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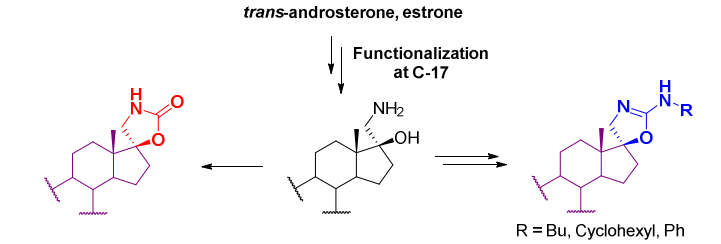

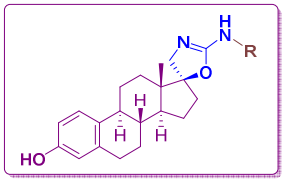

R = Bu GI_{50}= 0.34–2.9 μM

R = Cyclohexyl GI_{50}= 0.34–1.5 μM

Synthesis of unprecedented steroidal spiro heterocycles as potential antiproliferative drugs

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Abstract

Herein we report the straightforward preparation of novel conformationally-restricted steroids from *trans*-androsterone and estrone, decorated with spiranic oxazolidin-2-one or 2-aminooxazoline motifs at C-17 as potential antiproliferative agents. Such unprecedented pharmacophores were accessed using an aminomethylalcohol derivative at C-17 as the key intermediate; reaction of such functionality with triphosgene, or conversion into *N*-substituted thioureas, followed by an intramolecular cyclodesulfurization reaction promoted by yellow HgO, furnished such spirocycles in excellent yields.

Title compounds were tested *in vitro* against a panel of six human tumor cell lines, named A549 (non-small cell lung), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon), and the results were compared with steroidal chemotherapeutic agents (abiraterone and galeterone); the A-ring of the steroidal backbone, the nature of the heterocycle and the *N*-substituents proved to be essential motifs for establishing structure-activity relationships concerning not only the potency but also the selectivity against tumor cell lines. Estrone derivatives, particularly those bearing a spiranic 2-aminooxazoline scaffold were found to be the most active compounds, with GI_{50} values ranging from the low micromolar to the submicromolar level (0.34-1.5 μM). Noteworthy, the lead compounds showed a remarkable increase in activity against the resistant cancer cell lines (T-47D and WiDr) compared to the anticancer reference drugs (up to 120-fold).

Keywords: steroids; oxazoline; oxazolidine; heterocycles; antiproliferative activity.

Abbreviations: COSY (Correlation Spectroscopy), DEPT (Distortionless Enhancement by Polarization Transfer), EI (Electronic Impact), ESI (ElectroSpray Ionization), FAB (Fast Atom Bombardment), FDA (Food and Drug Administration), GI_{50} (Growth Inhibition of 50%), HMBC (Heteronuclear Multiple Bond Correlation), HSQC (Heteronuclear Single Quantum Correlation), NOESY (Nuclear Overhauser Effect Spectroscopy)

1. Introduction

Heterocyclic moieties are considered as privileged structures [1], endowed with a pivotal importance from a biological and pharmaceutical point of view [2-6]. They are promising targets in the drug discovery process [7-12], and are subsequently ubiquitous motifs in a plethora of commercially-available drugs for the treatment of numerous diseases; in fact, it is estimated that roughly 59% of small drugs approved by the US FDA contain a nitrogen-based heterocycle [13].

Heterocycles exhibit a great variety of pharmaceutical properties, including antidepressant [14], antimicrobial [15, 16], antibacterial [17], and anticancer [18, 19], among others [20]. The broad spectrum of biological activities of such molecules has encouraged the synthesis of new compounds with improved properties; heterocycles are widely-used for the isosteric replacement of a series of functional groups, as H-acceptors and H-donors, as metal chelators, or for modulating the polarity of the drug, thus enhancing its bioavailability [21].

In this context, the conjugation of a heterocyclic motif with a steroidal framework has allowed a synergic effect of both biologically-active pharmacophores, leading to unique properties, such as anti-inflammatory [22], antibacterial [23, 24], antiproliferative [25-28], aromatase [29] and 5α-reductase [30] inhibitors (these enzymes being involved in breast and prostate cancer processes, respectively). Moreover, the highly lipophilic nature of the steroidal skeleton might allow the penetration of the drug into the cytosol through the lipidic membrane, thus improving its bioavailability.

Until now, interesting steroidal heterocycles have been synthesized and are being used in clinical trials against different types of cancer. Abiraterone [31, 32] (17-(3-pyridyl) ring) and galeterone [33, 34] (17-(1-benzimidazole) substituent) are clear examples of this kind of drugs used in the treatment of prostate cancer, acting as potent and selective 17α-hydroxylase/17,20 lyase inhibitors to block androgen synthesis [35]. Shi and co-workers developed a new family of spirooxiindoles having the same steroidal nucleus as abiraterone and galeterone; such compounds exhibited a good antiproliverative profile, where multiple ROS processes seem to be involved in the tumor cells death [36]. Although a vast number of compounds and/or therapies have been developed against cancer in the last few years, a search of new compounds is still a key target in Medicinal Chemistry.

Therefore, stimulated by the activities shown by heterocyclic-containing compounds, herein we have focused on the design and synthesis of novel spiranic steroidal heterocycles derived from *trans*-androsterone and estrone as potential antiproliferative agents; the steroidal backbone, the nature of the heterocyclic motif, and substituents were modified in order to provide the structural diversity required for establishing structure-activity relationships.

2. Results and discussion

2.1. Chemistry

Incorporation of heterocycles into steroidal frameworks has usually been reported in the pendant side-chain [37], or by fusing or joining the heterocyclic moiety mainly to the A [38,39], B [40] or D [41-43] rings. Conversely, although the restriction of conformational flexibility has been demonstrated to enhance some biological properties, like the receptor selectivity and the binding affinity [44,45], the number of synthetic spiranic steroids is relatively scarce [46-49]. Taking this into consideration, we envisioned the possibility of preparing spiranic steroids bearing a oxazolidin-2-one or a 2-aminooxazoline scaffold starting from two structurally-related steroids (*trans*-androsterone and estrone). The synthesis of oxazolines, oxazolidines and related structures has been widely described in the literature [50], including steroidal derivatives [51,52].

The key step to synthesize these heterocycles on the steroidal backbone was the access to an aminomethyl alcohol on C-17; we designed the conversion of this functionality into the general structures depicted in Figure 1, upon its transformation into transient isocyanates and thioureas.

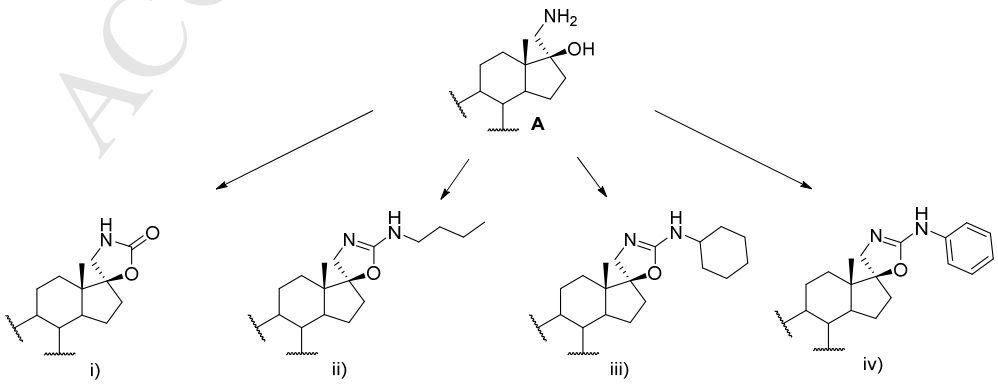


Figure 1. Synthesis of oxazolidin-2-one (**i)** and 2-aminooxazolines (**ii-iv)** from aminoalcohol **A**.

The synthesis of the aminoalcohol was accomplished in 3 steps from *trans*-androsterone and 4 steps from estrone. *Trans*-androsterone (**1**) was treated with trimethylsulfonium iodide under basic conditions to obtain epoxide **2** [53] in a total stereoselective fashion *via* a Corey-Chaykovsky reaction. Nucleophilic opening of epoxide **2** was carried out using sodium azide in the presence of boric acid to get azide **3** in a quantitative yield (Scheme 1); the absolute configuration of the new chiral carbon was assigned for compound **3** as the (*S*)-diastereomer using a 1D-NOESY experiment. The spatial correlation in NOESY was clearly observed between the protons CH_2N_3 and the protons H-12ax and H-16α, and the evidence in the configuration was given by the correlation with H-14 (Figure 2).

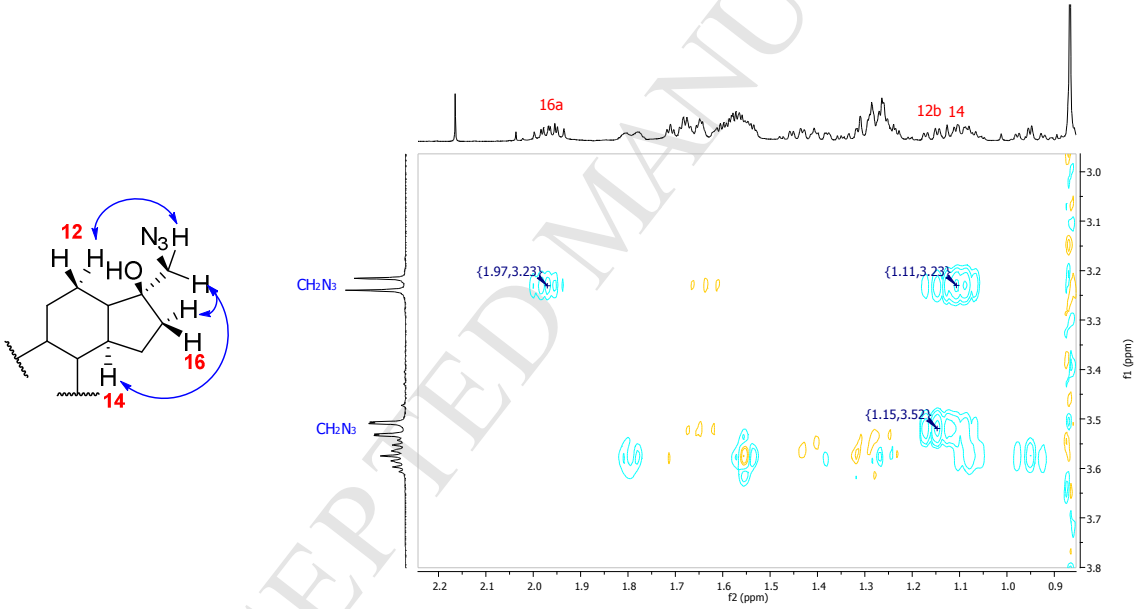
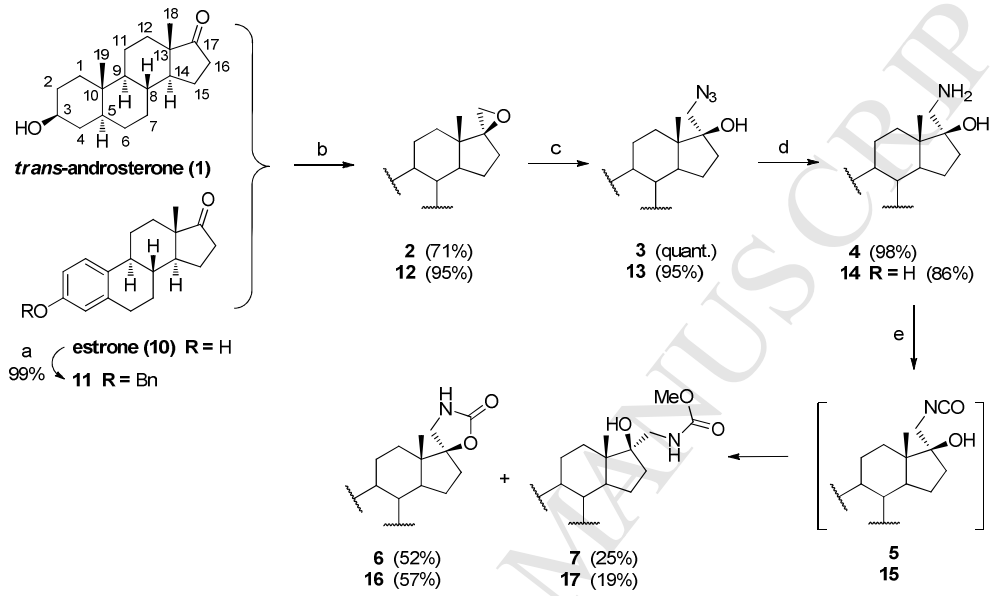


Figure 2. NOESY correlations between CH_2N_3 and H-12ax, H-14 and H-16α in **3**. Methyl group at C-13 has been omitted for clarity.

