Intramolecular approach to some new D-ring-fused steroidal isoxazolidines by 1,3-dipolar cycloaddition: synthesis, theoretical and *in vitro* pharmacological studies

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Intramolecular 1,3-dipolar cycloaddition of alkenyl oxime **5**, obtained from *trans*-3β-acetoxy-16,17-secopregna-5,17(20)-dien-16-al **2** with hydroxylamine hydrochloride, was carried out under reflux in toluene to furnish a *ca.* 3 : 2 mixture of D-ring-fused isoxazolidine diastereomers **8** and **11a** both with *cis* D/E ring junction stereochemistry. The corresponding reaction of **5** in the presence of a catalytic amount of $BF_3{\cdot}OEt_2$ gave a single isomer **8** under milder conditions with an improved yield. The experimental findings on the thermally induced and BF_3-catalysed transformations were supported by calculations of the proposed mechanism at the BLYP/6-31G(d) level of theory. Analogously, cyclization of D-secopregnene aldehyde **2** with *N*-substituted hydroxylamine derivatives (**10b–e**) under thermal conditions furnished *N*-functionalized isoxazolidines **11b–e** diastereoselectively, *via* the corresponding alkenyl nitrones **7b–e**. The activities of the 3-deacetylated compounds (**9**, **12b–e**) were tested *in vitro* on rat testicular $C_{17,20}$-lyase: the radioligand incubation assay revealed that **9** exerted a moderate enzyme-inhibitory effect (IC_{50} = 26 μM), while the other derivatives were found to decrease the enzyme activity by only 68–83%. The antiproliferative activities of the structurally related isoxazolidine derivatives were also determined *in vitro* on three malignant human cell lines (HeLa, MCF7, and A431) by the microculture tetrazolium assay. The highest cytotoxic activities were displayed by the *N*-benzyl-substituted derivative **12e** (IC_{50}: 14.00, 23.18 and 8.76 μM on HeLa, MCF7 and A341 cells, respectively).

1. Introduction

The 1,3-dipolar cycloadditions of nitrones with alkenes leading to isoxazolidine derivatives have been extensively studied and have found general application in organic synthesis.[1] In particular, the intramolecular reactions are of potential in the construction of heterocyclic frameworks, in consequence of their advantages of higher degrees of regio- and diastereoselectivity due to entropy factors and limited conformational mobility in the transition state, in contrast with the intermolecular version.[2] Most of the reactions are carried out under thermal conditions by heating the reactants in an inert solvent,[3] though several examples of Lewis acid-catalysed cyclizations[4] are also to be found in the literature. Thermally induced cycloadditions are believed to follow a concerted, but non-synchronous mechanism, *i.e.* C–C bond formation precedes C–O bond formation. The presence of a Lewis acid further enhances the asynchronous character of the process, and the nitrone and the alkene therefore probably behave more as an electrophile and a nucleophile than as a dipole/dipolarophile pair.[5] Direct ring closure of alkenyl oximes affords another possibility for the intramolecular synthesis of isoxazolidines. The thermal reaction presumably occurs through formation of the intermediate N–H nitrone from the oxime by a 1,2-prototropic shift. Grigg *et al.* postulated that, while the tautomerization between an oxime and a N–H nitrone is facile, dipolar cycloadditions of these types of nitrones, especially with non-activated alkene dipolarophiles, are relatively rare;[6] only a few examples have been reported.[7] In contrast with the thermal version, the mechanism of the Lewis acid-promoted reactions of oximes to isoxazolidines[8] has been less well studied.[9] The importance of isoxazolidines arises from their utility as precursors in the syntheses of 1,3-aminoalcohols, which are excellent starting materials for a wide variety of natural products and related compounds, such as alkaloids and nucleoside antibiotics.[10]

The intensive research on steroidal compounds has focused in recent years on the development of novel, potentially bioactive heterocyclic molecules.[11] Interestingly, only a few derivatives containing an isoxazolidine moiety (which may be of importance from a pharmacological aspect) have been described so far.[12] The thermally induced intramolecular 1,3-dipolar cycloadditions of steroidal (*Z*)- and (*E*)-1,10-unsaturated

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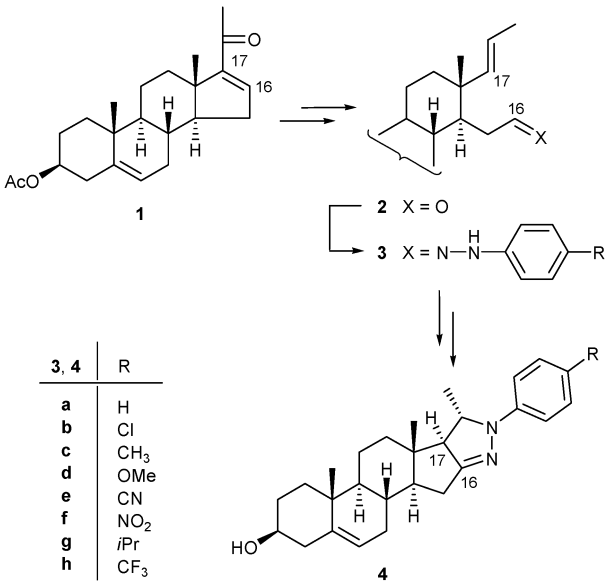


Fig. 1 Some androstene-fused arylpyrazolines (**4a–h**) synthesized earlier from D-secoaldehyde **2**.

5,10-seco-5-ketones with *N*-methylhydroxylamine hydrochloride yielded some novel bridged *N*-methyl isoxazolidines,[13] and the Lewis acid-promoted synthesis of an isoxazolidine condensed to ring D of the estrane skeleton by internal cycloaddition was reported earlier.[8]

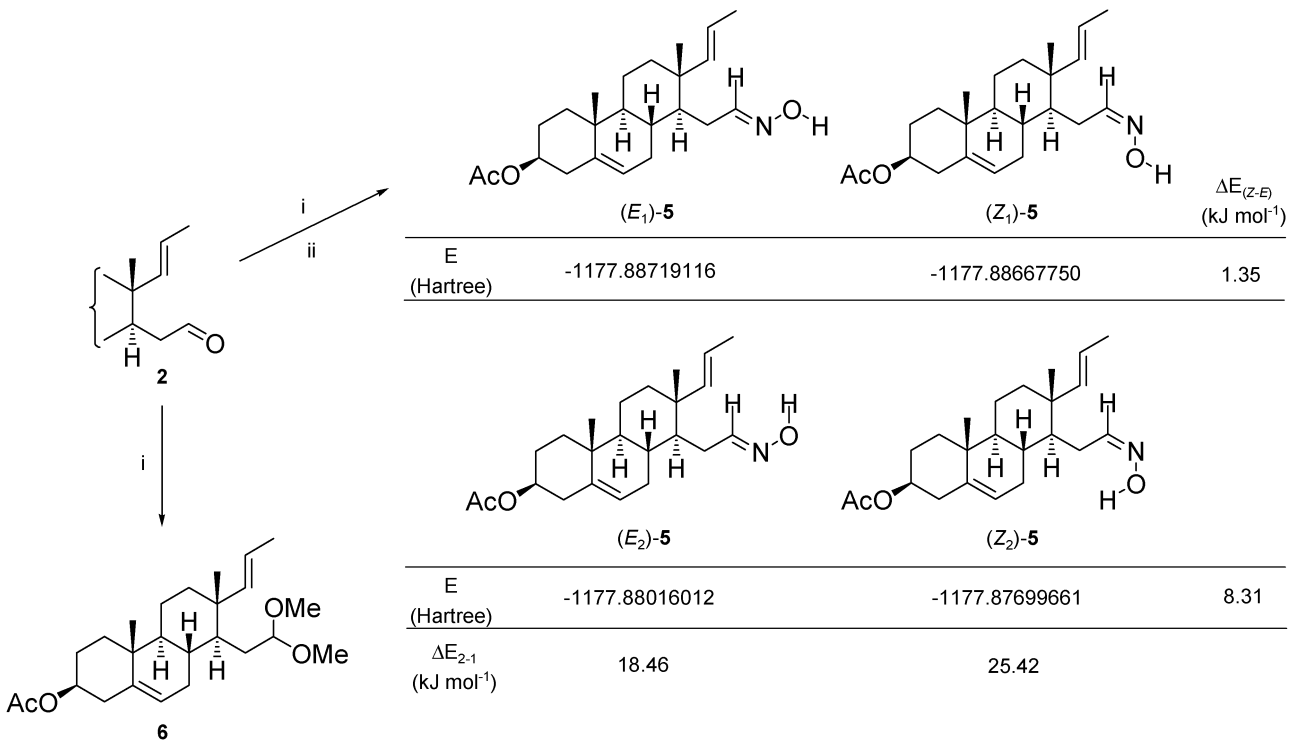
We describe here the synthesis of a series of novel D-ring-fused isoxazolidinoandrost-5-ene derivatives by [3 + 2] cycloaddition of olefinic nitrones formed *in situ* from a D-secopregnene aldoxime and its aldehyde precursor. The reaction mechanisms proposed for the thermally initiated and $BF{\cdot}OEt_2$-induced direct ring closure of the aldoxime were subjected to theoretical analysis by DFT (B3LYP) calculations with the intention of seeking support for the experimental findings. Moreover, the synthesized compounds were applied in *in vitro* pharmacological studies to investigate their inhibitory effects on rat testicular $C_{17,20}$-lyase and their antiproliferative activities on three malignant human cell lines.

