transformations were not accompanied by side-reactions (*e.g.* chlorination or Wagner–Meerwein rearrangements [35]) under the applied conditions, and after separation by column chromatography the corresponding products (**7a–k**) were obtained in yields of 85–92%.

The preparation of 17-isoxazole **7b** was also investigated in a reverse synthetic sequence. Mestranol **1** was first dehydrated to 3-methoxy-17-ethynylestra-1,3,5(10),16-tetraene **8**, and this was followed by Cu(I)-catalyzed cycloaddition to 2-methylbenzonitrile oxide **5b**, generated *in situ* from **4b** by using DIPEA (Scheme 1). The effectiveness of the initial elimination reaction was found to be quite similar to those of the preparations of **6a–k**, and **8** was obtained in 88% yield. However, the step of cycloaddition of **8** with **5b** appeared to be less chemoselective since the formation of by-products accompanying **7b** was detected by TLC, this eventually decreasing the isolated yield of **7b** from 98% to 38%. The observed side-reactions can be explained by the structural character of **8**, which contains both C=C and C≡C bonds, which display similar reactivities toward cycloaddition with nitrile oxides. The 1,3-cycloaddition of the dipole **5b** to the olephinic moiety, affording isoxazoline regioisomers (**9** and **10**), can therefore compete with the alkyne–dipole reaction and reduce the yield of the desired product **7b**. Accordingly, the 1,3-dipolar cycloaddition of mestranol **1** with nitrile oxides followed by dehydration was demonstrated to be a more efficient pathway for the preparation of **7a–k** than the latter method, with its low selectivity.

The structures of the synthetized compounds (**6a–k** and **7a–k**) were determined by NMR and MS measurements. In the ^{1}H NMR spectra of **6a–k**, the signals of the protons on the aromatic moiety introduced by the nitrile oxides (**5a–k**) appeared at 7.1–8.3 ppm, with appropriate multiplicities; an exception was **6g**, which contains a fully-substituted ring. The ^{1}H and ^{13}C NMR spectra indicated the 4′-H singlet of the newly formed hetero ring at 6.5 ppm, while the signal of the quaternary C-5′ was identified at around 178 ppm. The ^{1}H NMR spectra of the unsaturated derivatives **7a–k** confirmed the presence of the double bond between C-16 and C-17 since the multiplets of 16-H_2 had disappeared as compared with **6a–k**, and the signal of 16-H was at 6.5 ppm, together with the peak of 4′-H. The elimination of water and hence the development of conjugation with the isoxazole ring were also proved by ^{13}C NMR; the signals of C-16 and C-17 appeared at around 133 and 142 ppm, respectively.

The novel isoxazolyl derivatives (**6a–k** and **7a–k**) were applied in *in vitro* pharmacological studies in order to investigate their antiproliferative effects on three malignant human gynecological cell lines (Table 3). The cell growth-inhibitory potencies of the dehydrated analogs (**7a–k**) were generally found to be lower than those of their 17β-hydroxy counterparts (**6a–k**). Hence, the unsaturated isoxazoles (**7a–k**) may be considered to be practically ineffective; among the hydroxylated compounds, methylation of the aromatic ring (**6b–d**) did not cause relevant changes in the biological effects as compared with the unsubstituted derivative **6a**. The *p*-methoxy analog **6e** exerted slightly more pronounced antiproliferative action, while polysubstitution (**6f** and **6g**) was less favorable. The halogenated compounds (**6h–6j**) displayed the F > Cl > Br order of potency. The fluorinated analog (**6h**) and the *p*-nitro-substituted derivative (**6k**) proved to be the most promising compounds in the currently presented set.

The results demonstrate that the isoxazolyl moiety on the sterane framework deserves attention from a pharmacological aspect, motivating the search for further derivatives and their optimization for better activities.

4. Conclusions

In view of the lack of structural characteristics of estrogenic steroids contributing to their binding to the corresponding hormone receptors, the major aim of the present work was to synthetize novel steroidal 17-isoxazoles in order to investigate their cytostatic activities. The 17β-hydroxy-17α-isoxazolyl steroids in the estrone series were prepared efficiently via Cu(I)-catalyzed regioselective cycloadditions of different aryl nitrile oxides to mestranol. The transformations were carried out under two kinds of conditions, using different additives to furnish the desired products with similar high yields. The ring closures were affected by the substitution pattern of the nitrile oxide, the highest conversions being achieved with sterically hindered dipoles containing electron-donating substituents. Efficient dehydrations of the 17β-hydroxy derivatives led to $\Delta^{16,17}$ steroidal isoxazoles. All compounds were tested *in vitro* as concerns their antiproliferative activities on three malignant cell lines, and some hydroxylated analogs proved to exert promising cell growth-inhibitory effects. Although the antiproliferative activities of the tested compounds are moderate, the results suggest that steroidal isoxazoles may induce a disturbance in the cell division by a mode other than hormone receptor-based action, motivating the design of further anticancer lead candidates and optimization for better activities.

Acknowledgments

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