Azide **3** was reduced by catalytic hydrogenation at 1 atm and 3 h to get the aminomethylalcohol **4**, which in turn was treated with triphosgene, as a secure alternative to hazardous phosgene, in a MeOH-CH_2Cl_2 mixture to obtain transient isocyanate **5**; this compound underwent a spontaneous cyclization involving the nucleophilic attack of the free OH to the heterocumulene, affording the spirooxazolidin-2-one (spirocarbamate) **6**

(52%); the use of MeOH as solvent led also to carbamate **7** as a by-product (25%). Hence both products were tested to observe the differences in biological activity (Scheme 1). When the reaction was made using DMF as solvent, it did not proceed, even when the temperature and the base were changed.



a) BnBr, K_2CO_3, CH_3CN, reflux; b) SMe_3I, KO*t*Bu or NaH, DMF; c) NaN_3, H_3BO_3, DMF, reflux; d) Pd/C, H_2, 1 atm, MeOH-CH_2Cl_2; e) triphosgene, MeOH-$NaHCO_3$ aq or MeOH-CH_2Cl_2-$NaHCO_3$ aq.

Scheme 1. Synthesis of oxazolidin-2-ones (**6**,**16**) from *trans*-androsterone (**1**) and estrone (**10**).

The same reaction sequence was applied to get the estrone-derived spirocarbamate **16**; changing the nature of the A-ring (aromatic for estrone) might modulate the biological properties and thus, afford valuable structure-activity relationships.

For this purpose, the free OH on C-3 was first protected as its benzyl ether, and then, the functionalization of the C-17 was accomplished. Epoxide **12** [54] was obtained in a stereoselective fashion, using KO*t*Bu to form the sulfur ylide. Crystallization of **12** in a hexane-EtOAc mixture allowed the determination of the absolute configuration (*S*) of the new asymmetric carbon by single crystal X-ray diffraction (Cambridge Crystallographic Data Centre 1561885) (Figure 3).

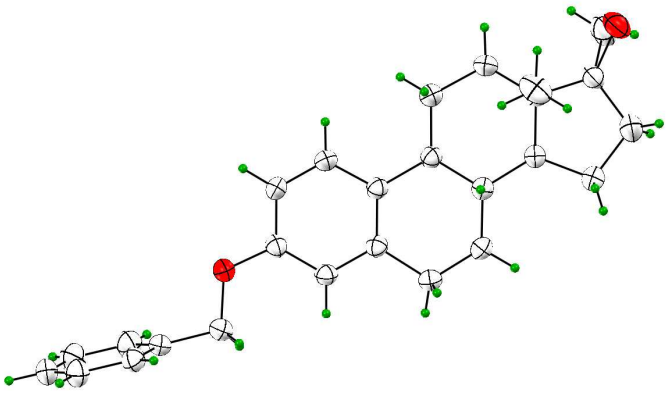


Figure 3. X-ray crystal structure of **12**.

The nucleophilic opening of **12** was carried out using sodium azide in DMF as indicated for the *trans*-androsterone derivative, but with a longer reaction time. The structure of azide **13** obtained by single crystal X-ray diffraction (hexane-EtOAc mixture), showed the configuration of C-17 as *S* (Figure 4) (Cambridge Crystallographic Data Centre 1561886).

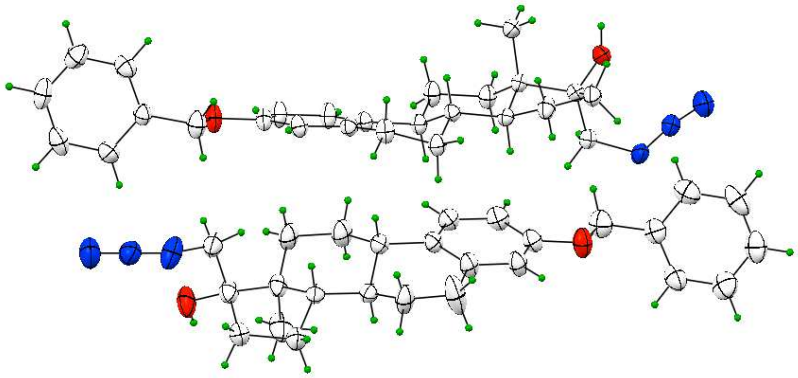


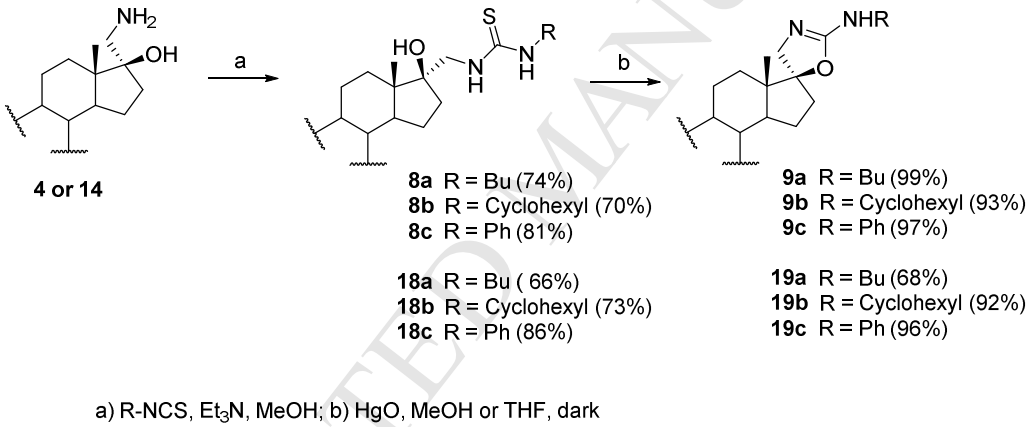
Figure 4. X-ray crystal structure of **13**.

Reduction of azide **13** under catalytic hydrogenation conditions also eliminated the benzyl group at C-3, giving amine **14** [54] in a 77% yield after 4 steps. Reaction between **14** and triphosgene gave isocyanate **15**, which was not isolated and spontaneously evolved to the final compound **16** and the by-product **17** in a 3:1 ratio (Scheme 1).

Aminoalcohols from *trans*-androsterone and estrone were also used to obtain 2-aminooxazolines. Derivatives **4** and **14** were treated separately with different isothiocyanates (butyl, cyclohexyl and phenyl) under basic conditions, giving thioureas **8** and **18** (Scheme 2). The cyclodesulfurization reaction of thioureas promoted by yellow

HgO afforded 2-aminooxazolines **9** and **19** with good to excellent yields (Scheme 2); a transient carbodiimide was reported [55] to be detected by TLC in the conversion of glycopyranosyl thioureas into the corresponding ureas, where a desulfurization reaction with HgO of the former was the key step. The same procedure was latter used for preparing spiranic isoureas and guanidines on a carbohydrate template [56]. Accordingly, it can be assumed that herein, HgO promotes the formation of a steroidal carbodiimide, which undergoes a nucleophilic attack of the free OH on C-17, thus leading to the spirocyclic moiety. To the best of our knowledge this is the first example of a spirocyclization reaction in steroids starting from thioureas.

Changes in the ^{13}C-NMR resonances of C-2' (roughly 180 ppm for **8**, **18** to roughly 160 ppm for **9**, **19**) supports such transformation).



Scheme 2. Synthesis of 2-aminooxazolines (**9**,**19**) from *trans*-androsterone (**1**) and estrone (**10**).

2.2. Antiproliferative activity

As a model to study the antiproliferative activity of compounds **3**,**4**, **6**-**9**, **13**, **14**, **16**-**19**, we selected the screening protocol of the National Cancer Institute [57]. The steroidal derivatives were tested against a panel of six human solid tumor cell lines A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast) and WiDr (colon). The results, expressed as 50% growth inhibition (GI_{50}), are given in Table 1. The anticancer drugs abiraterone and galeterone were also tested for comparison purposes.

According to the obtained GI_{50} values we can infer some structure activity relationships. Taking the data as a whole (Figure 5), we can observe that compounds from the estrone

series (**13-19**) were more potent than those from the androsterone set (**3-9**). In contrast, the GI_{50} ranges are larger in the estrone derivatives (denoting differences in selectivity against the cell lines) than in the androsterone compounds (indicating similar sensitivity of the cells to the compounds). The most potent compounds in both series were the 2-aminooxazoline derivatives **9** and **19** (low micromolar to submicromolar range), whilst compound **3** resulted inactive against all cell lines. When considering the side chain of the 2-aminooxazoline derivatives, the aliphatic substituents butyl (**9a**, **19a**) and cyclohexyl (**9b**, **19b**) are preferred over the aromatic phenyl group (**19c**). A similar activity pattern based on the side chain was observed for thioureas **8** and **18**. In both series, the order of activity according to the substituent at C-17 was established as 2-aminooxazoline (**9**, **19**) > spirocarbamate (**6**, **16**) > thiourea (**8**, **18**).

Noteworthy, the lead compounds **19a-b** showed more potent activity against the resistant cancer cell lines T-47D and WiDr, with GI_{50} values in the submicromolar range (0.34-0.71 µM). The relevance of this result is best understood if the GI_{50} values of the reference anticancer drugs abiraterone and galeterone against the same cell lines are compared.

In addition to tumor cell lines, we tested the most potent compounds of the series (**6**, **9a-b**, **19a-c**) against the (non-tumor) human fibroblasts cell line BJ-hTert in order to look for selectivity. Unfortunately, the normal cell line was sensitive to the compounds. This effect was more pronounced for the reference drugs, especially for abiraterone.

Table 1. Antiproliferative values of steroidal derivatives against human cancer cell lines.

	GI_{50} (µM)						
Compound	**A549**	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**	**BJ-hTert**
3	>100	>100	>100	>100	>100	>100	
4	17 ± 2.3	20 ± 1.9	22 ± 4.0	19 ± 4.7	21 ± 0.5	25 ± 3.9	
6	**3.7 ± 0.8**	**10 ± 4.5**	**4.0 ± 0.6**	**6.0 ± 3.2**	**4.5 ± 1.6**	**3.8 ± 1.9**	**<1**
7	38 ± 8.2	99 ± 1.3	70 ± 27	92 ± 9.5	41 ± 3.8	39 ± 13	
8a	12 ± 1.5	13 ± 2.5	14 ± 2.1	**9.4 ± 2.4**	**5.8 ± 1.0**	**4.5 ± 0.8**	
8b	16 ± 4.7	18 ± 3.4	21 ± 0.3	15 ± 0.3	18 ± 0.03	18 ± 1.8	
8c	29 ± 3.7	27 ± 5.9	27 ± 7.6	27 ± 6.7	34 ± 5.1	17 ± 6.5	
9a	**1.4 ± 0.4**	**2.1 ± 0.3**	**1.6 ± 0.1**	**1.2 ± 0.1**	**1.2 ± 0.3**	**0.87 ± 0.21**	**<1**
9b	**1.5 ± 0.2**	**2.0 ± 0.1**	**1.6 ± 0.1**	**1.5 ± 0.3**	**1.2 ± 0.2**	**0.96 ± 0.21**	**1.2 ± 0.3**

9c				[a]			
13	13 ± 4.0	22 ± 2.8	**2.9 ± 0.4**	29 ± 5.3	**4.1 ± 0.6**	**2.4 ± 0.2**	
14	39 ± 2.6	34 ± 3.7	27 ± 1.5	43 ± 10	30 ± 0.5	22 ± 2.2	
16	**5.4 ± 0.6**	**6.3 ± 0.5**	**1.3 ± 0.1**	18 ± 4.8	**4.4 ± 0.7**	**9.2 ± 1.7**	
17	**6.8 ± 0.6**	**4.0 ± 0.9**	**3.5 ± 0.2**	**5.9 ± 0.4**	**4.3 ± 1.1**	**4.9 ± 1.0**	
18a	**4.8 ± 1.8**	**7.2 ±2.0**	**4.2 ± 0.5**	15 ± 0.1	**3.9 ± 0.5**	**4.4 ± 0.8**	
18b	**4.5 ± 1.7**	**5.1 ± 1.4**	**3.3 ± 0.6**	14 ± 5.7	**3.9 ± 0.9**	**2.8 ± 0.2**	
18c	**9.8 ± 3.0**	15 ± 2.6	**6.3 ± 1.8**	21 ± 4.8	**5.7 ± 2.2**	**4.5 ± 0.2**	
19a	**1.4 ± 0.1**	**2.9 ± 0.4**	**2.1 ± 0.9**	**2.2 ± 0.8**	**0.45 ± 0.09**	**0.34 ± 0.13**	**<1**
19b	**1.1 ± 0.1**	**1.5 ± 0.2**	**1.3 ± 0.3**	**1.5 ± 0.2**	**0.71 ± 0.43**	**0.34 ± 0.21**	**<1**
19c	**2.6 ± 0.2**	**4.4 ± 0.6**	**3.3 ± 1.6**	**3.4 ± 0.7**	**2.6 ± 0.7**	**2.6 ± 0.8**	**1.4 ± 0.6**
Abiraterone	95 ± 8.0	>100	**7.9 ± 0.5**	85 ± 8.9	24 ± 4.5	42 ± 7.7	**4.5 ± 1.0**
Galeterone	**3.9 ± 1.3**	10 ± 0.9	**5.3 ± 0.4**	**3.9 ± 0.5**	**2.1 ± 0.1**	**2.7 ± 0.2**	**5.0 ± 1.3**