2. Results and discussion

2.1 Synthetic studies

During preliminary experiments, D-secopregnene aldehyde **2** was synthesized from the commercially available pregnadienolone acetate **1**[14] (Fig. 1). The presence of the formyl group and the unsaturated side-chain makes the molecule an excellent precursor for condensation and subsequent cyclization reactions to give heteroatom-containing derivatives *via* intramolecular sequences. Thus, the synthesis of D-ring-fused arylpyrazolines **4** by internal Lewis acid-induced 1,3-dipolar cycloaddition of phenylhydrazones **3**, derived from **2**, was recently reported.[15]

Our present goal was to obtain five-membered *N*,*O*- instead of *N*,*N*-heterocycles condensed to ring D of the sterane skeleton. Therefore, compound **2** was first reacted with hydroxylamine hydrochloride in the presence of NaOAc to furnish the corresponding aldoxime **5** as a mixture of (*E*) and (*Z*) isomers (Scheme 1). The output of the condensation reaction was observed to depend appreciably on the solvent applied: the use of methanol diminished the yield of the desired product **5**,



	(E_1)-**5**	(Z_1)-**5**	$\Delta E_{(Z\text{-}E)}$ (kJ mol^{-1})
E (Hartree)	-1177.88719116	-1177.88667750	1.35
	(E_2)-**5**	(Z_2)-**5**	
E (Hartree)	-1177.88016012	-1177.87699661	8.31
$\Delta E_{2\text{-}1}$ (kJ mol^{-1})	18.46	25.42	

Scheme 1 *Reagents and conditions*: (i) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, 65 °C, 6 h; (ii) $NH_2OH{\cdot}HCl$, NaOAc, *i*PrOH, 50 °C, 20 min. Computed absolute energies (in Hartree) and relative energies (kJ mol^{-1}) of the possible (*E*) and (*Z*) isomers of alkenyl oxime **5** at the BLYP/6-31G(d) level of theory.

and dimethylacetate **6** was formed as a minor side-product, while when methanol was replaced by isopropyl alcohol, a high conversion was achieved, with the formation of **5** alone in 96% yield within a shorter time. Although acetal formation is somewhat unusual in alkaline media, it is not unknown.[16] The larger size of the isopropyl group relative to the methyl group can preclude the solvent-induced side-reaction leading to the acetal **6**. The ^{1}H NMR spectra of the crude product **5** revealed a 3 : 4 mixture of two geometric isomers,[17] which did not undergo interconversion in solution[18] and could therefore be separated by column chromatography. Theoretically, the existence of two kinds of (*E*) and (*Z*) stereostructures must be considered for **5**, the computed energies of which are given in Scheme 1. Configurational analysis indicates that (E_1)-**5** and (Z_1)-**5** are energetically more favourable than (E_2)-**5** and (Z_2)-**5**. Moreover, the (E_1) isomer is 1.35 kJ mol^{-1} more stable than the corresponding (Z_1) isomer.

To determine the configurations of the products arising from the condensation, the chemical shifts of the 16-H signals of the related stereomers were compared with those calculated at the BLYP/6-31G(d) level of theory (Table 1). The chemical shifts computed for (E_1)-**5** and (Z_1)-**5** were in good agreement with the experimental data. The major formation of the (Z_1)-**5** isomer during the condensation, which seems obvious from the ^{1}H NMR spectrum of the isomeric mixture, may be attributed not only to thermodynamic, but also to kinetic control of the process. The crude oxime **5** was used for the following step without separation of the individual isomers,

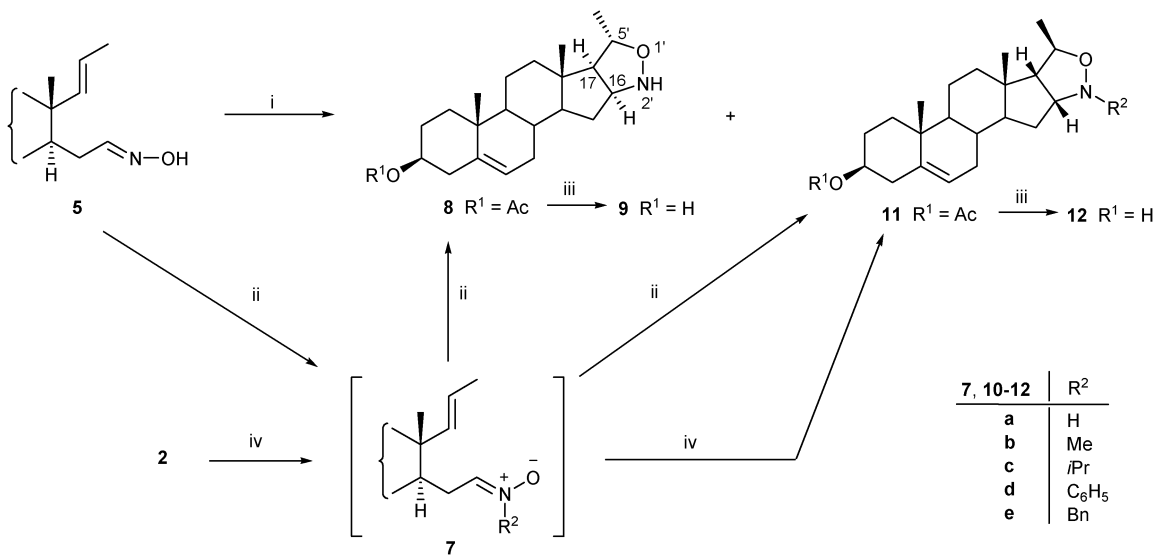
Table 1 Experimental and calculated ^{1}H NMR chemical shifts (in ppm) of the 16-H in oxime **5**

	Experimental	BLYP/6-31G(d)
(E_1)-**5**	7.39	7.59
(Z_1)-**5**	6.70	6.73
(E_2)-**5**	—	7.99
(Z_2)-**5**	—	6.97

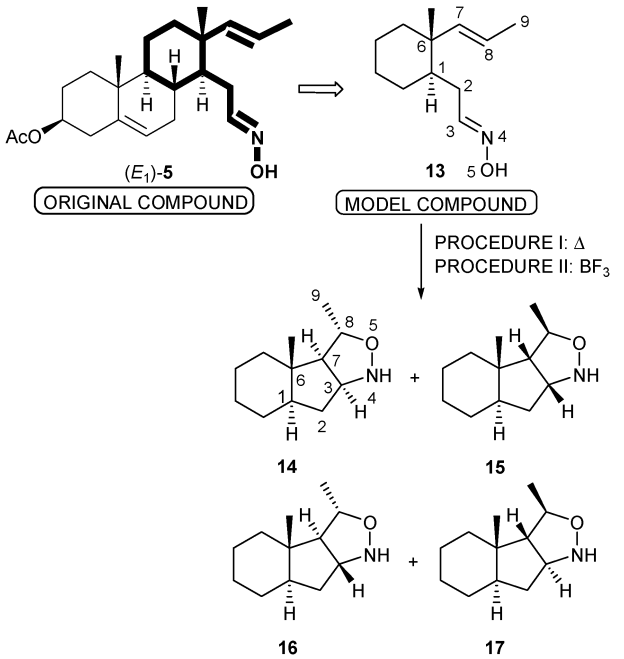
and was subjected to intramolecular cyclization in refluxing toluene. The ring closure of **5** furnished a *ca.* 3 : 2 mixture of D-ring-fused isoxazolidine diastereomers **8** and **11a** in a total yield of 51% together with unreacted oxime **5** (Scheme 2). The cyclization of **5** presumably occurs *via* its less stable nitrone tautomer **7a**, as the buttress effect[19] can facilitate the rate of cycloaddition due to restriction of the conformational space available to the dipole and the dipolarophile moieties, forcing them into closing proximity. The 3 : 4 ratio of (E_1)-**5** and (Z_1)-**5** remained unchanged during the thermally induced intramolecular reaction, which suggests their similar reactivity towards cyclization. However, the 1,3-cycloaddition proceeded in a stereoselective manner to furnish a single isoxazolidine diastereomer **8** in better yield (85%) when **5** was treated with a catalytic amount of $BF_3{\cdot}OEt_2$ at room temperature. In order to synthesize analogous *N*,*O*-heteroring-containing systems, secopregnene aldehyde **2** was reacted with different *N*-substituted hydroxylamines **10b–e** in methanol under reflux. The ring closures proved to be highly diastereoselective in giving *N*-substituted isoxazolidines **11b–e** as single diastereomers, with 5′-H in the α and 16-H and 17-H in the β position, *via* the nitrone intermediates **7b–e** formed *in situ*. Since the electrophilicity and hence the reactivity of nitrone dipoles in 1,3-dipolar cycloadditions may be drastically changed by the variation of substituent R,[2,5] different conversions were observed; the overall yields of the desired products decreased in the sequence **11e** (78%) > **11b** (67%) > **11c** (50%) > **11d** (31%). For pharmacological studies, the synthesized cycloadducts **8** and **11b–e** were deacetylated in alkaline MeOH to furnish the corresponding 3-OH derivatives **9** and **12b–e**, respectively.

2.2 Theoretical studies

In order to find support for the experimental findings during the transformations of **5** with hydroxylamine, computational analysis of the thermally induced (PROCEDURE I) and BF_3-catalysed reaction (PROCEDURE II) mechanisms was



Scheme 2 *Reagents and conditions:* (i) $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, N_2 atm, 6 h; (ii) toluene, 111 °C, 6 h; (iii) KOH, MeOH, rt, 2 h; (iv) $R_2NHOH{\cdot}HCl$ (**10**), NaOAc, MeOH, 65 °C, 2 h.