[a] Not soluble under the assay conditions

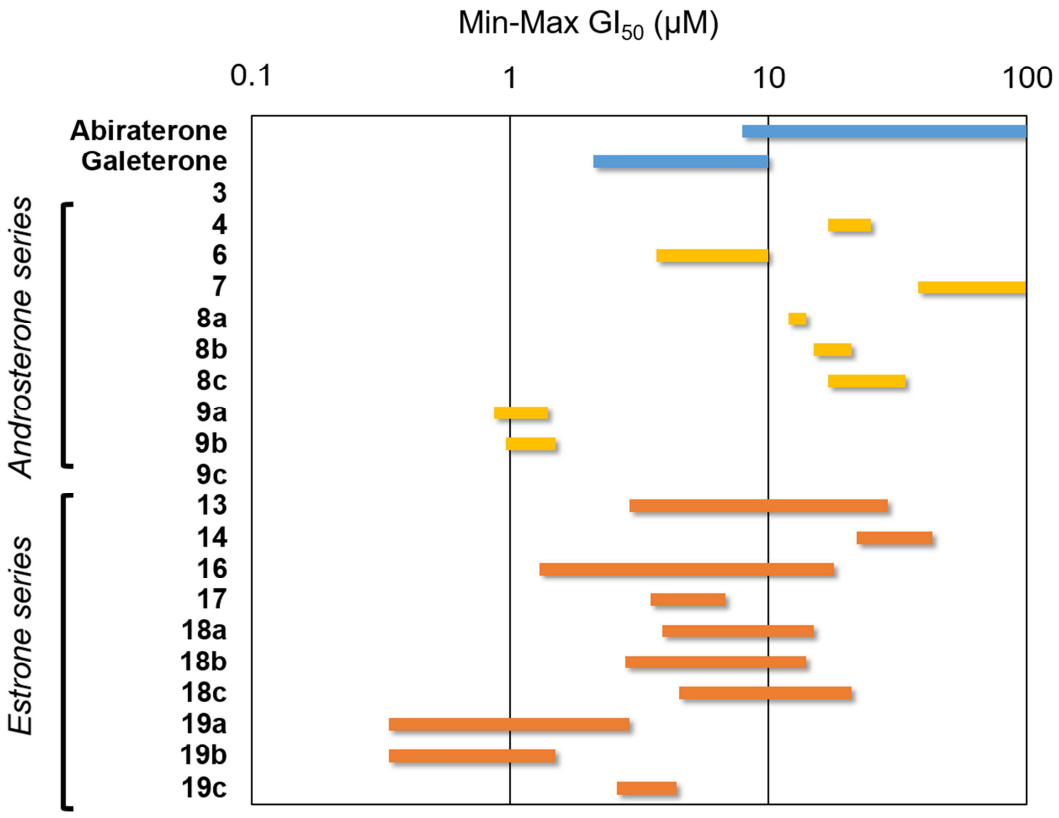


Figure 5. GI_{50} range plot of tested compounds.

3. Conclusions

A straightforward methodology has been developed to access the hitherto unknown spiranic steroids bearing oxazolidin-2-one and 2-aminooxazoline motifs at C-17, starting from *trans*-androsterone and estrone. The key intermediate was an aminomethyl alcohol functionality on C-17, which upon reaction with triphosgene, or with an alkyl/aryl isothiocyanate, followed by a cyclodesulfurization reaction, furnished title heterocyclic scaffolds.

Title compounds were tested as potential antiproliferative agents, and the order of activity was found to be aminooxazoline > spirocarbamate > thiourea. The lead compounds, bearing a spiranic aminooxazoline motif on estrone backbone, exhibited GI_{50} values in the low micromolar to submicromolar range (0.34-1.5 μM), with particular increase in activity against drug-resistant cell lines compared to steroidal chemotherapeutic agents (abiraterone and galeterone).

4. Experimental section

4.1. Materials and methods

4.1.1. General methods

Melting points were measured by the open capillary tube method on a Melt-temp apparatus and were not corrected. Optical rotations were measured on a Jasco P-2000 polarimeter. IR spectra were acquired on a Nicolet FT-IR 380 spectrophotometer ($\bar{\nu}_{max}$, cm^{-1}). NMR spectra were recorded on a Bruker Ascend 500 MHz instrument. Chemical shifts are reported in ppm (δ) and spectra were referenced to the residual protonated solvents: $CDCl_3$ (7.26 and 77.16 ppm), CD_3OD (3.31 and 49.0 ppm) and acetone-d_6 (2.05 and 29.84 ppm) for ^1H and ^{13}C NMR respectively. Coupling constants (*J*) are expressed in Hertz (Hz). ^1H and ^{13}C signals were assigned using 1D and 2D NMR experiments (DEPT, COSY, HSQC, HMBC and NOESY). High resolution mass spectra were obtained by EI, ESI and FAB using a Hewlett Packard 5989 A spectrometer coupled to a Hewlett Packard 5990 II gas chromatographer, a Micromass AutoSpec-Q and a JEOL JMS-700 MStation mass

spectrometers. The X-ray measurements were performed on a Xcalibur, Atlas, Gemini diffractometer. Column chromatography was performed using Merck silica gel 60 (230-400 mesh). TLC was performed using aluminum pre-coated silica gel plates 60 F_{254}; spots were visualized by UV light and charring with 5% H_2SO_4 (aq), 10% vanillin in EtOH containing 1% of H_2SO_4 or with 0.1% ninhydrin in EtOH.

4.1.2. Antiproliferative activity

The in vitro antiproliferative activity was evaluated using the sulforhodamine B (SRB) assay with slight modifications [58]. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Cells were inoculated onto 96-well plates in a volume of 100 µL per well at densities of 2 500 (A549, HBL-100, HeLa and SW1573), 5 000 (T-47D and WiDr) or 10 000 (BJ-hTert) cells per well, based on their doubling times. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicate at different dilutions in the range of 1–100 µM. The drug treatment was started on day 1 after plating. Drug incubation times comprised 48 h, after which time cells were precipitated with 25 µL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 ºC. Then the SRB assay was performed. The optical density (OD) of each well was measured at 530 nm, using BioTek's PowerWave XS microplate reader. Values were corrected for background OD from wells containing only medium. The percentage growth (PG) was calculated in relation to untreated control cells (C) at each of the drug concentration levels based on the difference in OD at the start (T_0) and end of drug exposure (T), according to NCI formulas Therefore, if T is greater than or equal to T_0, the calculation is 100 x $[(T–T_0)/(C–T_0)]$. If T is less than T_0, denoting cell killing, the calculation is 100 x $[(T–T_0)/(T_0)]$. The effect is defined as percentage of growth, where 50% growth inhibition (GI_{50}) represents the concentration at which PG is +50. With these calculations, a PG value of 0 represents no difference from the start of drug exposure, while negative PG values denote net cell death.

4.2 Chemistry

*4.2.1 (17S)-Spiro[5α-androstan-17,2'-oxiran]-3β-ol (**2**)* [53]. To a solution of SMe_3I (2.3 g, 11.27 mmol) in dry DMF (10 mL) was added NaH (1.0 g, 25.0 mmol, 60% in mineral oil) under Ar. The mixture was stirred for 20 min at room temperature and cooled to 0 °C. After that, a solution of *trans*-androsterone (**1)** (1.0 g, 3.44 mmol) in dry DMF (10 mL) was added. The mixture was stirred at 0 °C for 15 min and then at room temperature for 6 h. After that, it was cooled to 0°C and MeOH and H_2O were carefully added, the corresponding mixture was diluted with EtOAc and the organic layer was separated. Then it was washed with brine (4×10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. Column chromatography (9:1 hexane-EtOAc) gave **2** as a white solid (0.74 g, 71%); mp 165-167 °C. $[\alpha]_D^{20}$ +4 (*c* 1.0, $CHCl_3$). IR (cm^{-1}): 3452 (OH), 2917-2850 (C-H aliphatic), 1033 (C-O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.54 (m, 1H, H-3), 2.86 (d, 1H, J_{gem} = 5.1 Hz, CH_{2a} oxirane), 2.56 (d, 1H, J_{gem} = 5.1 Hz, CH_{2b} oxirane), 1.94 (ddd, 1H, $J_{H,H}$ = 14.2 Hz, $J_{H,H}$ = 11.7 Hz, $J_{H,H}$ = 2.0 Hz, H-16a), 1.76 (m, 1H, H-2a), 1.75 (m, 1H, H-16b), 1.69 (m, 1H, H-7a), 1.67 (m, 1H, H-15a), 1.66 (m, 1H, H-1a), 1.53 (m, 1H, H-4a), 1.52 (m, 1H, H-11a), 1.42 (m, 1H, H-8), 1.36 (m, 1H, H-2b), 1.35 (m, 1H, H-15b), 1.33 (m, 1H, H-12a), 1.25 (m, 3H, H-4b, H-6a, H-6b), 1.23 (m, 1H, H-11b), 1.20 (m, 1H, H-14), 1.07 (m, 1H, H-5), 0.98 (m, 1H, H-12b), 0.92 (m, 1H, H-1b), 0.87 (m, 1H, H-7b), 0.83 (s, 3H, H-18), 0.78 (s, 3H, H-19), 0.62 (ddd, 1H, $J_{H,H}$ = 12.3 Hz, $J_{H,H}$ = 10.7 Hz, $J_{H,H}$ = 4.1 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 71.4 (C-3), 70.7 (C-17), 54.5 (C-9), 53.8 (CH_2 oxirane), 53.0 (C-14), 45.0 (C-5), 40.3 (C-13), 38.3 (C-4), 37.1 (C-1), 35.8 (C-8), 35.7 (C-10), 34.1 (C-12), 31.6 (x2) (C-2, C-7), 29.2 (C-16), 28.7 (C-6), 23.7 (C-15), 20.7 (C-11), 14.5 (C-18), 12.4 (C-19). EI-MS *m*/*z* 304 ($[M]^+$, 14%); HREI-MS calcd for $C_{20}H_{32}O_2$ ($[M]^+$): 304.2397, found: 304.2406.

*4.2.2 17α-Azidomethyl-5α-androstane-3β,17β-diol (**3**).* To a solution of **2** (0.94 g, 3.09 mmol) in dry DMF (28 mL) were added NaN_3 (1.99 g, 30.61 mmol) and H_3BO_3 (0.8 g, 12.94 mmol) under Ar. The mixture was refluxed for 2 h, poured into water and extracted with EtOAc (3×20 mL). The organic layer was washed with brine (4×15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. Column chromatography (4:1 hexane-EtOAc) gave compound **3** as a white solid (1.07 g, quant.); mp 175-177 °C. $[\alpha]_D^{25}$ -13 (*c* 0.2, $CHCl_3$). IR (cm^{-1}): 3411, 3327 (OH), 2940-2847 (C-H aliphatic), 2107 (N=N=N), 1318, 1036 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.57 (m, 1H, H-3), 3.52 (dd, 1H, J_{gem} = 12.1 Hz, $J_{H,H}$ = 1.3 Hz, $CH_{2a}N_3$), 3.23 (d, 1H, J_{gem} = 12.1 Hz, $CH_{2b}N_3$), 1.97 (ddd, 1H, $J_{H,H}$ = 14.7 Hz, $J_{H,H}$ = 9.7 Hz, $J_{H,H}$ = 7.0 Hz, H-16a), 1.79 (m, 1H, H-2a), 1.70 (m, 1H, H-1a), 1.66 (m, 1H, H-7a), 1.65 (m, 1H, H-16b), 1.59 (m, 1H, H-12a), 1.58 (m, 1H, H-11a), 1.56 (m, 2H, H-15a, H-4a), 1.45 (m, 1H, H-8), 1.39 (m, 1H, H-2b), 1.30 (m, 1H, H-11b), 1.29 (m, 1H, H-15b), 1.28 (m, 1H, H-4b), 1.27 (m, 2H, H-6), 1.15 (m, 1H, H-12b), 1.10 (m, 1H, H-14),

1.08 (m, 1H, H-5), 0.95 (td, 1H, $J_{H,H}$ = 13.5 Hz, $J_{H,H}$ = 3.8 Hz, H-1b), 0.87 (s, 3H, H-18), 0.83 (m, 1H, H-7b), 0.81 (s, 3H, H-19), 0.59 (ddd, 1H, $J_{H,H}$ = 12.3 Hz, $J_{H,H}$ = 10.8 Hz, $J_{H,H}$ = 4.0 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 83.6 (C-17), 71.3 (C-3), 58.5 (CH_2N_3), 54.3 (C-9), 51.4 (C-14), 46.1 (C-13), 45.0 (C-5), 38.2 (C-4), 37.1 (C-1), 36.3 (C-8), 35.7 (C-10), 34.8 (C-16), 32.1 (C-12), 31.9 (C-7), 31.6 (C-2), 28.6 (C-6), 23.7 (C-15), 20.9 (C-11), 14.3 (C-18), 12.4 (C-19). HRFAB-MS calcd for $C_{20}H_{32}N_3O_2$ ($[M-H]^+$): 346.2489, found: 346.2491.

*4.2.3 17α-Aminomethyl-5α-androstane-3β,17β-diol (**4**).* A solution of **3** (1.07 g, 3.08 mmol) in a 7:3 MeOH-CH_2Cl_2 mixture (60 mL) was hydrogenated over 20% $Pd(OH)_2$/C (0.64 g) at 1 atm and room temperature for 3 h. The catalyst was removed by filtration through a pad of Celite® and the filtrate was concentrated to dryness. The residue was washed with cooled EtOAc to give **4** as a white solid (0.97 g, 98%); mp 260 °C (dec.). $[\alpha]_D^{25}$ +11 (*c* 0.2, MeOH). IR (cm^{-1}): 3288 (OH), 2921-2862 (C-H aliphatic), 1612 (NH), 1037 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD-$CDCl_3$) δ 3.51 (m, 1H, H-3), 2.97 (dd, 1H, J_{gem} = 12.6 Hz, $J_{H,H}$ = 1.2 Hz, CH_{2a}NH$_2$), 2.92 (d, 1H, J_{gem} = 12.6 Hz, CH_{2b}NH$_2$), 1.90 (ddd, 1H, $J_{H,H}$ = 16.1 Hz, $J_{H,H}$ = 9.5 Hz, $J_{H,H}$ = 6.6 Hz, H-16a), 1.76 (m, 1H, H-2a), 1.71 (m, 1H, H-1a), 1.70 (m, 1H, H-7a), 1.69 (m, 1H, H-16b), 1.66 (m, 1H, H-15a), 1.62 (m, 1H, H-11a), 1.61 (m, 1H, H-12a), 1.52 (m, 1H, H-4a), 1.49 (m, 1H, H-8), 1.39 (m, 1H, H-2b), 1.37 (m, 1H, H-15b), 1.35 (m, 1H, H-11b), 1.28 (m, 2H, H-6), 1.27 (m, 1H, H-4b), 1.14 (m, 1H, H-12b), 1.10 (m, 1H, H-5), 0.97 (td, 1H, $J_{H,H}$ = 14.0 Hz, $J_{H,H}$ = 4.1 Hz, H-1b), 0.89 (s, 3H, H-18), 0.84 (s, 3H, H-19), 0.65 (td, 1H, $J_{H,H}$ = 11.7 Hz, $J_{H,H}$ = 3.9 Hz, H-9). ^{13}C NMR (125.7 MHz, MeOD-$CDCl_3$) δ 81.6 (C-17), 71.5 (C-3), 55.2 (C-9), 52.0 (C-14), 47.0 (C-13), 46.4 (CH_2NH_2), 45.9 (C-5), 38.5 (C-4), 37.9 (C-1), 37.3 (C-8), 36.4 (C-10), 33.3 (C-16), 32.6 (C-7), 32.4 (C-12), 31.7 (C-2), 29.5 (C-6), 24.3 (C-15), 21.7 (C-11), 14.5 (C-18), 12.7 (C-19). FAB-MS *m*/*z* 322 ($[M+H]^+$, 32%); HRFAB-MS calcd for $C_{20}H_{36}NO_2$ ($[M+H]^+$): 322.2741, found: 322.2749.

*4.2.4 (17S)-2'-Oxo-spiro[3β-hydroxy-5α-androstan-17,5'-oxazolidine] (**6**) and methyl N-[(3β,17β-dihydroxy-5α-androstan-17α-yl)methyl]carbamate (**7**).* To a solution of **4** (0.1 g, 0.31 mmol) in a MeOH-CH_2Cl_2 mixture (2:1, 15 mL) were added saturated aqueous $NaHCO_3$ (5 mL) and triphosgene (37 mg, 0.12 mmol) at 0 °C. The suspension was vigorously stirred for 25 min at 0 °C, filtered and the solvent was evaporated. Column chromatography (1:1 hexane-EtOAc) gave **6** (56 mg, 52%) and **7** (29 mg, 25%) as white solids. Data for compound **6**: mp 220 °C (dec.). $[\alpha]_D^{22}$ -28 (*c* 0.4, MeOH). IR (cm^{-1}): 3427 (NH), 3200 (OH), 2925-2854 (C-H aliphatic), 1729 (C=O), 1284-1045 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD-$CDCl_3$) δ 3.57 (d, 1H, $J_{4'a,4'b}$ = 9.1 Hz, H-4'a),

3.42 (m, 1H, H-3), 3.11 (d, 1H, $J_{4'b,4'a}$ = 9.1 Hz, H-4'b), 2.14 (ddd, 1H, $J_{16a,16b}$ = 14.9 Hz, $J_{16a,H}$ = 12.8 Hz, $J_{16a,H}$ = 3.7 Hz, H-16a), 1.73 (ddd, 1H, $J_{16b,16a}$ = 14.9 Hz, $J_{16b,H}$ = 9.3 Hz, $J_{16b,H}$ = 5.8 Hz, H-16b), 1.65 (m, 1H, H-2a), 1.59 (m, 1H, H-1a), 1.57 (m, 1H, H-7a), 1.52 (m, 1H, H-15a), 1.49 (m, 1H, H-11a), 1.47 (m, 1H, H-12a), 1.42 (m, 1H, H-4a), 1.32 (m, 1H, H-8), 1.27 (m, 1H, H-2b), 1.23 (m, 1H, H-15b), 1.18 (m, 1H, H-11b), 1.18 (m, 1H, H-12b), 1.16 (m, 2H, H-6a, H-6b), 1.15 (m, 1H, H-4b), 0.97 (m, 1H, H-5), 0.84 (m, 1H, H-14), 0.83 (m, 1H, H-1b), 0.80 (s, 3H, H-18), 0.74 (m, 1H, H-7b), 0.69 (s, 3H, H-19), 0.49 (m, 1H, H-9). ^{13}C NMR (125.7 MHz, MeOD-$CDCl_3$) δ 160.4 (C-2'), 92.8 (C-17), 70.6 (C-3), 53.9 (C-9), 49.4 (C-4'), 49.3 (C-14), 45.3 (C-13), 44.7 (C-5), 37.5 (C-4), 36.9 (C-1), 35.7 (C-8), 35.4 (C-10), 35.3 (C-16), 31.4 (C-7), 31.0 (C-12), 30.8 (C-2), 28.3 (C-6), 22.4 (C-15), 20.4 (C-11), 14.2 (C-18), 12.1 (C-19). FAB-MS *m/z* 348 ($[M+H]^+$, 17%); HRFAB-MS calcd for $C_{21}H_{34}NO_3$ ($[M+H]^+$): 348.2533, found: 348.2541. Data for compound **7**: mp 75-77 °C. $[\alpha]_D^{20}$ -3 (*c* 0.1, $CHCl_3$). IR (cm^{-1}): 3404 (OH), 2920-2851 (C-H aliphatic), 1702 (C=O), 1038 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.19 (m, 1H, NH), 3.66 (s, 3H, OCH_3), 3.58 (m, 1H, H-3), 3.31 (dd, 1H, J_{gem} = 13.4 Hz, J = 8.1 Hz, CH_{2a}NH), 3.18 (d, 1H, J_{gem} = 13.4 Hz, C$H_{2b}NH_2$), 1.90 (ddd, 1H, $J_{H,H}$ = 14.1 Hz, $J_{H,H}$ = 9.9 Hz, $J_{H,H}$ = 6.8 Hz, H-16a), 1.79 (m, 1H, H-2a), 1.68 (td, 1H, $J_{H,H}$ = 13.2 Hz, $J_{H,H}$ = 3.4 Hz, H-1a), 1.64 (m, 1H, 7a), 1.56 (m, 1H, H-15a), 1.56 (m, 1H, H-11a), 1.54 (m, 1H, H-4a), 1.52 (m, 1H, H-12a), 1.48 (m, 1H, H-16b), 1.42 (m, 1H, H-8), 1.38 (m, 1H, H-2b), 1.28 (m, 1H, H-11b), 1.27 (m, 1H, H-4b), 1.25 (m, 2H, H-6a, H-6b), 1.23 (m, 1H, H-15b), 1.22 (m, 1H, H-12b), 1.21 (m, 1H, H-14), 1.08 (m, 1H, H-5), 0.95 (td, 1H, $J_{H,H}$ = 13.2 Hz, $J_{H,H}$ = 3.6 Hz, H-1b), 0.84 (m, 1H, H-7b), 0.84 (s, 3H, H-18), 0.80 (s, 3H, H-19), 0.61 (td, 1H, $J_{H,H}$ = 11.2 Hz, $J_{H,H}$ = 4.1 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 158.1 (C=O), 83.6 (C-17), 71.3 (C-3), 54.2 (C-9), 52.4 (OCH_3), 50.8 (C-14), 47.1 (CH_2NH), 45.8 (C-13), 44.9 (C-5), 38.2 (C-4), 37.1 (C-1), 36.4 (C-8), 35.6 (C-10), 34.2 (C-16), 31.8 (C-7), 31.7 (C-12), 31.5 (C-2), 28.7 (C-6), 23.6 (C-15), 20.9 (C-11), 14.2 (C-18), 12.4 (C-19). FAB-MS *m/z* 380 ($[M+H]^+$, 13%); HRFAB-MS calcd for $C_{22}H_{38}NO_4$ ($[M+H]^+$): 380.2796, found: 380.2795.

*4.2.5 General procedure for the synthesis of steroidal thioureas **8***

To a solution of **4** (0.2 g, 0.62 mmol) in MeOH (10 mL) were added the corresponding isothiocyanate (0.75 mmol) and Et_3N (50 μL, 0.36 mmol). The corresponding mixture was stirred during the time and at the temperature indicated in each case and the solvent was evaporated to dryness. The residue was purified by flash column chromatography to give compounds **8a-c** as white solids.

*4.2.5.1 17α-[(3'-Butylthioureido)methyl]-5α-androstane-3β,17β-diol (**8a**).* Butyl isothiocyanate was used (90 μL) and the reaction proceeded for 3 days at 45 °C. Column chromatography (3:2 hexane-EtOAc) afforded **8a** (0.20 g, 74%); mp 134-136 °C. $[\alpha]_D^{20}$ -7 (*c* 1.0, MeOH). IR (cm^{-1}): 3279 (OH), 2929-2857 (C-H aliphatic), 1551 (C-NH), 1522, 1348, 1037 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD) δ 3.96 (brs, 1H, CH_{2a}NH), 3.52 (m, 1H, H-3), 3.48 (m, 2H, NH-CH_2-CH_2), 3.34 (brs, 1H, CH_{2b}NH), 1.89 (m, 1H, H-16a), 1.76 (m, 1H, H-2a), 1.74 (m, 1H, H-1a), 1.71 (m, 1H, H-7a), 1.61 (m, 1H, H-11a), 1.60 (m, 1H, H-15a), 1.59 (m, 1H, H-12a), 1.56 (m, 3H, NH-CH_2-CH_2, H-16b), 1.53 (m, 1H, H-4a), 1.49 (m, 1H, H-8), 1.40 (m, 1H, H-2b), 1.38 (m, 3H, CH_2-CH_3, H-11b), 1.32 (m, 1H, H-14), 1.30 (m, 3H, H-6, H-12b), 1.29 (m, 2H, H-4b, H-15b), 1.14 (m, 1H, H-5), 1.02 (m, 1H, H-1b), 0.95 (t, 3H, $J_{H,H}$ = 7.4 Hz, CH_2-CH_3), 0.94 (m, 1H, H-7b), 0.87 (s, 3H, H-18), 0.86 (s, 3H, H-19), 0.70 (td, 1H, $J_{H,H}$ = 11.7 Hz, $J_{H,H}$ = 3.5 Hz, H-9). ^{13}C NMR (125.7 MHz, MeOD) δ 184.3 (C=S), 84.4 (C-17), 71.8 (C-3), 55.7 (C-9), 52.2 (C-14), 51.6 (CH_2NH), 47.2 (C-13), 46.2 (C-5), 45.1 (NH-CH_2-CH_2), 38.9 (C-4), 38.3 (C-1), 37.7 (C-8), 36.7 (C-10), 33.9 (C-16), 33.0 (C-7), 32.7 (C-12), 32.4 (NH-CH_2-CH_2), 32.1 (C-2), 29.9 (C-6), 24.6 (C-15), 22.0 (C-11), 21.1 (CH_2-CH_3), 14.9 (C-18), 14.2 (CH_2-CH_3), 12.8 (C-19). FAB-MS *m/z* 437 ($[M+H]^+$, 75%); HRFAB-MS calcd for $C_{25}H_{45}N_2O_2S$ ($[M+H]^+$): 437.3196, found: 437.3210.