Scheme 3 A simplified model (**13**) of the starting sterane skeleton (**5**), and the four possible products (**14**, **15**, **16** and **17**), with the atom numbering.

performed to compare the energy profiles of the two processes. For the calculations, a simplified alkenyl oxime model structure **13** (derived from the original sterane framework **5**) was used, assuming that the part ignored does not have a significant effect on the reaction (Scheme 3). The atoms directly involved in the cycloaddition or situated near the reaction centre in **13** are arbitrarily numbered. Theoretically, the ring closure of **13** can lead to four isoxazolidine diastereomers (**14**, **15**, **16**, and **17**) in both PROCEDURES I and II, in view of the fact that the *trans* configuration of the olefinic dipolarophile moiety is conserved during the reaction.[20] The formation of compounds **16** and **17** seems less favourable due to their *trans* D/E ring junction stereochemistry. During PROCEDURE I, an initial isomerization is presumed to occur around the C-1–C-2 σ-bond, with equilibration between the *anti* (**13**) and *gauche* isomers (**18**), the latter existing in four conformers (I, II, II and IV) through twisting around the C-2–C-3 and/or C-6–C-7 axis (Scheme 4). Oxime **18** is proposed to be in thermal tautomeric equilibrium with the nitrone form **19**,[21] which then undergoes an intramolecular 1,3-dipolar cycloaddition to result in the four possible diastereomeric products

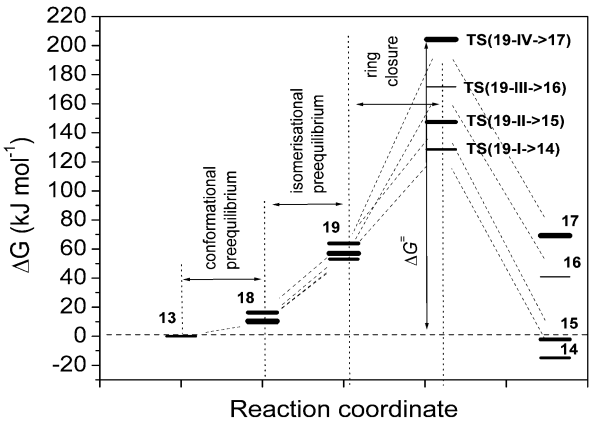
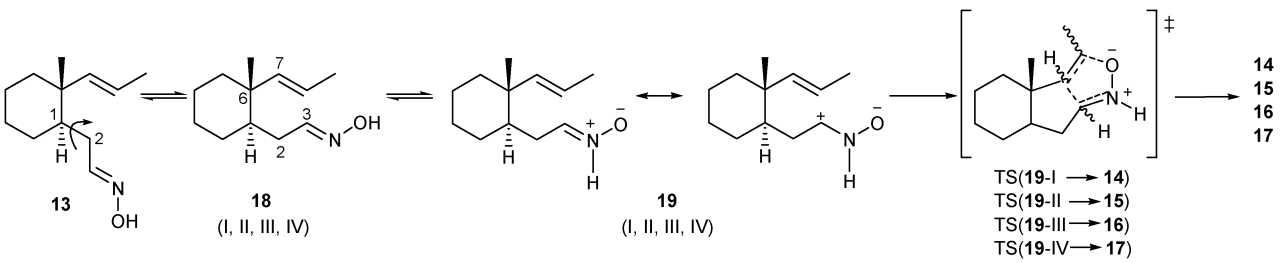


Fig. 2 Energy diagram of the possible routes for PROCEDURE I leading to isoxazolidine diastereomers (**14–17**).

(**14**, **15**, **16** and **17**). In PROCEDURE I, the activation energies leading to **14** and **15** are lower than those leading to **16** and **17**, and formation of the former compounds is therefore predicted to be more favourable (Fig. 2, Table 2). However, for the ring closures towards **14** and **15** to occur, relatively high activation energies ($\Delta G^{\ddagger}$ = 128.5 and 147.4 kJ mol^{-1}, respectively) are needed, suggesting that the cycloadditions require an elevated reaction temperature. The difference between the $\Delta G^{\ddagger}$ values of **14** and **15** is 18.9 kJ mol^{-1} and the reactions leading to these products are exothermic ($\Delta H < 0$), which allows the formation of both diastereomers under thermal conditions, although the formation of **14** is preferred. During PROCEDURE II, the complexation of N-4 in **13** with BF_3 to furnish **20**, and a subsequent conformational change around the C-1–C-2 bond to give **21** are presumed to occur as initiating steps (Scheme 5). Intermediates **20** and **21** can exist in four conformers (I, II, III and IV) by rotation around the C-2–C-3 and/or C-6–C-7 bond axis. Next, an additional isomerization may take place, involving a proton/BF_3 interchange between N-4 and O-5 to afford a *quasi*-nitrone **22** with four conformational states (**22**-I, **22**-II, **22**-III and **22**-IV). The following cyclization leads to intermediates **23**, **24**, **25** and **26**, which can further transform to the isomeric products (**14**, **15**, **16** and **17**) by loss of BF_3 during the work-up. The computed energy profiles of the hypothetical PROCEDURE I (Fig. 2) and PROCEDURE II (Fig. 3) suggest that the latter would be more favourable, due to the lower energies of all the transition states *via* which the isoxazolidine diastereomers (**14**, **15**, **16** and **17**) can be produced (Table 2 and Table 3). Moreover, the lowest

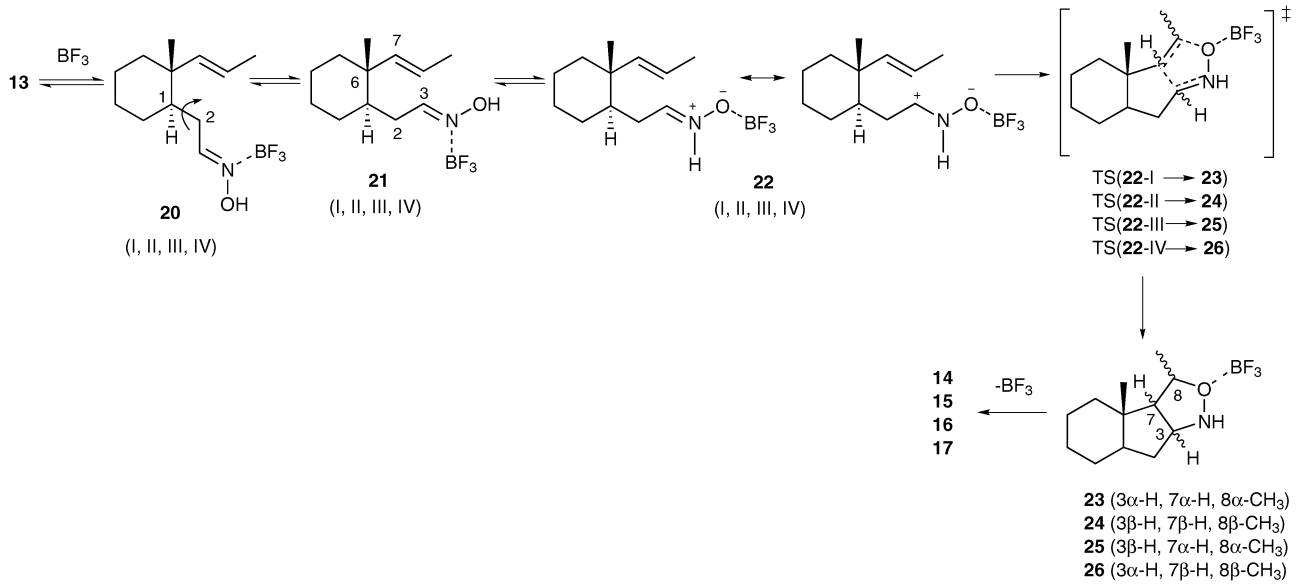


Scheme 4 Mechanism suggested for the formation of isoxazolidines (**14–17**) under thermal conditions (PROCEDURE I); TS = transition state.

Table 2 Computed enthalpies and Gibbs free energies (kJ mol^{-1}) of the individual intermediates (I–IV) relative to **13** for PROCEDURE I

	13	**18**				**19**			
		I	II	III	IV	I	II	III	IV
H	0.00	7.19	17.21	9.70	12.41	50.10	61.73	51.27	57.07
G	0.00	9.00	16.28	8.62	10.43	53.12	63.90	52.42	56.99

	TS				Product			
	19-I→14	**19-II→15**	**19-III→16**	**19-IV→17**	**14**	**15**	**16**	**17**
H	110.94	130.80	155.95	187.77	−34.87	−23.40	23.05	48.76
G	128.50	147.40	171.64	204.21	−14.84	−2.18	40.95	69.21



Scheme 5 The possible mechanism of the BF_3·OEt_2-induced formation of isoxazolidines (**14–17**); TS = transition state.

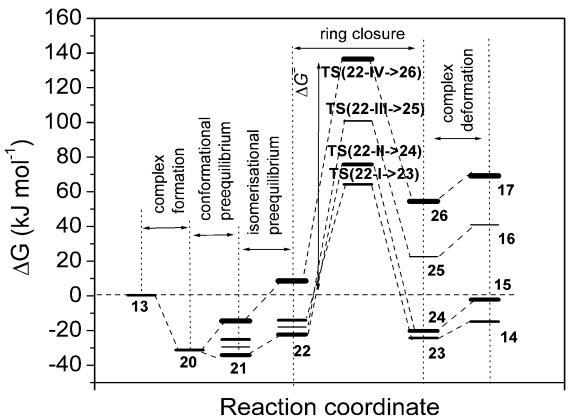


Fig. 3 Energy diagram of the possible routes for PROCEDURE II leading to isoxazolidine diastereomers (**14–17**).

threshold energy is required for the formation of **14** during PROCEDURE II, which means that the reaction may be expected to occur in a stereoselective manner at room temperature. This is in good agreement with the experimental finding that the BF_3-induced ring closure of **5** was diastereoselective, furnishing **8** exclusively. The overall activation energy difference between PROCEDURE I and PROCEDURE II for **14** is about 64 kJ mol^{-1}, which makes the latter preferable and allows it to occur under milder conditions with the formation of only one diastereomer.

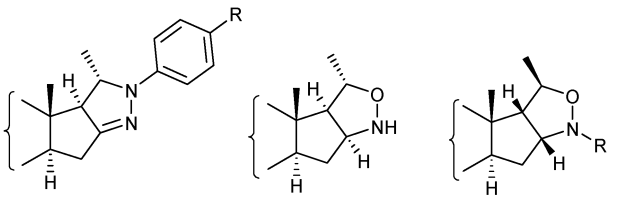
2.3 Pharmacological studies

In recent years, considerable interest has been focused on steroidal heterocycles in view of the broad spectrum of their biological activities. However, D-ring-fused isoxazolidines have received less attention from both synthetic and pharmacological aspects.[22] Since isoxazolidines **9** and **12b–e** were formed within a project aimed at the synthesis of novel five-membered *N*,*N*- and *N*,*O*-heterocyclic frameworks from **2** by 1,3-dipolar cycloaddition, *in vitro* pharmacological studies of all the products seemed obvious. Investigation of the inhibitory effects of arylpyrazolines (**4a–h**, Fig. 1) on $C_{17,20}$-lyase was justified by the fact that similar compounds have been reported to possess significant antiandrogenic activity.[23] Moreover, their antiproliferative efficacy was suggested by the structural similarity to the natural alkaloid solanidine, which has certified cytotoxic activity. The same *in vitro* assays were also performed for isoxazolidines (**9**, **12b–e**) in order to compare the results with those obtained for pyrazolines (**4a–h**). The inhibitory effects of all these compounds on rat testicular $C_{17,20}$-lyase were tested by means of a radiosubstrate incubation technique. Among the *N*,*N*-heterocycles (**4a–h**), only the *p*-methyl-substituted arylpyrazoline **4c** exhibited

Table 3 Computed enthalpies and Gibbs free energies (kJ mol^{-1}) of the individual intermediates (I–IV) relative to **13** for PROCEDURE II

	13	**20** complex form.	**21** conf. preequil.				**22** isom. preequil.					
			I	II	III	IV	I	II	III	IV		
H	0.00	−40.70	−32.11	−40.90	−36.31	−20.12	−23.07	−27.02	−23.07	1.03		
G	0.00	−31.10	−25.20	−34.20	−29.53	−14.50	−14.02	−22.31	−18.04	8.61		
	TS				Complex product				Product			
	22-I → 23	**22-II → 24**	**22-III → 25**	**22-IV → 26**	**23**	**24**	**25**	**26**	**14**	**15**	**16**	**17**
H	43.22	55.32	82.57	115.64	−45.68	−41.22	0.60	32.50	−34.87	−23.40	23.05	48.76
G	64.29	75.76	100.87	136.57	−24.32	−20.24	22.55	54.54	−14.84	−2.18	40.95	69.21

Table 4 Inhibition of $C_{17,20}$-lyase by arylpyrazolines (**4a–h**) and isoxazolidines (**9**, **12b–e**)



Compound	R	Relative conversion[a] (%)	IC_{50}/μM
4a	H	86	—
4b	Cl	89	—
4c	CH_3	—	5.8
4d	OMe	77	—
4e	CN	79	—
4f	NO_2	NI[b]	—
4g	*i*Pr	85	—
4h	CF_3	80	—
9	—	42	26
12b	Me	68	—
12c	*i*Pr	77	—
12d	C_6H_5	78	—
12e	Bn	83	
Ketoconazole (ref.)		—	0.75

[a] Measured in the presence of 50 μM of the compounds tested; control incubation with no inhibition is taken as 100%. [b] No inhibition.

noteworthy inhibitory action (IC_{50} = 5.8 μM), the other derivatives proving to be weaker inhibitors (Table 4), decreasing the enzyme activity by only 77–89% at 50 μM. All of the D-ring-fused isoxazolidines with a [16α,17α]-*cis* D/E ring junction (**12b–e**) also exerted some inhibition. The enzyme activity was reduced by 68–83%. However, compound **9**, containing its isoxazolidine moiety in the 16β,17β orientation, was a more potent enzyme inhibitor, with IC_{50} = 26 μM. Arylpyrazolines **4a–h** performed much better in the *in vitro* cytotoxic assays, as several of the derivatives exerted pronounced antiproliferative effects on the three malignant human cell lines (HeLa, MCF7 and A431).[24] However, the cell growth-inhibitory potencies of the investigated isoxazolidines (**9**, **12b–e**) were found to be lower than those of *N*,*N*-heterocycles. Compounds **9**, **12c** and **12d** did not display any substantial activity up to the final concentration of 30 μM, while **12b** exerted moderate cytotoxic activity on HeLa and A431 cells (Table 5). The introduction of a benzyl group instead of a methyl group into the isoxazolidine moiety (**12e**) resulted in an increase in potency on all three cell lines, with IC_{50} values close to those of cisplatin.

Table 5 Cytotoxic activity of izoxazolidines (**9**, **12b–e**)

		IC_{50}/μM		
Compound	R	HeLa	MCF-7	A431
9	—	> 30	> 30	> 30
12b	Me	21.89	> 30	15.89
12c	*i*Pr	> 30	> 30	> 30
12d	C_6H_5	> 30	> 30	> 30
12e	Bn	14.00	23.18	8.76
Doxorubicin (ref.)		0.15	0.28	0.15
Cisplatin (ref.)		12.43	9.63	2.84

3. Conclusions

In summary, novel androst-5-ene-fused isoxazolidines were prepared *via* thermally induced and Lewis acid-catalysed intramolecular [3 + 2] cycloaddition of a steroidal alkenyl oxime. The catalytic reaction was carried out under mild conditions and proved highly stereoselective, while the thermal version resulted in a mixture of two diastereomers in lower overall yield. The experimental findings were supported by computational calculations on simplified model compounds structurally related to the experimentally applied steroidal oxime. Similar derivatives with their isoxazolidine moiety in the 16α,17α spatial orientation were also prepared diastereoselectively, by thermal ring closure of a D-secopregnene aldehyde with different *N*-substituted hydroxylamines. Among the synthesized analogues, compound **9** displayed the best, but still moderate *in vitro* inhibition on $C_{17,20}$-lyase, while from the related *N*,*N*-heterocycles **4c** was found to be more effective. **12e** exerted *in vitro* cytotoxic activity on three malignant human cell lines.

4. Experimental

4.1 General

Melting points were determined on a Kofler block and are uncorrected. EI mass spectra were obtained with a Varian MAT 311A spectrometer with an ionization energy of 70 eV. The IR spectra were recorded in KBr pellets with a Bio-Rad FTS-60A spectrometer. ^{1}H NMR spectra were obtained in $CDCl_3$ or in DMSO-d_6 solution at 400 MHz (Bruker DRX 400) or 500 MHz (Bruker DRX 500), and the ^{13}C NMR spectra at 100 MHz with the same instruments. Chemical shifts are reported relative to TMS; *J* values are given in Hz. ^{13}C NMR spectra are ^{1}H-decoupled. For

determination of the multiplicities, the J-MOD pulse sequence was used. Elemental analyses were carried out with a Perkin-Elmer CHN Analyzer (Model 2400). All solvents were distilled and dried 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 254F) 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 spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm.

4.2 *trans*-3β-Acetoxy-16,17-secopregna-5,17(20)-dien-16-aldoxime (5)

Compound **2** (718 mg, 2.00 mmol) and hydroxylamine hydrochloride (139 mg, 2.00 mmol) were dissolved together in *i*PrOH (10 cm^3), a solution of anhydrous NaOAc (250 mg, 3.00 mmol) in *i*PrOH (10 cm^3) was added, and the mixture was stirred at 50 °C for 20 min. The solution was then diluted with water and much of the organic solvent was evaporated off *in vacuo*. The resulting precipitate was filtered off, washed with water and dried to give **5** as a 3 : 4 mixture of (*E*) and (*Z*) isomers (717 mg, 96%), which were separated by column chromatography (EtOAc–CH_2Cl_2 = 2 : 98).