*4.2.5.2 17α-[(3'-Cyclohexylthioureido)methyl]-5α-androstane-3β,17β-diol (**8b**).* Cyclohexyl isothiocyanate was used (0.11 mL) and the reaction proceeded for 56 h at 45 °C. Column chromatography (3:2 hexane-EtOAc) afforded **8b** (0.2 g, 70%); mp 165-167 ºC. $[\alpha]_D^{20}$ -7 (*c* 0.9, MeOH). IR (cm^{-1}): 3253 (OH), 2923-2854 (C-H aliphatic), 1554 (C-NH), 1523, 1348, 1024 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD) δ 4.06 (brs, 1H, H-1''), 3.98 (brs, 1H, CH_{2a}NH), 3.52 (m, 1H, H-3), 3.33 (m, 1H, CH_{2b}NH), 1.97 (m, 2H, H-2''a, H-6''a), 1.89 (m, 1H, H-16a), 1.76 (m, 1H, H-2a), 1.73 (m, 2H, H-3''a, H-5''a), 1.72 (m, 1H, H-1a), 1.70 (m, 1H, H-7a), 1.62 (m, 1H, H-4''a), 1.60 (m, 1H, H-11a), 1.59 (m, 1H, H-15a), 1.57 (m, 1H, H-12a), 1.53 (m, 1H, H-16b), 1.52 (m, 1H, H-4a), 1.47 (m, 1H, H-8), 1.39 (m, 1H, H-2b), 1.37 (m, 2H, H-3''b, H-5''b), 1.33 (m, 1H, H-11b), 1.30 (m, 1H, H-14), 1.29 (m, 1H, H-12b), 1.28 (m, 3H, H-6, H-15b), 1.27 (m, 1H, H-4b), 1.20 (m, 3H, H-2''b, H-6''b, H-4''b), 1.12 (m, 1H, H-5), 0.99 (m, 1H, H-1b), 0.92 (m, 1H, H-7b), 0.87 (s, 3H, H-18), 0.85 (s, 3H, H-19), 0.69 (td, 1H, $J_{H,H}$ = 11.6 Hz, $J_{H,H}$ = 3.5 Hz, H-9). ^{13}C NMR (125.7 MHz, MeOD) δ 183.1 (C=S), 84.3 (C-17), 71.6 (C-3), 55.4 (C-9), 53.7 (C-1''), 51.9 (C-14), 51.4 (CH_2NH), 47.0 (C-13), 46.0 (C-5), 38.7 (C-4), 38.1 (C-1), 37.5 (C-8), 36.5 (C-10), 33.7 (x2) (C-2'', C-6''), 33.7 (C-16), 32.8 (C-7), 32.5 (C-12), 31.9 (C-2), 29.7 (C-6), 26.5 (C-4''), 25.9 (x2) (C-3'', C-5''), 24.5 (C-15), 21.8 (C-11), 14.7 (C-18), 12.7 (C-19). HRFAB-MS calcd for $C_{27}H_{47}N_2O_2S$ ($[M+H]^+$): 463.3353, found: 463.3386.

*4.2.5.3 17α-[(3'-Phenylthioureido)methyl]-5α-androstane-3β,17β-diol (**8c**).* Phenyl isothiocyanate was used (90 μL) and the reaction proceeded for 30 min at room temperature. Column chromatography (7:3 hexane-EtOAc) afforded **8c** (0.23 g, 81%); mp 280 °C (dec.). $[\alpha]_D^{20}$ -11 (*c* 0.5, MeOH). IR (cm^{-1}): 3204 (OH), 2920-2840 (C-H aliphatic), 1598 (C=C), 1528 (C-NH), 1523, 1026 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD) δ 7.36 (m, 4H, Ar-H*o*, Ar-H*m*), 7.22 (m, 1H, Ar-H*p*), 4.04 (d, 1H, J_{gem} = 13.2 Hz, CH_{2a}NH), 3.52 (m, 1H, H-3), 3.40 (d, 1H, J_{gem} = 13.2 Hz, CH_{2b}NH), 1.90 (m, 1H, H-16a), 1.77 (m, 1H, H-2a), 1.73 (m, 1H, H-1a), 1.72 (m, 1H, H-7a), 1.63 (m, 1H, H-15a), 1.62 (m, 1H, H-11a), 1.58 (m, 1H, H-16b), 1.56 (m, 1H, H-12a), 1.53 (m, 1H, H-4a), 1.50 (m, 1H, H-8), 1.40 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.33 (m, 1H, H-14), 1.30 (m, 4H, H-6a, H-6b, H-15b, H-12b), 1.28 (m, 1H, H-4b), 1.15 (m, 1H, H-5), 1.01 (m, 1H, H-1b), 0.95 (m, 1H, H-7b), 0.85 (s, 6H, H-18, H-19), 0.70 (td, 1H, $J_{H,H}$ = 11.3 Hz, $J_{H,H}$ = 3.2 Hz, H-9). ^{13}C NMR (125.7 MHz, MeOD) δ 182.2 (C=S), 139.3 (Ar-C*ipso*), 130.5 (x2) (Ar-C), 127.0 (Ar-C*p*), 125.5 (x2) (Ar-C), 84.3 (C-17), 71.8 (C-3), 55.7 (C-9), 52.2 (CH_2NH), 52.2 (C-14), 47.1 (C-13), 46.2 (C-5), 38.9 (C-4), 38.2 (C-1), 37.7 (C-8), 36.7 (C-10), 34.1 (C-16), 33.0 (C-7), 32.9 (C-12), 32.1 (C-2), 29.9 (C-6), 24.6 (C-15), 22.0 (C-11), 14.8 (C-18), 12.7 (C-19). HRFAB-MS calcd for $C_{27}H_{41}N_2O_2S$ ($[M+H]^+$): 457.2883, found: 457.2892.

*4.2.6 General procedure for the synthesis of steroidal spiranic isoureas **9***

To a solution of the corresponding thiourea **8** (0.25 mmol) in dry THF (10 mL) was added yellow HgO (4.50 mmol for compounds **8a**,**b** and 2.29 mmol for compound **8c**) under Ar. The mixture was stirred at room temperature in the dark during the time indicated in each case, filtered over a pad of Celite® and evaporated to dryness. The residue was washed with hexane or purified by flash column chromatography, as indicated in each case, to give compounds **9a-c** as white foams.

*4.2.6.1 (17S)-2'-n-Butylamino-spiro[3β-hydroxy-5α-androstan-17,5'-oxazoline] (**9a**).* Thiourea **8a** was used (0.11 g) and the reaction proceeded for 6 days to give **9a** (0.10 g, 99%). IR (cm^{-1}): 3226 (OH), 2928-2856 (C-H aliphatic), 1650 (C=N), 1046 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.05 (brs, 1H, NH), 3.81 (d, 1H, $J_{4'a,4'b}$ = 12.2 Hz, H-4'a), 3.51 (m, 1H, H-3), 3.28 (d, 1H, $J_{4'b,4'a}$ = 12.2 Hz, H-4'b), 3.10 (m, 2H, H-1''), 2.14 (ddd, 1H, $J_{H,H}$ = 14.4 Hz, $J_{H,H}$ = 12.5 Hz, $J_{H,H}$ = 3.6 Hz, H-16a), 1.74 (m, 2H, H-16b, H-2a), 1.65 (m, 1H, H-1a), 1.63 (m, 1H, H-7a), 1.55 (m, 1H, H-15a), 1.53 (m, 1H, H-11a), 1.50 (m, 1H, H-4a), 1.45 (m, 2H, H-2''), 1.39 (m, 1H, H-12a), 1.34 (m, 1H, H-2b), 1.33 (m, 1H, H-8), 1.29 (m, 2H, H-3''), 1.28 (m, 1H, H-12b), 1.23 (m, 2H, H-15b, H-4b), 1.22 (m, 3H, H-11b, H-6), 1.04 (m, 1H, H-5), 0.91 (m, 1H, H-1b), 0.90 (m, 1H, H-14), 0.86 (t, 3H,

$J_{4'',3''}$ = 7.4 Hz, H-4''), 0.82 (s, 3H, H-18), 0.81 (m, 1H, H-7b), 0.76 (s, 3H, H-19), 0.56 (td, 1H, $J_{H,H}$ = 11.9 Hz, $J_{H,H}$ = 4.0 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 160.8 (C-2'), 96.0 (C-17), 70.8 (C-3), 60.5 (C-4'), 54.2 (C-9), 49.6 (C-14), 45.0 (C-13), 45.0 (C-5), 42.4 (C-1''), 38.1 (C-4), 37.2 (C-1), 35.9 (C-8), 35.6 (C-10), 35.2 (C-16), 32.0 (C-2''), 31.6 (C-7), 31.5 (C-2), 31.2 (C-12), 28.5 (C-6), 22.6 (C-15), 20.6 (C-11), 20.0 (C-3''), 14.6 (C-18), 13.8 (C-4''), 12.4 (C-19). HRFAB-MS calcd for $C_{25}H_{43}N_2O_2$ ($[M+H]^+$): 403.3319, found: 403.3313.

*4.2.6.2. (17S)-2'-Cyclohexylamino-spiro[3β-hydroxy-5α-androstan-17,5'-oxazoline] (**9b**).* Thiourea **8b** was used (0.12 g) and the reaction proceeded for 3 days to give **9b** (0.1 g, 93%). $[\alpha]_D^{20}$ -21 (*c* 1.0, $CHCl_3$). IR (cm^{-1}): 3286 (OH), 2924-2853 (C-H aliphatic), 1654 (C=N), 1044 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (d, 1H, $J_{4'a,4'b}$ = 12.4 Hz, H-4'a), 3.56 (m, 1H, H-3), 3.35 (m, 1H, H-1''), 3.32 (d, 1H, $J_{4'b,4'a}$ = 12.4 Hz, H-4'b), 2.17 (ddd, 1H, $J_{H,H}$ = 14.0 Hz, $J_{H,H}$ = 12.4 Hz, $J_{H,H}$ = 3.8 Hz, H-16a), 1.96 (m, 2H, H-2''a, H-6''a), 1.78 (m, 2H, H-2a, H-16b), 1.69 (m, 1H, H-1a), 1.68 (m, 2H, H-3''a, H-5''a), 1.67 (m, 1H, H-7a), 1.58 (m, 1H, H-15a), 1.57 (m, 1H, H-4''a), 1.56 (m, 1H, H-11a), 1.55 (m, 1H, H-4a), 1.43 (m, 1H, H-12a), 1.38 (m, 1H, H-2b), 1.36 (m, 1H, H-8), 1.34 (m, 1H, H-12b), 1.31 (m, 2H, H-3''b, H-5''b), 1.27 (m, 2H, H-15b, H-4b), 1.25 (m, 3H, H-6a, H-6b, H-11b), 1.13 (m, 1H, H-4''b), 1.10 (m, 2H, H-2''b, H-6''b), 1.08 (m, 1H, H-5), 0.95 (m, 1H, H-1b), 0.94 (m, 1H, H-14), 0.85 (s, 3H, H-18), 0.84 (m, 1H, H-7b), 0.79 (s, 3H, H-19), 0.60 (td, 1H, $J_{H,H}$ = 11.8 Hz, $J_{H,H}$ = 4.1 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 159.8 (C-2'), 95.8 (C-17), 71.2 (C-3), 60.9 (C-4'), 54.2 (C-9), 51.5 (C-1''), 49.6 (C-14), 45.1 (C-13), 45.0 (C-5), 38.2 (C-4), 37.2 (C-1), 36.0 (C-8), 35.6 (C-10), 35.3 (C-16), 33.8 (C-2'' or C-6''), 33.7 (C-2'' or C-6''), 31.6 (C-7), 31.6 (C-2), 31.2 (C-12), 28.6 (C-6), 25.7 (C-4''), 25.0 (x2) (C-3'', C-5''), 22.7 (C-15), 20.7 (C-11), 14.7 (C-18), 12.5 (C-19). FAB-MS *m/z* 429 ($[M+H]^+$, 100%); HRFAB-MS calcd for $C_{27}H_{45}N_2O_2$ ($[M+H]^+$): 429.3476, found: 429.3508.