(*E*)-**5**: white crystals (Found: C, 74.05; H, 9.36. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44%); mp 140–142 °C (from CH_2Cl_2–hexane); R_f 0.65 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.95 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.05–1.15 (m, 2H), 1.21 (m, 1H), 1.34–1.45 (m, 3H), 1.48–1.60 (m, 4H), 1.68 (d, 3H, J = 6.1 Hz, 21-H_3), 1.87 (m, 2H), 2.03 (s, 3H, Ac-CH_3), 2.10 (m, 1H), 2.18–2.36 (m, 4H), 4.60 (m, 1H, 3-H), 5.25 (d, 1H, J = 15.7 Hz, 17-H), 5.35–5.43 (m, 2H, 6-H and 20-H), 7.39 (t, 1H, J = 6.3 Hz, 16-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 16.9 (C-18), 18.2 (C-21), 19.2 (C-19), 20.2 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH_2), 32.7 (CH_2), 33.7 (CH), 36.7 (CH_2), 36.8 (C-10), 37.8 (CH_2), 39.7 (CH_2), 39.9 (C-13), 49.2 (CH), 49.5 (CH), 73.8 (C-3), 122.1 (C-6), 122.2 (C-20), 139.0 (C-5), 142.5 (C-17), 153.6 (C-16), 170.5 (Ac–C); ν_{max} (KBr)/cm^{-1}: 3381, 1641; m/z (EI) (%): 373 [M^+], 356 (28), 311 (66), 296 (100).

(*Z*)-**5**: white crystals (Found: C, 73.88; H, 9.32. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44%); mp 157–159 °C (from EtOAc–CH_2Cl_2); R_f 0.48 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.95 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.03–1.14 (m, 2H), 1.18 (m, 1H), 1.35–1.45 (m, 3H), 1.48–1.60 (m, 4H), 1.66 (d, 3H, J = 6.3 Hz, 21-H_3), 1.88 (m, 2H), 2.03 (s, 3H, Ac-CH_3), 2.15 (m, 1H), 2.23–2.33 (m, 3H), 2.41 (m, 1H), 4.60 (m, 1H, 3-H), 5.27 (d, 1H, J = 15.7 Hz, 17-H), 5.35–5.43 (m, 2H, 6-H and 20-H), 6.70 (dd, 1H, J = 6.2, 4.2 Hz, 16-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 16.6 (C-18), 18.2 (C-21), 19.2 (C-19), 20.2 (CH_2), 21.4 (Ac-CH_3), 26.0 (CH_2), 27.7 (CH_2), 32.1 (CH_2), 33.8 (CH), 36.7 (CH_2), 36.8 (C-10), 37.8 (CH_2), 39.7 (CH_2), 39.9 (C-13), 49.4 (CH), 49.5 (CH), 73.8 (C-3), 122.0 (C-6), 122.2 (C-20), 139.1 (C-5), 142.5 (C-17), 154.1 (C-16), 170.5 (Ac–C); ν_{max} (KBr)/cm^{-1}: 3373, 1651; m/z (EI) (%): 373 [M^+], 313 (100), 296 (51), 255 (32), 145 (33), 43 (31).

4.3 *trans*-3β-Acetoxy-16,17-secopregna-5,17(20)-dien-16-al dimethyl acetal (6)

Compound **2** (718 mg, 2.00 mmol) and hydroxylamine hydrochloride (139 mg, 2.00 mmol) were dissolved together in MeOH (10 cm^3), a solution of anhydrous NaOAc (250 mg, 3.00 mmol) in MeOH (10 cm^3) was added, and the mixture was refluxed for 6 h. The solution was then diluted with water (50 cm^3) and extracted with CH_2Cl_2 (2 × 20 cm^3). The combined organic phases were dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by column chromatography (EtOAc–CH_2Cl_2 = 2 : 98) to give **5** as a mixture of (*E*) and (*Z*) isomers (560 mg, 75%) and **6** (81 mg, 10%). **6**: white crystals (Found: C, 74.25; H, 10.05. Calc. for $C_{25}H_{40}O_4$: C, 74.22; H, 9.97%); mp 103–105 °C (from EtOAc–CH_2Cl_2); R_f 0.37 (EtOAc–CH_2Cl_2 = 2 : 98); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.90 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 1.02–1.15 (m, 3H), 1.31–1.64 (m, 9H), 1.66 (d, 3H, J = 4.9 Hz, 21-H_3), 1.86 (m, 2H), 2.02 (s, 3H, Ac-CH_3), 2.19 (m, 1H), 2.27–2.35 (m, 2H), 3.23 (s, 3H, one of 16-OMe), 3.30 (s, 3H, the other 16-OMe), 4.38 (t, 1H, J = 5.9 Hz, 16-H), 4.59 (m, 1H, 3-H), 5.31–5.38 (m, 3H, 6-H, 17-H and 20-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 16.4 (C-18), 18.2 (C-21), 19.2 (C-19), 20.2 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 32.7 (CH_2), 33.7 (CH_2), 34.2 (CH), 36.7 (CH_2), 36.9 (C-10), 37.8 (CH_2), 39.4 (C-13), 39.7 (CH_2), 46.4 (CH), 49.5 (CH), 52.1 (one of 16-OMe), 54.0 (the other 16-OMe), 73.9 (C-3), 105.5 (C-16), 120.6 (C-6), 122.5 (C-20), 139.0 (C-5), 143.5 (C-17), 170.4 (Ac–C); ν_{max} (KBr)/cm^{-1}: 1724, 1242; m/z (EI) (%): 404 [M^+], 312 (100), 280 (34).

4.4 (5′*S*,16*S*,17*R*)-3β-Acetoxy-5′-methylisoxazolidino-[3′,4′ : 16,17]androst-5-ene (8)

Oxime **5** (374 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 cm^3), and $BF_3{\cdot}OEt_2$ (a 48% solution in diethyl ether, 0.09 cm^3, 0.3 mmol) was added dropwise at room temperature under a nitrogen atmosphere. The mixture was stirred for 6 h, then quenched by the addition of 1 M $NaHCO_3$ (10 cm^3). The organic phase was separated, the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3), and the combined organic phases were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc–CH_2Cl_2 = 20 : 80) to give **8** (318 mg, 85%) as a white solid (Found: C, 73.80; H, 9.55. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44%); mp 242–244 °C (from EtOAc–CH_2Cl_2); R_f 0.48 (EtOAc–CH_2Cl_2 = 50 : 50); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.87 (s, 3H, 18-H_3), 0.94–1.01 (m, 2H), 1.04 (s, 3H, 19-H_3), 1.07–1.26 (m, 3H), 1.14 (d, 3H, J = 6.3 Hz, 5′-CH_3), 1.45–1.67 (m, 5H), 1.78 (m, 1H), 1.86 (m, 2H) 1.97–2.01 (m, 2H), 2.03 (s, 3H, Ac-CH_3), 2.13 (dd, 1H, J = 9.4, 2.0 Hz, 17-H), 2.30–2.33 (m, 2H), 3.95 (m, 1H, 16-H), 4.22 (m, 1H, 5′-H), 4.60 (m, 1H, 3-H), 5.37 (d, 1H, J = 5.0 Hz, 6-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 19.0 (5′-CH_3), 19.3 (2C, C-18 and C-19), 20.7 (2C, 2 × CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.8 (C-8), 32.0 (CH_2), 36.7 (C-10), 36.9 (CH_2), 38.0 (CH_2), 39.9 (CH_2), 40.8 (C-13), 49.9 (2C, C-9 and C-14), 67.3 (CH), 73.8 (2C, 2 × CH), 76.5 (CH), 122.2 (C-6), 139.7 (C-5), 170.4 (Ac–C); ν_{max} (KBr)/cm^{-1}: 3199, 1732, 1248; m/z (EI) (%): 373 (100) [M^+], 313 (42), 157 (24), 86 (32), 43 (33).

4.5 (5′*S*,16*S*,17*R*)-3β-Hydroxy-5′-methylisoxazolidino-[3′,4′ : 16,17]androst-5-ene (9)

3β-Acetoxyisoxazolidine (**8**) (187 mg, 0.50 mmol) was dissolved in MeOH (10 cm^3) and KOH (100 mg, 1.8 mmol) was added. The mixture was stirred for 2 h at room temperature, and then diluted with water. The resulting precipitate was filtered off, washed with water and dried to give **9** (157 mg, 95%) as a white solid (Found: C, 76.12; H, 9.98. Calc. for $C_{21}H_{33}NO_2$: C, 76.09; H, 10.03%); mp 222–224 °C (from MeOH); R_f 0.45 (EtOAc–CH_2Cl_2 = 50 : 50); δ_H (400 MHz, DMSO-d_6, [ppm]): 0.77 (s, 3H, 18-H_3), 0.85–0.93 (m, 3H), 0.95 (s, 3H, 19-H_3), 1.02 (m, 4H, 5′-CH_3 + 1H), 1.14 (m, 1H), 1.29–1.43 (m, 2H), 1.47–1.52 (m, 3H), 1.65–1.83 (m, 4H), 1.92 (m, 1H), 2.02–2.17 (m, 3H), 3.25 (m, 1H), 3.81 (m, 1H), 4.05 (m, 1H), 4.57 (bs, 1H, OH), 5.25 (d, 1H, J = 4.6 Hz, 6-H); ν_{max} (KBr)/cm^{-1}: 3433, 3225; m/z (EI) (%): 331 (100) [M^+], 86 (45).

4.6 (5′*R*,16*R*,17*S*)-3β-Acetoxy-5′-methylisoxazolidino-[3′,4′ : 16,17]androst-5-ene (11a)

Oxime **5** (374 mg, 1.00 mmol) was dissolved in toluene (10 cm^3), and the solution was refluxed for 6 h, and the solvent was then evaporated off *in vacuo*. The crude product was purified by column chromatography (EtOAc–CH_2Cl_2 = 20 : 80) to give **8** (115 mg, 31%) and **11a** (76 mg, 20%) as white solids.

11a (Found: C, 73.92; H, 9.62. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44%); mp 194–197 °C (from EtOAc–CH_2Cl_2); R_f 0.46 (EtOAc–CH_2Cl_2 = 50 : 50); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.83 (s, 3H, 18-H_3), 0.98 (m, 1H), 1.01 (s, 3H, 19-H_3), 1.14 (m, 1H), 1.23 (d, 3H, J = 6.1 Hz, 5′-CH_3), 1.33–1.43 (m, 2H), 1.49–1.86 (m, 10H), 1.98 (m, 1H), 2.01 (s, 3H, Ac-CH_3), 2.10 (t, 1H, J = 7.7 Hz, 17-H), 2.31–2.33 (m, 2H), 3.74 (m, 1H, 16-H), 3.99 (m, 1H, 5′-H), 4.60 (m, 1H, 3-H), 5.35 (d-like m, 1H, 6-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 18.6 (5′-CH_3), 19.3 (C-19), 20.4 (CH_2), 21.2 and 21.4 (C-18 and Ac-CH_3), 27.7 (CH_2), 31.5 (C-8), 32.0 (CH_2), 32.1 (CH_2), 33.6 (CH_2), 36.6 (C-10), 37.0 (CH_2), 38.0 (CH_2), 39.9 (C-13), 49.9 (2C, C-9 and C-14), 65.6 (C-17), 66.9 (C-5′), 73.7 (2C, C-3 and C-16), 122.1 (C-6), 139.6 (C-5), 170.4 (Ac–C).

4.7 General procedure for the synthesis of steroidal isoxazolidines (11b–e)

To a mixture of **2** (359 mg, 1.00 mmol) and *N*-substituted hydroxylamine hydrochloride (**10b–e**, 1.00 mmol) in MeOH (10 cm^3), a solution of NaOAc (125 mg, 1.50 mmol) in MeOH (10 cm^3) was added and the mixture was refluxed for 2 h. The solution was then poured into water (20 cm^3) and extracted with CH_2Cl_2 (3 × 10 cm^3). The combined organic phases were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product (**11b–e**) was purified by column chromatography (EtOAc–CH_2Cl_2 = 10 : 90).

4.7.1 (5′*R*,16*R*,17*S*)-3β-Acetoxy-2′,5′-dimethylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (11b).

Compound **10b** (84 mg) was used for the synthesis as described in the General procedure. Yield (**11b**): 260 mg (67%); white solid (Found: C, 74.52; H, 9.71. Calc. for $C_{24}H_{37}NO_3$: C, 74.38; H, 9.62%); mp 186–187 °C (from CH_2Cl_2–hexane); R_f 0.34 (EtOAc–CH_2Cl_2 = 20 : 80); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.81 (s, 3H, 18-H_3), 0.98 (m, 1H), 1.02 (s, 3H, 19-H_3), 1.14 (m, 1H), 1.22 (d, 3H, J 6.0, 5′-CH_3), 1.34–1.43 (m, 2H), 1.45–1.72 (m, 8H), 1.84–1.87 (m, 2H), 1.96 (m, 1H), 2.02 (s, 3H, Ac-CH_3), 2.07 (t, 1H, J = 8.7 Hz, 17-H), 2.29–2.33 (m, 2H), 2.68 (s, 3H, *N*-Me), 3.13 (dd, 1H, J = 8.4, 6.4 Hz, 16-H), 3.85 (m, 1H, 5′-H), 4.60 (m, 1H, 3-H), 5.37 (d, 1H, J = 4.9 Hz, 6-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 18.4 (5′-CH_3), 19.3 (C-19), 20.5 (CH_2), 21.4 (2C, Ac-CH_3 and C-18), 27.7 (CH_2), 31.4 (C-8), 32.0 (CH_2), 32.1 (CH_2), 32.8 (CH_2), 36.7 (C-10), 37.0 (CH_2), 38.1 (CH_2), 41.6 (C-13), 44.5 (*N*-Me), 49.5 and 49.9 (C-9 and C-14), 66.8 (C-17), 73.8 (2C, C-3 and C-16), 74.3 (C-5′), 122.5 (C-6), 139.5 (C-5), 170.4 (Ac–C); ν_{max} (KBr)/cm^{-1}: 2862, 1726, 1244; m/z (EI) (%): 387 (100) [M^+].

4.7.2 (5′*R*,16*R*,17*S*)-3β-Acetoxy-2′-isopropyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (11c).

Compound **10c** (112 mg) was used for the synthesis as described in the General procedure. Yield (**11c**): 208 mg (50%); white solid (Found: C, 75.06; H, 10.02. Calc. for $C_{26}H_{41}NO_3$: C, 75.14; H, 9.94%); mp 163–165 °C (from EtOAc–CH_2Cl_2); R_f 0.38 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.80 (s, 3H, 18-H_3), 0.96 (m, 1H), 1.01 (s, 3H, 19-H_3), 1.05 (d, 3H, J = 6.4 Hz, 5′-CH_3), 1.12 (m, 1H), 1.17 (d, 3H, J = 6.2 Hz) and 1.20 (d, 3H, J = 5.9 Hz): 2 × *i*Pr-CH_3, 1.33–1.73 (m, 10H), 1.85 (m, 2H), 1.94 (m, 1H), 2.02 (s, 3H, Ac-CH_3 and t-like m, 1H, 17-H), 2.26–2.36 (m, 2H), 2.83 (m, 1H, *N*-CH), 3.35 (t-like m, 1H, 16-H), 3.81 (m, 1H, 5′-H), 4.60 (m, 1H, 3-H), 5.36 (d-like m, 1H, 6-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 18.3 (5′-CH_3), 19.3 (C-19), 20.3 (C-18), 20.5 (CH_2), 21.1 and 21.2 (2 × *i*Pr-CH_3), 21.4 (Ac-CH_3), 27.7 (CH_2), 31.6 (C-8), 32.0 (CH_2), 33.1 (CH_2), 34.1 (CH_2), 36.7 (C-10), 37.0 (CH_2), 38.1 (CH_2), 41.2 (C-13), 49.0 and 49.9 (C-9 and C-14), 58.2 (*N*-CH), 66.7 (C-17), 69.5 (C-16), 73.2 and 73.8 (C-3 and C-5′), 122.5 (C-6), 139.4 (C-5), 170.4 (Ac–C); ν_{max} (KBr)/cm^{-1}: 1728, 1248; m/z (EI) (%): 415 (57) [M^+], 400 (100).

4.7.3 (5′*R*,16*R*,17*S*)-3β-Acetoxy-2′-cyclohexyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (11d).

Compound **10d** (152 mg) was used for the synthesis as described in the General procedure. Yield (**11d**): 142 mg (31%); white solid (Found: C, 76.35; H, 10.07. Calc. for $C_{29}H_{45}NO_3$: C, 76.44; H, 9.95%); mp 120–123 °C (from MeOH); R_f 0.62 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.80 (s, 3H, 18-H_3), 0.86 (m, 1H), 0.97 (m, 1H), 1.01 (s, 3H, 19-H_3), 1.13 (m, 1H), 1.20 (d, 3H, J = 5.9 Hz, 5′-CH_3), 1.23–1.26 (m, 3H), 1.33–1.42 (m, 2H), 1.45–1.65 (m, 9H), 1.68–1.77 (m, 4H), 1.85 (m, 2H), 1.93 (m, 1H), 2.00 (t, 1H, J = 9.0 Hz, 17-H), 2.02 (s, 3H, Ac-CH_3), 2.17 (m, 1H), 2.29–2.34 (m, 2H), 2.50 (m, 1H), 3.37 (t-like m, 1H, 16-H), 3.79 (m, 1H, 5′-H), 4.60 (m, 1H, 3-H), 5.36 (d-like m, 1H, 6-H); δ_C (100 MHz, $CDCl_3$, [ppm]): 18.3 (5′-CH_3), 19.3 (C-19), 20.5 (CH_2), 21.2 and 21.4 (C-18 and Ac-CH_3), 24.8 (CH_2), 25.2 (CH_2), 26.1 (CH_2), 27.7 (CH_2), 30.8 (CH_2), 31.5 (CH_2), 31.6 (C-8), 32.0 (CH_2), 33.1 (CH_2), 34.0 (CH_2), 36.7 (C-10), 37.0 (CH_2), 38.1 (CH_2), 41.1 (C-13), 48.9 and 49.9 (C-9 and C-14), 66.4 (C-17), 67.0 (*N*-CH), 69.7 (C-16), 73.0 and 73.8 (C-3 and C-5′), 122.5 (C-6), 139.4 (C-5), 170.4 (Ac–C); ν_{max}

(KBr)/cm^{-1}: 1736, 1246; *m*/*z* (EI) (%): 455 (12) [M$^+$], 69 (34), 56 (100), 41 (74).