*4.2.6.3. (17S)-2'-Phenylamino-spiro[3β-hydroxy-5α-androstan-17,5'-oxazoline] (**9c**).* Thiourea **8c** was used (0.12 g) and the reaction proceeded for 19 h. Column chromatography (CH_2Cl_2 → 20:1 CH_2Cl_2-Isopropanol) afforded **9c** (0.10 g, 97%). $[\alpha]_D^{20}$ +2 (*c* 1.0, $CHCl_3$). IR (cm^{-1}): 3282 (OH), 2920-2853 (C-H aliphatic), 1658 (C=N), 1594-1447 (C=C), 1040 (C-O, C-N), 748-691 (=C-H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (m, 4H, Ar-H*o*, Ar-H*m*), 7.0 (m, 1H, Ar-H*p*), 4.85 (brs, 1H, NH), 3.91 (d, 1H, $J_{4'a,4'b}$ = 11.1 Hz, H-4'a), 3.59 (m, 1H, H-3), 3.40 (d, 1H, $J_{4'b,4'a}$ = 11.1 Hz, H-4'b), 2.29 (ddd, 1H, $J_{16a,16b}$ = 14.6 Hz, $J_{16a,H}$ = 12.4 Hz, $J_{16a,H}$ = 3.7 Hz, H-16a), 1.88 (ddd, 1H, $J_{16b,16a}$ = 14.6 Hz, $J_{16b,H}$ = 9.5 Hz, $J_{16b,H}$ = 5.8 Hz, H-16b), 1.80 (m, 1H, H-2a), 1.72 (m, 1H, H-1a), 1.71 (m, 1H, H-7a), 1.65 (m, 1H, H-15a), 1.60 (m, 1H, H-11a), 1.57 (m, 2H, H-12a, H-4a), 1.43 (m, 1H, H-8),

1.41 (m, 1H, H-2b), 1.36 (m, 1H, H-12b), 1.35 (m, 1H, H-15b), 1.31 (m, 1H, H-11b), 1.30 (m, 1H, H-4b), 1.28 (m, 2H, H-6), 1.10 (m, 1H, H-5), 0.99 (m, 1H, H-14), 0.97 (m, 1H, H-1b), 0.96 (s, 3H, H-18), 0.87 (qd, 1H, $J_{H,H}$ = 12.1 Hz, $J_{H,H}$ = 5.3 Hz, H-7b), 0.82 (s, 3H, H-19), 0.63 (td, 1H, $J_{H,H}$ = 11.6 Hz, $J_{H,H}$ = 3.9 Hz, H-9). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.1 (C-2'), 129.1 (x3) (Ar-C), 122.7 (x2) (Ar-C), 120.0 (Ar-C), 96.5 (C-17), 71.2 (C-3), 56.4 (C-4'), 54.1 (C-9), 49.9 (C-14), 45.4 (C-13), 44.9 (C-5), 38.2 (C-4), 37.1 (C-1), 36.0 (C-8), 35.6 (C-10), 35.4 (C-16), 31.6 (C-7), 31.5 (C-2), 31.4 (C-12), 28.5 (C-6), 22.8 (C-15), 20.6 (C-11), 14.8 (C-18), 12.5 (C-19). HRFAB-MS calcd for $C_{27}H_{39}N_2O_2$ ($[M+H]^+$): 423.3006, found: 423.3004.

*4.2.7. (17S)-Spiro[3-benzyloxyestra-1,3,5(10)-trien-17,2'-oxirane] (**12**).* SMe_3I (0.63 g, 3.09 mmol) and KO*t*Bu (0.47 g, 4.24 mmol) were added to a solution of **11** (0.69 g, 1.91 mmol) in dry DMF (16 mL) under Ar. The reaction was stirred at 0 °C for 10 min and then at room temperature for 45 min. After this time, cold water was added and the product was extracted with diethyl ether. The organic phase was washed with brine (3×20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. Column chromatography (19:1 hexane-EtOAc) gave compound **12** as white crystals (0.68 g, 95%); mp 154-156 °C (lit. 155-157 °C) [54]. $[\alpha]_D^{25}$ +45 (*c* 0.4, $CHCl_3$). IR (cm^{-1}): 3027 (C-H aromatic), 2919-2862 (C-H aliphatic), 1602-1495 (C=C), 1233-1016 (C-O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (m, 2H, Ar-H*o*), 7.40 (m , 2H, Ar-H*m*), 7.34 (m, 1H, Ar-H*p*), 7.21 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 6.80 (dd, 1H, $J_{2,1}$ = 8.5 Hz, $J_{2,4}$ = 2.7 Hz, H-2), 6.75 (d, 1H, $J_{4,2}$ = 2.7 Hz, H-4), 5.05 (s, 2H, CH_2-Ph), 2.98 (d, 1H, J_{gem} = 5.1 Hz, CH_{2a} oxirane), 2.90 (m, 2H, H-6), 2.66 (d, 1H, J_{gem} = 5.1 Hz, CH_{2b} oxirane), 2.32 (m, 1H, H-11a), 2.24 (m, 1H, H-9), 2.06 (m, 1H, H-16a), 1.96 (m, 1H, H-7a), 1.87 (m, 1H, H-16b), 1.86 (m, 1H, H-15a), 1.55 (m, 1H, H-12a), 1.53 (m, 1H, H-8), 1.53 (m, 1H, H-15b), 1.52 (m, 1H, H-14), 1.50 (m, 1H, H-11b), 1.40 (m, 1H, H-7b), 1.28 (m, 1H, H-12b), 0.94 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.9 (C-3), 138.1 (C-5), 137.4 (Ar-C*ipso*), 132.8 (C-10), 128.7 (x2) (Ar-C*m*), 128.0 (Ar-C*p*), 127.6 (x2) (Ar-C*o*), 126.4 (C-1), 114.9 (C-4), 112.4 (C-2), 70.7 (C-17), 70.0 (CH_2-Ph), 53.8 (CH_2 oxirane), 51.9 (C-14), 44.0 (C-9), 40.5 (C-13), 39.0 (C-8), 34.0 (C-12), 29.9 (C-6), 29.2 (C-16), 27.3 (C-7), 26.1 (C-11), 23.4 (C-15), 14.4 (C-18). HRESI-MS calcd for $C_{26}H_{31}O_2$ ($[M+H]^+$): 375.2319, found: 375.2311.

*4.2.8. 17α-Azidomethyl-3-benzyloxyestra-1,3,5(10)-trien-17β-ol (**13**).* To a solution of **12** (1.84 g, 4.91 mmol) in dry DMF (50 mL) were added NaN_3 (3.19 g, 49.07 mmol) and H_3BO_3 (1.28 g, 20.70 mmol) under Ar. The mixture was refluxed for 4 h, poured into water and extracted with diethyl ether (4×25 mL). The organic phase was washed with brine (3×15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. Column chromatography (19:1 hexane-EtOAc) gave

compound **13** as white crystals (1.95 g, 95%); mp 92-93 °C (lit 96-98 °C) [54]. $[\alpha]_D^{25}$ +26 (*c* 1.0, $CHCl_3$). IR (cm^{-1}): 3434 (OH), 3020 (C-H aromatic), 2928-2865 (C-H aliphatic), 2093 (N=N=N), 1609-1450 (C=C), 1237-1018 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (m, 2H, Ar-H*o*), 7.41 (m, 2H, Ar-H*m*), 7.35 (m, 1H, Ar-H*p*), 7.22 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 6.82 (dd, 1H, $J_{2,1}$ = 8.6 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.75 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 5.06 (s, 2H, CH_2-Ph), 3.62 (d, 1H, J_{gem} = 12.1 Hz, $CH_{2a}N_3$), 3.32 (d, 1H, J_{gem} = 12.1 Hz, $CH_{2b}N_3$), 2.89 (m, 2H, H-6a, H-6b), 2.35 (m, 1H, H-11a), 2.19 (m, 1H, H-9), 2.08 (ddd, 1H, $J_{H,H}$ = 15.8 Hz, $J_{H,H}$ = 9.2 Hz, $J_{H,H}$ = 5.8 Hz, H-16a), 1.91 (m, 1H, H-7a), 1.78 (dt, 1H, $J_{H,H}$ = 12.5 Hz, $J_{H,H}$ = 3.1 Hz, H-12a), 1.74 (m, 1H, H-16b), 1.72 (m, 1H, H-15a), 1.55 (m, 1H, H-11b), 1.53 (m, 1H, H-8), 1.45 (m, 1H, H-15b), 1.42 (m, 1H, H-12b), 1.38 (m, 1H, H-14), 1.35 (m, 1H, H-7b), 0.95 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.8 (C-3), 138.0 (C-5), 137.3 (Ar-C*ipso*), 132.6 (C-10), 128.6 (x2) (Ar-C*m*), 128.0 (Ar-C*p*), 127.5 (x2) (Ar-C*o*), 126.4 (C-1), 114.8 (C-4), 112.3 (C-2), 83.6 (C-17), 70.0 (CH_2Ph), 58.4 (CH_2N_3), 50.3 (C-14), 46.2 (C-13), 43.8 (C-9), 39.4 (C-8), 34.8 (C-16), 32.0 (C-12), 29.8 (C-6), 27.5 (C-7), 26.2 (C-11), 23.3 (C-15), 14.2 (C-18). HRESI-MS calcd for $C_{26}H_{31}N_3NaO_2$ ($[M+Na]^+$): 440.2308, found: 440.2298.

*4.2.9. 17α-Aminomethylestra-1,3,5(10)-triene-3,17β-diol (**14**).* A solution of **13** (0.5 g, 1.20 mmol) in a 7:3 MeOH-CH_2Cl_2 mixture (30 mL) was hydrogenated over 10% Pd/C (0.25 g) at 1 atm and room temperature for 25 h. The catalyst was removed by filtration through a pad of Celite® and the filtrate was concentrated to dryness, dissolved with MeOH and precipitated with CH_2Cl_2. The compound was filtered and washed with cooled diethyl ether to give **14** as a white solid (0.31 g, 86%); mp 254 °C (dec.) (lit. 242-243 °C) [54]. $[\alpha]_D^{25}$ +48 (*c* 0.3, MeOH). IR (cm^{-1}): 3471 (NH), 3200 (OH), 3060 (C-H aromatic), 2920-2860 (C-H aliphatic), 1614 (NH), 1600-1494 (C=C), 1241-1018 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD) δ 7.07 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.54 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.48 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 3.04 (s, 2H, CH_2NH$_2$), 2.78 (m, 2H, H-6a, H-6b), 2.33 (m, 1H, H-11a), 2.12 (m, 1H, H-9), 1.98 (ddd, 1H, $J_{H,H}$ = 14.3 Hz, $J_{H,H}$ = 9.2 Hz, $J_{H,H}$ = 5.3 Hz, H-16a), 1.88 (m, 1H, H-7a), 1.78 (m, 1H, H-15a), 1.76 (m, 1H, H-16b), 1.74 (m, 1H, H-12a), 1.49 (m, 1H, H-11b), 1.46 (m, 1H, H-15b), 1.45 (m, 1H, H-8), 1.44 (m, 1H, H-14), 1.40 (m, 1H, H-12b), 1.30 (m, 1H, H-7b), 0.95 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, MeOD) δ 156.0 (C-3), 138.7 (C-5), 132.2 (C-10), 127.2 (C-1), 116.1 (C-4), 113.8 (C-2), 81.9 (C-17), 51.1 (C-14), 47.5 (C-13), 46.7 (CH_2NH_2), 44.9 (C-9), 41.1 (C-8), 33.6 (C-16), 32.6 (C-12), 30.6 (C-6), 28.6 (C-7), 27.4 (C-11), 24.2 (C-15), 14.6 (C-18). HRESI-MS calcd for $C_{19}H_{28}NO_2$ ($[M+H]^+$): 302.2115, found: 302.2110.