4.7.4 (5′*R*,16*R*,17*S*)-3β-Acetoxy-2′-benzyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (11e). Compound **10e** (160 mg) was used for the synthesis as described in the General procedure. Yield (**11e**): 361 mg (78%); white solid (Found: C, 77.58; H, 9.05. Calc. for $C_{30}H_{41}NO_3$: C, 77.71; H, 8.91%); mp 163–165 °C (from EtOAC/CH_2Cl_2); R_f 0.46 (EtOAc–CH_2Cl_2 = 5 : 95); δ_H (400 MHz, $CDCl_3$, [ppm]): 0.79 (s, 3H, 18-H_3), 0.97 (m, 1H), 1.01 (s, 3H, 19-H_3), 1.13 (m, 1H), 1.20 (m, 1H), 1.21 (d, 3H, J = 5.8 Hz, 5′-CH_3), 1.31–1.65 (m, 9H), 1.85 (m, 2H), 1.92 (m, 1H), 2.02 (s, 3H, Ac-CH_3), 2.07 (t, 1H, J = 8.6 Hz, 17-H), 2.28–2.32 (m, 2H), 3.37 (t-like m, 1H, 16-H), 3.86 (m, 2H, one of benzyl-CH_2 and 5′-H), 4.04 (d, 1H, J = 13.2 Hz, the other benzyl-CH_2), 4.60 (m, 1H, 3-H), 5.35 (d-like m, 1H, 6-H), 7.22–7.38 (m, 5H); δ_C (100 MHz, $CDCl_3$, [ppm]): 18.5 (5′-CH_3), 19.3 (C-19), 20.5 (CH_2), 21.4 (2C, C-18 and Ac-CH_3), 27.7 (CH_2), 31.4 (C-8), 32.0 (CH_2), 32.3 (CH_2), 32.9 (CH_2), 36.6 (C-10), 37.0 (CH_2), 38.1 (CH_2), 41.4 (C-13), 49.3 and 49.9 (C-9 and C-14), 62.3 (benzyl-CH_2), 66.4 (C-17), 71.5 (C-16), 73.8 and 74.0 (C-3 and C-5′), 122.4 (C-6), 127.1 (C-4′′), 128.1 (2C, C-2′′ and C-6′′), 129.2 (2C, C-3′′ and C-5′′), 137.5 (C-1′′), 139.4 (C-5), 170.4 (Ac–C); ν_{max} (KBr)/cm^{-1}: 1730, 1240, 742; *m*/*z* (EI) (%): 463 (100) [M$^+$], 91 (22).

4.8 General procedure for the synthesis of steroidal isoxazolidines (12b–e)

3β-Acetoxyisoxazolidine (**11b–e**) (0.50 mmol) was dissolved in MeOH (10 cm^3), and KOH (100 mg, 1.8 mmol) was added. The mixture was stirred for 2 h at room temperature, and then diluted with water. The resulting precipitate was filtered off, washed with water and dried.

4.8.1 (5′*R*,16*R*,17*S*)-3β-Hydroxy-2′,5′-dimethylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (12b). Compound **11b** (194 mg) was used for the synthesis as described in the General procedure. Yield (**12b**): 168 mg (97%); white solid (Found: C, 76.62; H, 10.15. Calc. for $C_{22}H_{35}NO_2$: C, 76.47; H, 10.21%); mp 212–215 °C (from (MeOH); R_f 0.21 (EtOAc–CH_2Cl_2 = 20 : 80); δ_H (400 MHz, DMSO-d_6, [ppm]): 0.79 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 0.95–1.03 (m, 2H), 1.27 (d, 3H, J = 5.6 Hz, 5′-CH_3), 1.30–1.70 (m, 10H), 1.77 (m, 1H), 1.90–1.98 (m, 2H), 2.09 (m, 1H), 2.17 (m, 1H), 2.42 (m, 1H), 3.03 (bs, 3H, *N*-Me), 3.26 (m, 1H), 4.17 (bs, 2H), 5.27 (d, 1H, J = 4.5 Hz, 6-H); ν_{max} (KBr)/cm^{-1}: 3375, 2846; *m*/*z* (EI) (%): 345 (100) [M$^+$].

4.8.2 (5′*R*,16*R*,17*S*)-3β-Hydroxy-2′-isopropyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (12c). Compound **11c** (208 mg) was used for the synthesis as described in the General procedure. Yield (**12c**): 175 mg (94%); white solid (Found: C, 77.02; H, 10.58. Calc. for $C_{24}H_{39}NO_2$: C, 77.16; H, 10.52%); mp 168–170 °C (from MeOH); R_f 0.13 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, DMSO-d_6, [ppm]): 0.77 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 0.97 (m, 2H), 0.98 (d, 3H, J = 6.3 Hz, 5′-CH_3), 1.04 (d, 3H, J = 6.1 Hz) and 1.10 (d, 3H, J = 5.9 Hz): 2 × *i*Pr-CH_3, 1.24–1.55 (m, 10H), 1.68 (m, 1H), 1.77 (m, 1H), 1.92–1.96 (m, 2H), 2.03–2.18 (m, 2H), 2.73 (m, 1H, *N*-CH), 3.26 (m, 1H), 3.31 (m, 1H), 3.67 (m, 1H), 4.57 (bs, 1H, OH), 5.26 (d-like m, 1H, 6-H); ν_{max} (KBr)/cm^{-1}: 3410; *m*/*z* (EI) (%): 373 (54) [M$^+$], 358 (100).

4.8.3 (5′*R*,16*R*,17*S*)-3β-Hydroxy-2′-cyclohexyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (12d). Compound **11d** (228 mg) was used for the synthesis as described in the General procedure. Yield (**12d**): 196 mg (95%); white solid (Found: C, 78.36; H, 10.56. Calc. for $C_{27}H_{43}NO_2$: C, 78.40; H, 10.48%); mp 98–111 °C (from MeOH); R_f 0.16 (EtOAc–CH_2Cl_2 = 10 : 90); δ_H (400 MHz, DMSO-d_6, [ppm]): 0.77 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 0.95–1.02 (m, 2H), 1.10 (d, 3H, J = 5.9 Hz, 5′-CH_3), 1.12–1.70 (m, 20H), 1.76 (m, 1H), 1.90–1.95 (m, 2H), 2.02 (m, 1H), 2.08–2.18 (m, 2H), 2.41 (m, 1H), 3.25 (m, 1H), 3.32 (m, 1H), 3.65 (m, 1H), 4.56 (d, 1H, J = 4.5 Hz, OH), 5.26 (d-like m, 1H, 6-H); ν_{max} (KBr)/cm^{-1}: 3433; *m*/*z* (EI) (%): 413 (100) [M$^+$], 370 (41).

4.8.4 (5′*R*,16*R*,17*S*)-3β-Hydroxy-2′-benzyl-5′-methylisoxazolidino[4′,5′ : 17 : 16]androst-5-ene (12e). Compound **11e** (232 mg) was used for the synthesis as described in the General procedure. Yield (**12e**): 202 mg (96%); white solid (Found: C, 79.55; H, 9.42. Calc. for $C_{28}H_{39}NO_2$: C, 79.76; H, 9.32%); mp 78–80 °C (from MeOH); R_f 0.18 (EtOAc–CH_2Cl_2 = 5 : 95); δ_H (400 MHz, DMSO-d_6, [ppm]): 0.77 (s, 3H, 18-H_3), 0.94 (s, 3H, 19-H_3), 0.95–1.01 (m, 2H), 1.10 (d, 3H, J = 5.8 Hz, 5′-CH_3), 1.21–1.54 (m, 10H), 1.68 (m, 1H), 1.76 (m, 1H), 1.90 (m, 1H), 1.99–2.17 (m, 3H), 3.24 (m, 1H), 3.32 (m, 1H), 3.35 (m, 1H), 3.72 (m, 1H), 3.81 (d, 1H, J = 13.7 Hz) and 3.90 (d, 1H, J = 13.7 Hz): benzyl-CH_2, 4.57 (d, 1H, J = 4.4 Hz, OH), 5.25 (d-like m, 1H, 6-H), 7.21–7.32 (m, 5H); ν_{max} (KBr)/cm^{-1}: 3404, 739; *m*/*z* (EI) (%): 421 (100) [M$^+$], 91 (17).

4.9 Theoretical studies

All computations were carried out with the Gaussian03 program package.[25] Geometry optimizations and subsequent frequency calculations were performed by applying the B3LYP/6-31G(d) basis set.[26] Thermodynamic parameters (*H*, *G*) were computed at 298.15 K, applying a quantum chemical rather than a conventional thermodynamic scale.

4.10 Determination of $C_{17,20}$-lyase activity and its inhibition in the rat testis

The inhibitory effects exerted on $C_{17,20}$-lyase activity by the newly synthesized isoxazolidine derivatives were determined *via* an *in vitro* radiosubstrate incubation method described in detail earlier.[27] In brief, testicular tissue from adult Wistar rats was homogenized with an Ultra-Turrax in Krebs-Ringer phosphate buffer containing glucose. Aliquots of this homogenate were incubated with 1 μM [^{3}H]17-hydroxyprogesterone in the presence of 1 mM NADPH at 37 °C for 20 min. The enzymatic reaction was stopped by the addition of ethyl acetate and freezing. After extraction, the radioactive 17α-hydroxyprogesterone and the product androst-4-ene-3,17-dione were separated by TLC and a Packard Radiochromatogram Scanner was used to trace the separated steroids. Spots were scraped off and extracted, and the radioactivity of the androst-4-ene-3,17-dione formed and the 17α-hydroxyprogesterone remaining was measured by means of liquid scintillation counting. $C_{17,20}$-lyase

activity was calculated in picomoles of androst-4-ene-3,17-dione formed. Test compounds were applied at 50 μM. Control incubates without test substances and incubates with the reference compound ketoconazole were also prepared in every series. At least two experiments were performed with each test compound; the standard deviations of the mean enzyme activity results were within ±10%. IC_{50} values were determined for the more potent inhibitors. In this case, conversion was measured at five or six different concentrations of the test compound. IC_{50} results were calculated by linear regression analysis following a logit-log transformation of the data.