*4.2.10. (17S)-2'-Oxo-spiro[3-hydroxyestra-1,3,5(10)-trien-17,5'-oxazolidine] (**16**) and methyl N-[(3,17β-dihydroxyestra-1,3,5(10)-trien-17α-yl)methyl]carbamate (**17**).* To a solution of **14** (0.15 g, 0.51 mmol) in MeOH (20 mL) were added a saturated aqueous solution of $NaHCO_3$ (5 mL) and triphosgene (53 mg, 0.18 mmol) at 0 °C. The resulting suspension was vigorously stirred for 15 min at 0 °C and then filtered. The filtrate was evaporated and the residue was purified by flash column chromatography (7:3 hexane-EtOAc) to give **16** (96 mg, 57%) and **17** (35 mg, 19%) as white solids. Data for compound **16**: mp 270 °C (dec.). $[\alpha]_D^{22}$ -2 (*c* 0.6, MeOH). IR (cm^{-1}): 3371 (NH), 3259 (OH), 2922-2862 (C-H aliphatic), 1730 (C=O), 1618-1492 (C=C), 1285-1063 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD-$CDCl_3$) δ 7.07 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.58 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.1 Hz, H-2), 6.52 (d, 1H, $J_{4,2}$ = 2.1 Hz, H-4), 3.74 (d, 1H, $J_{4'a,4'b}$= 9.3 Hz, H-4'a), 3.27 (d, 1H, $J_{4'b,4'a}$ = 9.3 Hz, H-4'b), 2.78 (m, 2H, H-6a, H-6b), 2.31 (m, 1H, H-11a), 2.27 (m, 1H, H-16a), 2.11 (td, 1H, $J_{H,H}$ = 11.2 Hz, $J_{H,H}$ = 4.0 Hz, H-9), 1.90 (ddd, 1H, $J_{H,H}$ = 14.8 Hz, $J_{H,H}$ = 9.4 Hz, $J_{H,H}$ = 5.6 Hz, H-16b), 1.85 (m, 1H, H-7a), 1.73 (m, 1H, H-15a), 1.69 (m, 1H, H-12a), 1.51 (m, 1H, H-12b), 1.47 (m, 1H, H-11b), 1.43 (m, 1H, H-15b), 1.42 (m, 1H, H-8), 1.28 (m, 1H, H-7b), 1.18 (m, 1H, H-14), 0.94 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, MeOD-$CDCl_3$) δ 161.2 (C-2'), 155.0 (C-3), 138.3 (C-5), 131.4 (C-10), 126.8 (C-1), 115.7 (C-4), 113.3 (C-2), 93.4 (C-17), 50.1 (C-4'), 48.8 (C-14), 46.2 (C-13), 44.0 (C-9), 39.7 (C-8), 35.8 (C-16), 31.6 (C-12), 30.0 (C-6), 27.7 (C-7), 26.5 (C-11), 22.7 (C-15), 14.6 (C-18). HRESI-MS calcd for $C_{20}H_{25}NNaO_3$ ($[M+Na]^+$): 350.1726, found: 350.1724. Data for compound **17**: mp 164-166 °C. $[\alpha]_D^{23}$ +49 (*c* 0.5, MeOH). IR (cm^{-1}): 3292 (OH), 2920-2870 (C-H aliphatic), 1700 (C=O), 1602-1523 (C=C), 1212-1058 (C-O, C-N). ^{1}H NMR (500 MHz, MeOD) δ 7.07 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.54 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.48 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 3.66 (s, 3H, OCH_3), 3.36 (d, 1H, J_{gem} = 13.6 Hz, CH_{2a}NH), 3.18 (dd, 1H, J_{gem} = 13.6 Hz, $J_{H,H}$ = 1.1 Hz, CH_{2b}NH), 2.77 (m, 2H, H-6a, H-6b), 2.29 (m, 1H, H-11a), 2.12 (m, 1H, H-9), 1.91 (ddd, 1H, $J_{H,H}$ = 16.1 Hz, $J_{H,H}$ = 9.6 Hz, $J_{H,H}$ = 6.6 Hz, H-16a), 1.87 (m, 1H, H-7a), 1.70 (m, 1H, H-12a), 1.69 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.49 (m, 1H, H-14), 1.48 (m, 1H, H-12b), 1.46 (m, 1H, H-11b), 1.45 (m, 1H, H-8), 1.39 (m, 1H, H-15b), 1.30 (m, 1H, H-7b), 0.91 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, MeOD) δ 160.3 (C=O), 155.9 (C-3), 138.8 (C-5), 132.5 (C-10), 127.2 (C-1), 116.1 (C-4), 113.7 (C-2), 84.2 (C-17), 52.7 (OCH_3), 51.2 (C-14), 48.2 (CH_2NH), 47.3 (C-13), 45.1 (C-9), 41.2 (C-8), 33.5 (C-16), 32.6 (C-12), 30.7 (C-6), 28.7 (C-7), 27.5 (C-11), 24.2 (C-15), 14.8 (C-18). HRESI-MS calcd for $C_{21}H_{29}NNaO_4$ ($[M+Na]^+$): 382.1989, found: 382.1987.

*4.2.11. General procedure for the synthesis of steroidal thioureas **18***

To a solution of **14** (0.25 g, 0.83 mmol) in MeOH (15 mL) were added the corresponding isothiocyanate (1.0 mmol) and Et_3N (50 μL, 0.36 mmol). The corresponding mixture was stirred during the time and at the temperature indicated in each case and, after that, it was evaporated to dryness. The residue was purified by flash column chromatography to give compounds **18a-c** as colorless oils.

*4.2.11.1 17α-[(3'-Butylthioureido)methyl]estra-1,3,5(10)-triene-3,17β-diol (**18a**).* Butyl isothiocyanate was used (0.12 mL) and the reaction proceeded for 4 h at 45 °C. Column chromatography (hexane → 7:3 hexane-EtOAc) afforded **18a** (0.23 mg, 66%). $[\alpha]_D^{20}$ +34 (*c* 0.4, acetone). IR (cm^{-1}): 3286 (OH), 2928-2872 (C-H aliphatic), 1551-1498 (C-NH), 1250 (C=S), 1215-1024 (C-O, C-N). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.20 (brs, 1H, NH), 7.04 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.77 (brs, 1H, NH), 6.56 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.49 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 3.88 (brs, 1H, CH_{2a}NH), 3.49 (brs, 1H, CH_{2b}NH), 3.48 (m, 2H, NH-CH_2-CH_2), 2.72 (m, 2H, H-6a, H-6b), 2.24 (m, 1H, H-11a), 2.05 (m, 1H, H-9), 1.95 (m, 1H, H-16a), 1.82 (m, 1H, H-7a), 1.65 (m, 1H, H-12a), 1.62 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.51 (m, 2H, NH-CH_2-CH_2), 1.47 (m, 1H, H-14), 1.46 (m, 1H, H-12b), 1.39 (m, 1H, H-8), 1.37 (m, 1H, H-11b), 1.32 (m, 2H, CH_2-CH_3), 1.31 (m, 1H, H-15b), 1.24 (m, 1H, H-7b), 0.88 (s, 3H, H-18), 0.88 (t, 3H, $J_{H,H}$ = 7.4 Hz, CH_2-CH_3). ^{13}C NMR (125.7 MHz, acetone-d_6) δ 184.5 (C=S), 155.9 (C-3), 138.3 (C-5), 131.9 (C-10), 127.0 (C-1), 115.9 (C-4), 113.5 (C-2), 83.9 (C-17), 51.4 (CH_2NH), 50.5 (C-14), 46.9 (C-13), 44.8 (NH-CH_2-CH_2), 44.5 (C-9), 40.7 (C-8), 34.3 (C-16), 32.3 (C-12), 32.1 (NH-CH_2-CH_2), 30.3 (C-6), 28.3 (C-7), 27.1 (C-11), 24.0 (C-15), 20.7 (CH_2-CH_3), 14.7 (C-18), 14.1 CH_2-CH_3). FAB-MS *m/z* 417 ($[M+H]^+$, 36%); HRFAB-MS calcd for $C_{24}H_{37}N_2O_2S$ ($[M+H]^+$): 417.2570, found: 417.2574.

*4.2.11.2 17α-[(3'-Cyclohexylthioureido)methyl]estra-1,3,5(10)-triene-3,17β-diol (**18b**).* Cyclohexyl isothiocyanate was used (0.14 mL) and the reaction proceeded for 22 h at room temperature. Column chromatography (alumina, hexane → 4:1 hexane-EtOAc) afforded **18b** (0.27 g, 73%). $[\alpha]_D^{20}$ +28 (*c* 0.1, acetone). IR (cm^{-1}): 3293 (OH), 2930-2855 (C-H aliphatic), 1609-1449 (C=C), 1546-1498 (C-NH), 1252 (C=S), 1225-1020 (C-O, C-N). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.96 (brs, 1H, NH), 7.05 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 6.72 (brs, 1H, NH), 6.55 (dd, 1H, $J_{2,1}$ = 8.5 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.49 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 4.10 (brs, 1H, H-1''), 3.92 (brs, 1H, CH_{2a}NH), 3.47 (d, 1H, J_{gem} = 11.4 Hz, CH_{2b}NH), 2.73 (m, 2H, H-6a, H-6b), 2.25 (m, 1H, H-11a), 2.08 (m, 1H, H-9), 1.96 (m, 2H, H-2''a, H-6''a), 1.95 (m, 1H, H-16a), 1.82 (m, 1H, H-7a), 1.67 (m, 2H, H-3''a, H-5''a), 1.65 (m, 1H, H-12a), 1.63 (m, 1H, H-15a), 1.58 (m, 1H, H-16b), 1.55 (m, 1H, H-4''a), 1.49 (m, 1H, H-12b), 1.49 (m, 1H, H-14), 1.40 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.32 (m, 1H, H-

15b), 1.31 (m, 2H, H-3''b, H-5''b), 1.26 (m, 1H, H-7b), 1.19 (m, 2H, H-2''b, H-6''b), 1.15 (m, 1H, H-4''b), 0.88 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, acetone-d_6) δ 183.6 (C=S), 155.9 (C-3), 138.4 (C-5), 132.0 (C-10), 127.0 (C-1), 115.9 (C-4), 113.6 (C-2), 84.0 (C-17), 53.3 (C-1''), 51.3 (CH_2NH), 50.6 (C-14), 46.9 (C-13), 44.5 (C-9), 40.7 (C-8), 34.3 (C-16), 33.5 (C-2'' or C-6''), 33.5 (C-2'' or C-6''), 32.3 (C-12), 30.3 (C-6), 28.3 (C-7), 27.1 (C-11), 26.3 (C-4''), 25.6 (x2) (C-3'', C-5''), 24.0 (C-15), 14.7 (C-18). FAB-MS *m/z* 443 ([M+H]$^+$, 15%); HRFAB-MS calcd for $C_{26}H_{39}N_2O_2S$ ([M+H]$^+$): 443.2727, found: 443.2709.

*4.2.11.3 17α-[(3'-Phenylthioureido)methyl]estra-1,3,5(10)-triene-3,17β-diol (**18c**).* Phenyl isothiocyanate was used (0.12 mL) and the reaction proceeded for 3 h at room temperature. Column chromatography (hexane → 7:3 hexane-EtOAc) afforded **18c** (0.31 g, 86%). $[\alpha]_D^{20}$ +32 (*c* 0.9, acetone). IR (cm^{-1}): 3304 (OH), 2921-2873 (C-H aliphatic), 1596-1449 (C=C), 1541-1496 (C-NH), 1246 (C=S), 1227-1022 (C-O, C-N). ^{1}H NMR (500 MHz, acetone-d_6) δ 9.11 (brs, 1H, NH), 7.43 (d, 2H, $J_{o-,m-}$ = 7.4 Hz, Ar-H*o*), 7.34 (t, 2H, $J_{H,H}$ = 7.4 Hz, Ar-H*m*), 7.14 (m, 1H, Ar-H*p*), 7.11 (brs, 1H, NH), 7.05 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.57 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.6 Hz, H-2), 6.50 (d, 1H, $J_{4,2}$ = 2.6 Hz, H-4), 4.01 (brs, 1H, CH_{2a}NH), 3.58 (dd, 1H, J_{gem} = 13.3 Hz, $J_{H,H}$ = 2.5 Hz, CH_{2b}NH), 2.72 (m, 2H, H-6a, H-6b), 2.25 (m, 1H, H-11a), 2.07 (td, 1H, $J_{H,H}$ = 11.4 Hz, $J_{H,H}$ = 4.4 Hz, H-9), 1.96 (m, 1H, H-16a), 1.82 (m, 1H, H-7a), 1.66 (m, 1H, H-15a), 1.65 (m, 1H, H-12a), 1.64 (m, 1H, H-16b), 1.50 (m, 1H, H-14), 1.49 (m, 1H, H-12b), 1.39 (m, 1H, H-8), 1.38 (m, 1H, H-11b), 1.34 (m, 1H, H-15b), 1.26 (m, 1H, H-7b), 0.88 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, acetone-d_6) δ 181.9 (C=S), 155.8 (C-3), 139.3 (Ar-C*ipso*), 138.3 (C-5), 131.9 (C-10), 130.0 (x2) (Ar-C*m*), 127.0 (C-1), 126.0 (Ar-C*p*), 124.5 (x2) (Ar-C*o*), 115.9 (C-4), 113.5 (C-2), 83.7 (C-17), 51.9 (CH_2NH), 50.5 (C-14), 46.9 (C-13), 44.4 (C-9), 40.6 (C-8), 34.5 (C-16), 32.4 (C-12), 30.3 (C-6), 28.2 (C-7), 27.0 (C-11), 23.9 (C-15), 14.6 (C-18). FAB-MS *m/z* 437 ([M+H]$^+$, 23%); HRFAB-MS calcd for $C_{26}H_{33}N_2O_2S$ ([M+H]$^+$): 437.2257, found: 437.2241.

*4.2.12 General procedure for the synthesis of steroidal spiranic isoureas **19***

To a solution of the corresponding thiourea **18** (0.25 mmol) in dry MeOH or THF (10 mL) was added yellow HgO (4.5 mmol for compounds **18a**,**b** and 2.25 mmol for compound **18c**) under Ar. The mixture was stirred at room temperature in the dark during the time indicated in each case, filtered over a pad of Celite® and evaporated to dryness. The residue was purified by flash column chromatography and washed with cooled diethyl ether to give compounds **19a-c** as colorless oils.