4.11 Determination of antiproliferative activities

Cytotoxic effects were measured *in vitro* on three human cell lines (ECACC; Salisbury, UK): HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma). The cells were cultivated in minimal essential medium (Gibco BRL; Paisley, UK) supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. The cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. Near-confluent cells were seeded into a 96-well plate (5000 cells/well) and, after overnight standing, the medium containing the tested compound was added. Following a 72 h incubation the cytotoxicity was measured with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay.[28] The precipitated formazan crystals were solubilized in dimethyl sulfoxide (DMSO), and the absorbance was read at 545 nm with a microplate reader. The assay was repeated in the concentration range 0.3–30 μM when the compound exerted at least 50% inhibition of the cell proliferation at 30 μM. Sigmoidal concentration-response curves were fitted to the measured points, and the IC_{50} values were calculated with GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). Reported values are the averages of the results of 2 independent experiments with 4 parallels. The highest DMSO concentration of the medium, 0.1%, did not have any significant effect on cell proliferation. Doxorubicin and cisplatin were used as reference compounds.

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Notes and references

1 J. J. Tufariello, in *1,3-Dipolar cycloaddition chemistry*, ed. A. Padwa, J. Wiley & Sons Inc., New York, 1984, pp. 83–168; J. N. Martin and R. C. F. Jones, in *Synthetic applications of 1,3-dipolar cycloaddition chemistry toward heterocycles and natural products*, ed. A. Padwa, W. H. Pearson, J. Wiley & Sons Inc., Hoboken, New Jersey, 2003, pp. 1–81; K. V. Gothelf and K. A. Jørgensen, *Chem. Rev*., 1998, **98**, 863–909.
2 V. Nair and T. D. Suja, *Tetrahedron*, 2007, **63**, 12247–12275; A. Padwa, in *1,3-Dipolar cycloaddition chemistry*, ed. A. Padwa, J. Wiley & Sons Inc., New York, 1984, pp. 279–304..
3 M. Frederickson, *Tetrahedron*, 1997, **53**, 403–425.
4 P. Merino, T. Tejero, M. Laguna, E. Cerrada, A. Moreno and J. A. Lopez, *Org. Biomol. Chem*., 2003, **1**, 2336–2342; D. Carmona, M. P. Lamata, F. Viguri, R. Rodríguez, L. A. Oro, A. I. Balana, F. J. Lahoz, T. Tejero, P. Merino, S. Franco and I. Montesa, *J. Am. Chem. Soc*., 2004, **126**, 2716–2717; L. R. Domingo, W. Benchouk and S. M. Mekelleche, *Tetrahedron*, 2007, **63**, 4464–4471; H. Pellissier, *Tetrahedron*, 2007, **63**, 3235–3285; T. Kano, T. Hashimoto and K. Maruoka, *J. Am. Chem. Soc*., 2005, **127**, 11926–11927; S. Minakata, T. Ezoe, K. Nakamura, I. Ryu and M. Komatsu, *Tetrahedron Lett*., 1998, **39**, 5205–5208.
5 P. Pérez, L. R. Domingo, M. J. Aurell and R. Contreras, *Tetrahedron*, 2003, **59**, 3117–3125.
6 R. Grigg, F. Heaney, J. Markandu, S. Surendrakumar, M. Thorton-Pett and W. J. Warnock, *Tetrahedron*, 1991, **47**, 4007–4030.
7 A. Padwa and S. K. Bur, *Tetrahedron*, 2007, **63**, 5341–5378; A. Padwa, U. Chiacchio, D. C. Dean, A. M. Schoffstall, A. Hassner and K. S. K. Murthy, *Tetrahedron Lett*., 1988, **29**, 4169–4172.
8 É. Frank, J. Wölfling, B. Aukszi, V. König, T. R. Schneider and Gy. Schneider, *Tetrahedron*, 2002, **58**, 6843–6849; Y. Wu, G.-F. Dai, J.-H. Yang, Y.-X. Zhang, Y. Zhu and J.-C. Tao, *Bioorg. Med. Chem. Lett*., 2009, **19**, 1818–1821.
9 J. Tanaka and S. Kanemasa, *Tetrahedron*, 2001, **57**, 899–905.
10 L. F. de Oliveira and V. E. U. Costa, *Tetrahedron: Asymmetry*, 2004, **15**, 2583–2590.
11 H. Singh, D. P. Jindal, M. R. Yadav and M. Kumar, *Prog. Med. Chem*., 1991, **28**, 233–300; L. F. Tietze, Gy. Schneider, J. Wölfling, A. Fecher, T. Nöbel, S. Petersen, I. Schuberth and C. Wulff, *Chem.–Eur. J*., 2000, **6**, 3755–3760.
12 L. B. Lorenc, I. O. Juranić, M. M. Dabović and M. Lj. Mihailović, *Tetrahedron*, 1991, **47**, 6389–6398; R. K. Varma, N. J. Belle Mead, C.M. Cimarusti and N. J. Hamilton, *US Pat*., 4 018 774, Elsevier, Newyork, 1977; S. Colombi, G. Vecchio, G. Gottarelli, B. Samori, A. M. Manotti Lanfredi and A. Tiripicchio, *Tetrahedron*, 1978, **34**, 2967–2976; Ch. Camoutsis and S. Nikolaropoulos, *J. Heterocycl. Chem*., 1998, **35**, 731–759.
13 M. Lj. Mihailović, M. M. Rajković, L. B. Lorenc, V. D. Pavlović, A. Ž. Milovanović, B. Tinant and J.-P. Declercq, *Tetrahedron*, 1996, **52**, 11995–12008.
14 J. Wölfling, A. Magyar and Gy. Schneider, *Monatsh. Chem*., 2003, **134**, 1387–1393.
15 É. Frank, Zs. Kardos, J. Wölfling and Gy. Schneider, *Synlett*, 2007, 1311–1313.
16 P. Boontheung, P. Perlmutter and E. Puniani, *ARKIVOC*, 2001, (viii), 95–103.
17 P. J. Dunn, A. B. Graham, R. Grigg, P. Higginson and I. S. Saba, *Chem. Commun*., 2000, 2033–2034.
18 M. Noguchi, H. Okada, S. Nishimura, Y. Yamagata, S. Takamura, M. Tanaka, A. Kakehi and H. Yamamoto, *J. Chem. Soc., Perkin Trans. 1*, 1999, 185–191.
19 B. S. Orlek, P. G. Sammes and D. J. Weller, *J. Chem. Soc., Chem. Commun*., 1993, 1412–1413.
20 K. V. Gothelf and K. A. Jørgensen, in *Synthetic applications of 1,3-dipolar cycloaddition chemistry toward heterocycles and natural products*, ed. A. Padwaand W. H. Pearson, J. Wiley & Sons Inc., Hoboken, New Jersey, 2003, pp. 818–822.
21 A. Hassner, E. Falb, A. Nudelman, A. Albeck and H. E. Gottlieb, *Tetrahedron Lett*., 1994, **35**, 2397–2400.
22 T. P. Culbertson, G. W. Moersch and W. A. Neuklis, *J. Heterocycl. Chem*., 1964, **1**, 280–287; R. K. Varma and C. M. Cimarusti, *US Pat*., 4 018 774, 1977; M. J. Green, R. L. Tiberi, R. Friary, B. N. Lutsky, J. Berkenkoph, X. Fernandez and M. Monahan, *J. Med. Chem*., 1982, **25**, 1492–1495.
23 A. E. E. Amr, N. A. Abdel-Latif and M. M. Abdalla, *Bioorg. Med. Chem*., 2006, **14**, 373–384.
24 É. Frank, Z. Mucsi, I. Zupkó, B. Réthy, G. Falkay, Gy. Schneider and J. Wölfling, *J. Am. Chem. Soc*., 2009, **131**, 3894–3904.
25 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski,

J. A. Montgomery Jr, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle and J. Pople, *A. Gaussian 03 6.0*, Gaussian, Inc., Pittsburgh PA, 2003.

26 A. D. Beke, *J. Chem. Phys*., 1993, **98**, 5648–5652; M. Head-Gordon, J. A. Pople and M. J. Frisch, *Chem. Phys. Lett*., 1988, **153**, 503–506; Z. Mucsi, A. Szabó, I. Hermecz, Á. Kucsman and I. Csizmadia, *J. Am. Chem. Soc*., 2005, **127**, 7615–7631.

27 J. Wölfling, E. A. Oravecz, D. Ondré, E. Mernyák, Gy. Schneider, I. Tóth, M. Szécsi and J. Julesz, *Steroids*, 2006, **71**, 809–816; D. Ondré, J. Wölfling, Z. Iványi, Gy. Schneider, I. Tóth, M. Szécsi and J. Julesz, *Steroids*, 2008, **73**, 1375–1384.

28 T. Mossman, *J. Immunol. Methods*, 1983, **65**, 55–63; P. Csomós, I. Zupkó, B. Réthy, L. Fodor, G. Falkay and G. Bernáth, *Bioorg. Med. Chem. Lett*., 2006, **16**, 6273–6276; B. Réthy, I. Zupkó, R. Minorics, J. Hohmann, I. Ocsovszki and G. Falkay, *Planta Med*., 2007, **73**, 41–48.