*4.2.12.1 (17S)-2'-Butylamino-spiro[3-hydroxyestra-1,3,5(10)-trien-17,5'-oxazoline] (**19a**).* Thiourea **18a** was used (0.10 g) and the reaction proceeded for 4 days. Column chromatography (CH_2Cl_2 → 5:1 CH_2Cl_2-MeOH) afforded **19a** (64 mg, 68%). $[\alpha]_D^{20}$ -10 (*c* 0.4, $CHCl_3$). IR (cm^{-1}): 3231 (OH), 2928-2867 (C-H aliphatic), 1654 (C=N), 1600-1449 (C=C), 1242-1060 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.79 (brs, 1H, NH), 6.70 (dd, 1H, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.2 Hz, H-2), 6.62 (d, 1H, $J_{4,2}$ = 2.2 Hz, H-4), 3.91 (d, 1H, $J_{4'a,4'b}$ = 11.7 Hz, H-4'a), 3.42 (d, 1H, $J_{4'b,4'a}$ = 11.7 Hz, H-4'b), 3.21 (m, 2H, H-1''), 2.79 (m, 2H, H-6), 2.30 (m, 1H, H-16a), 2.27 (m, 1H, H-11a), 2.04 (m, 1H, H-9), 1.89 (ddd, 1H, $J_{H,H}$ = 14.7 Hz, $J_{H,H}$ = 9.4 Hz, $J_{H,H}$ = 5.6 Hz, H-16b), 1.84 (m, 1H, H-7a), 1.71 (m, 1H, H-15a), 1.58 (m, 1H, H-12a), 1.53 (m, 2H, H-2''), 1.47 (m, 1H, H-12b), 1.46 (m, 1H, H-11b), 1.42 (m, 1H, H-15b), 1.41 (m, 1H, H-8), 1.34 (m, 2H, H-3''), 1.27 (m, 1H, H-7b), 1.09 (m, 1H, H-14), 0.91 (s, 3H, H-18), 0.89 (t, 3H, $J_{4'',3''}$= 7.4 Hz, H-4''). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 161.1 (C-2'), 155.4 (C-3), 137.8 (C-5), 130.5 (C-10), 126.3 (C-1), 115.8 (C-4), 113.3 (C-2), 97.6 (C-17), 57.5 (C-4'), 48.4 (C-14), 45.6 (C-13), 43.5 (C-9), 42.5 (C-1''), 39.2 (C-8), 35.1 (C-16), 31.9 (C-2''), 31.1 (C-12), 29.7 (C-6), 27.3 (C-7), 26.0 (C-11), 22.3 (C-15), 19.9 (C-3''), 14.5 (C-18), 13.8 (C-4''). FAB-MS *m/z* 383 ($[M+H]^+$, 80%); HRFAB-MS calcd for $C_{24}H_{35}N_2O_2$ ($[M+1]^+$): 383.2693, found: 383.2700.

*4.2.12.2 (17S)-2'-Cyclohexylamino-spiro[3-hydroxyestra-1,3,5(10)-trien-17,5'-oxazoline] (**19b**).* Thiourea **18b** was used (0.11 g) and the reaction proceeded for 7 days. Column chromatography (CH_2Cl_2 → 20:1 CH_2Cl_2-Isopropanol) afforded **19b** (95 mg, 92%). $[\alpha]_D^{20}$ -19 (*c* 0.7, $CHCl_3$). IR (cm^{-1}): 3284 (OH), 2924-2854 (C-H aliphatic), 1653 (C=N), 1600-1448 (C=C), 1240-1016 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 6.68 (dd, 1H, $J_{2,1}$ = 8.5 Hz, $J_{2,4}$ = 2.2 Hz, H-2), 6.61 (d, 1H, $J_{4,2}$ = 2.2 Hz, H-4), 3.93 (d, 1H, $J_{4'a,4'b}$ = 12.2 Hz, H-4'a), 3.41 (d, 1H, $J_{4'b,4'a}$ = 12.2 Hz, H-4'b), 3.41 (m, 1H, H-1''), 2.80 (m, 2H, H-6), 2.29 (m, 1H, H-11a), 2.28 (m, 1H, H-16a), 2.10 (td, 1H, $J_{H,H}$ = 10.7 Hz, $J_{H,H}$ = 3.0 Hz, H-9), 1.97 (m, 2H, H-2''a, H-6''a), 1.86 (m, 1H, H-16b), 1.85 (m, 1H, H-7a), 1.70 (m, 1H, H-15a), 1.67 (m, 2H, H-3''a, H-5''a), 1.59 (m, 1H, H-12a), 1.57 (m, 1H, H-4''a), 1.54 (m, 1H, H-12b), 1.46 (m, 1H, H-11b), 1.42 (m, 1H, H-8), 1.40 (m, 1H, H-15b), 1.29 (m, 3H, H-7b, H-3''b, H-5''b), 1.15 (m, 1H, H-14), 1.13 (m, 3H, H-4''b, H-2''b, H-6''b), 0.92 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 160.2 (C-2'), 155.6 (C-3), 137.8 (C-5), 130.6 (C-10), 126.4 (C-1), 115.9 (C-4), 113.4 (C-2), 96.2 (C-17), 59.7 (C-4'), 51.5 (C-1''), 48.4 (C-14), 45.5 (C-13), 43.6 (C-9), 39.3 (C-8), 35.2 (C-16), 33.7 (C-2'' or C-6''), 33.6 (C-2'' or C-6''), 31.2 (C-12), 29.8 (C-6), 27.3 (C-7), 26.2 (C-11), 25.6 (C-4''), 24.8 (x2) (C-3'', C-5''), 22.3 (C-15), 14.6 (C-18). FAB-MS *m/z* 409 ($[M+H]^+$, 100%); HRFAB-MS calcd for $C_{26}H_{37}N_2O_2$ ($[M+H]^+$): 409.2850, found: 409.2813.

*4.2.12.3 (17S)-2'-Phenylamino-spiro[3-hydroxyestra-1,3,5(10)-trien-17,5'-oxazoline] (**19c**).* Thiourea **18c** was used (0.11 g) and the reaction proceeded for 21 h. Column chromatography (CH_2Cl_2 → 20:1 CH_2Cl_2-Isopropanol) afforded **19c** (96 mg, 96%). $[\alpha]_D^{20}$ +22 (*c* 0.2, $CHCl_3$). IR (cm^{-1}): 3280 (OH), 2923-2871 (C-H aliphatic), 1656 (C=N), 1592-1447 (C=C), 1224-1062 (C-O, C-N). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (m, 4H, Ar-H*o*, Ar-H*m*), 7.02 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 6.93 (m, 1H, Ar-H*p*), 6.59 (dd, 1H, $J_{2,1}$ = 8.5 Hz, $J_{2,4}$ = 1.9 Hz, H-2), 6.51 (d, 1H, $J_{4,2}$ = 1.9 Hz, H-4), 5.54 (brs, 1H, NH), 3.83 (d, 1H, $J_{4'a,4'b}$ = 11.0 Hz, H-4'a), 3.34 (dd, 1H, $J_{4'b,4'a}$ = 11.0 Hz, H-4'b), 2.72 (m, 2H, H-6a, H-6b), 2.28 (ddd, 1H, $J_{16a,16b}$ = 14.8 Hz, $J_{16a,H}$ = 12.6 Hz, $J_{16a,H}$ = 3.5, H-16a), 2.21 (m, 1H, H-11a), 2.04 (m, 1H, H-9), 1.84 (ddd, 1H, $J_{16b,16a}$ = 14.8 Hz, $J_{16b,H}$ = 9.4 Hz, $J_{16b,H}$ = 5.8 Hz, H-16b), 1.79 (m, 1H, H-7a), 1.66 (m, 1H, H-15a), 1.62 (m, 1H, H-12a), 1.45 (m, 1H, H-12b), 1.41 (m, 1H, H-11b), 1.37 (m, 2H, H-15b, H-8), 1.23 (m, 2H, H-7b, OH), 1.11 (m, 1H, H-14), 0.90 (s, 3H, H-18). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.3 (C-2'), 154.5 (C-3), 138.0 (C-5), 131.3 (C-10), 129.1 (x3) (Ar-C), 126.5 (C-1), 122.8 (Ar-C*p*), 120.5 (x2) (Ar-C), 115.7 (C-4), 113.2 (C-2), 96.4 (C-17), 56.3 (C-4'), 48.7 (C-14), 45.7 (C-13), 43.6 (C-9), 39.2 (C-8), 35.2 (C-16), 31.4 (C-12), 29.7 (C-6), 27.3 (C-7), 26.1 (C-11), 22.4 (C-15), 14.7 (C-18). FAB-MS *m*/*z* 403 ($[M+H]^+$, 28%); HRFAB-MS calcd for $C_{26}H_{31}N_2O_2$ ($[M+H]^+$): 403.2380, found: 403.2376.

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Table 1. Antiproliferative values of steroidal derivatives against human cancer cell lines.

	GI_{50} (μM)						
Compound	**A549**	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**	**BJ-hTert**
3	>100	>100	>100	>100	>100	>100	
4	17 ± 2.3	20 ± 1.9	22 ± 4.0	19 ± 4.7	21 ± 0.5	25 ± 3.9	
6	**3.7 ± 0.8**	**10 ± 4.5**	**4.0 ± 0.6**	**6.0 ± 3.2**	**4.5 ± 1.6**	**3.8 ± 1.9**	**<1**
7	38 ± 8.2	99 ± 1.3	70 ± 27	92 ± 9.5	41 ± 3.8	39 ± 13	
8a	12 ± 1.5	13 ± 2.5	14 ± 2.1	**9.4 ± 2.4**	**5.8 ± 1.0**	**4.5 ± 0.8**	
8b	16 ± 4.7	18 ± 3.4	21 ± 0.3	15 ± 0.3	18 ± 0.03	18 ± 1.8	
8c	29 ± 3.7	27 ± 5.9	27 ± 7.6	27 ± 6.7	34 ± 5.1	17 ± 6.5	
9a	**1.4 ± 0.4**	**2.1 ± 0.3**	**1.6 ± 0.1**	**1.2 ± 0.1**	**1.2 ± 0.3**	**0.87 ± 0.21**	**<1**
9b	**1.5 ± 0.2**	**2.0 ± 0.1**	**1.6 ± 0.1**	**1.5 ± 0.3**	**1.2 ± 0.2**	**0.96 ± 0.21**	**1.2 ± 0.3**
9c				[a]			
13	13 ± 4.0	22 ± 2.8	**2.9 ± 0.4**	29 ± 5.3	**4.1 ± 0.6**	**2.4 ± 0.2**	
14	39 ± 2.6	34 ± 3.7	27 ± 1.5	43 ± 10	30 ± 0.5	22 ± 2.2	
16	**5.4 ± 0.6**	**6.3 ± 0.5**	**1.3 ± 0.1**	18 ± 4.8	**4.4 ± 0.7**	**9.2 ± 1.7**	
17	**6.8 ± 0.6**	**4.0 ± 0.9**	**3.5 ± 0.2**	**5.9 ± 0.4**	**4.3 ± 1.1**	**4.9 ± 1.0**	
18a	**4.8 ± 1.8**	**7.2 ±2.0**	**4.2 ± 0.5**	15 ± 0.1	**3.9 ± 0.5**	**4.4 ± 0.8**	
18b	**4.5 ± 1.7**	**5.1 ± 1.4**	**3.3 ± 0.6**	14 ± 5.7	**3.9 ± 0.9**	**2.8 ± 0.2**	
18c	**9.8 ± 3.0**	15 ± 2.6	**6.3 ± 1.8**	21 ± 4.8	**5.7 ± 2.2**	**4.5 ± 0.2**	
19a	**1.4 ± 0.1**	**2.9 ± 0.4**	**2.1 ± 0.9**	**2.2 ± 0.8**	**0.45 ± 0.09**	**0.34 ± 0.13**	**<1**
19b	**1.1 ± 0.1**	**1.5 ± 0.2**	**1.3 ± 0.3**	**1.5 ± 0.2**	**0.71 ± 0.43**	**0.34 ± 0.21**	**<1**
19c	**2.6 ± 0.2**	**4.4 ± 0.6**	**3.3 ± 1.6**	**3.4 ± 0.7**	**2.6 ± 0.7**	**2.6 ± 0.8**	**1.4 ± 0.6**
Abiraterone	95 ± 8.0	>100	**7.9 ± 0.5**	85 ± 8.9	24 ± 4.5	42 ± 7.7	**4.5 ± 1.0**
Galeterone	**3.9 ± 1.3**	10 ± 0.9	**5.3 ± 0.4**	**3.9 ± 0.5**	**2.1 ± 0.1**	**2.7 ± 0.2**	**5.0 ± 1.3**

[a] Not soluble under the assay conditions

Highlights

- Stereoselective synthesis of conformationally-restricted spiranic heterocycles from *trans*-androsterone and estrone
- Treatment of aminomethyl alcohol at C-17 with triphosgene furnishes spiranic oxazolin-2-ones
- Cyclodesulfurization reaction of hydroxyl thioureas at C-17 furnishes spiranic 2-aminooxazolines
- Observed order of activity: aminooxazoline > spirocarbamate > thiourea
- Spiro-heterocycles from estrone are the lead antiproliferative agents (0.34-1.5 μM)