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20-Aminosteroids as a novel class of selective and complete androgen receptor antagonists and inhibitors of prostate cancer cell growth

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ABSTRACT

Here, the synthesis and the evaluation of novel 20-aminosteroids on androgen receptor (AR) activity is reported. Compounds **11** and **18** of the series inhibit both the wild type and the T877A mutant AR-mediated transactivation indicating AR antagonistic function. Interestingly, minor structural changes such as stereoisomers of the amino lactame moiety exhibit preferences for antagonism among wild type and mutant AR. Other tested nuclear receptors are only weakly or not affected. In line with this, the prostate cancer cell growth of androgen-dependent but not of cancer cells lacking expression of the AR is inhibited. Further, the expression of the prostate specific antigen used as a diagnostic marker is also repressed. Finally steroid **18** enhances cellular senescence that might explain in part the growth inhibition mediated by this derivative. Steroids **11** and **18** are the first steroids that act as complete AR antagonists and exhibit AR specificity.

1. Introduction

Benign prostate hyperplasia (BPH) and prostate cancer (PCa) is a serious health problem worldwide. PCa has advanced to the second leading cause of cancer mortality of men in western countries.[1] Many factors affect risk of developing PCa, important being age.[2] With advancing age the prostate gland increases in size leading to BPH. In addition older men develop more chances for having a diagnosis for PCa. Notably, both the proliferation of prostate and also PCa is enhanced by androgens.[1,3]

Androgens mediate effects through the androgen receptor (AR), a ligand-regulated transcription factor and member of the nuclear receptor superfamily.[4] Activation of AR either by endogenous androgens such as testosterone (T) and 5α-dehydrotestorenone (DHT) or by exogenous compounds induces conformational changes of the receptor resulting to its translocation to the nucleus and androgen-induced gene expression. The AR plays a fundamental role in the development and maintenance of primary and secondary male sexual characteristics including the growth of normal prostate gland.[5] However, excessive stimulation of the AR signaling is related with pathological disorders such as BPH and PCa, thus AR comprises primary therapeutic target for the treatment of these diseases.[6]

Antiandrogens (AR-antagonists) are structurally classified as steroidal and non-steroidal compounds. Structural modifications of T and DHT afforded many steroidal AR antagonists for PCa treatment like cyproterone acetate, a partial antiandrogen.[7] On the other hand, currently approved antiandrogens for PCa, are flutamide (Eulexin®), bicalutamide (Casodex®), and nilutamide (Nilandron®) are of non-steroidal nature.

Despite the initial tumor regression and the overall clinical improvement of patients, eventually the tumor develops resistance to antiandrogens and becomes hormone refractory and castration resistant. However, the AR is expressed, still remains active and promotes growth of prostate cells despite androgen ablation and antagonist treatments.[2,8] Particularly, point mutations often lead to a receptor that has gained the ability to promiscuously bind also to non-androgenic ligands, be activated by other ligands[9] and thus bypassing the androgen ablation or hormone therapy by androgen antagonists. For example, the mutation T877A of human AR, isolated from PCa patients, renders the complete non-steroidal antagonist hydroxyflutamide (OH-Fl) to an agonist that activates AR.[10] Unambiguously, the development of novel, more potent antiandrogens with pure antagonistic activities against both the wild-type (wt) and the mutated forms of AR, would be of a great therapeutic impact for the treatment of PCa.

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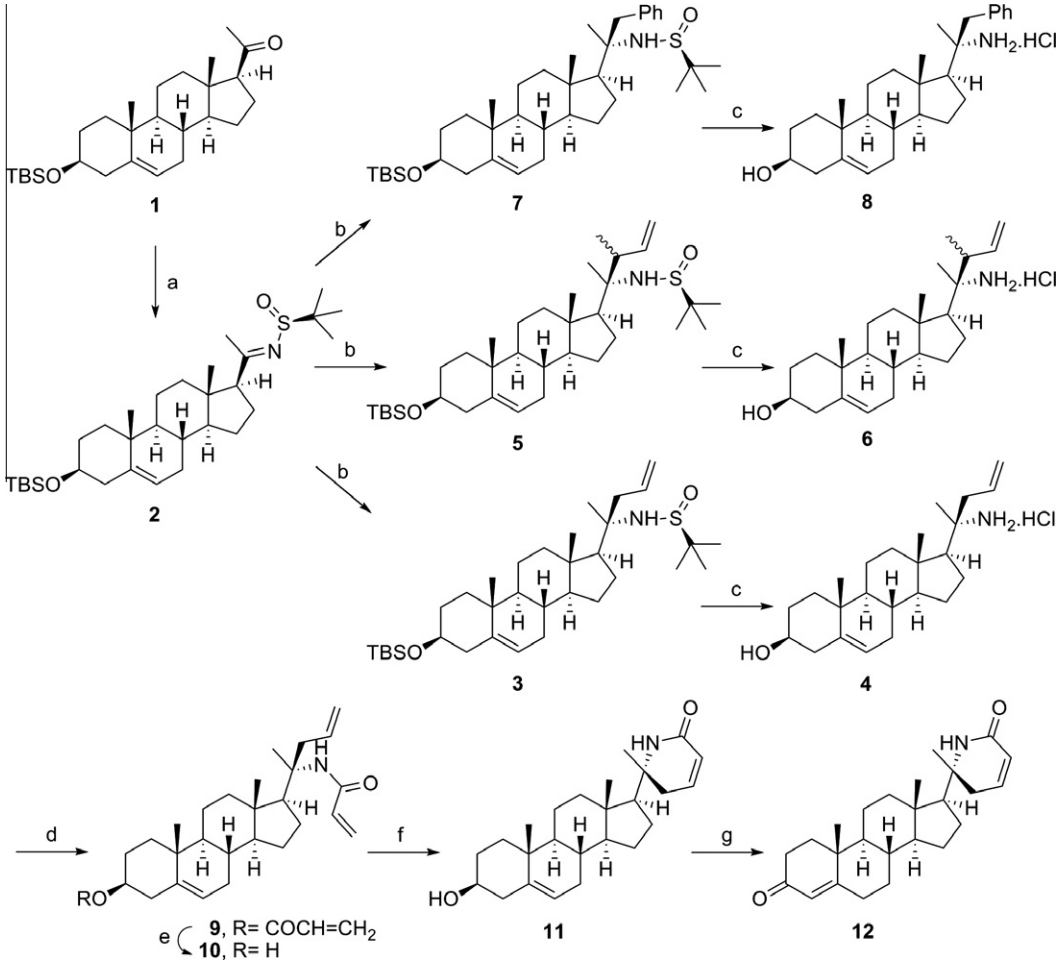
Be prompted by this need, we focused our attempts in the field of steroidal antiandrogens. Especially, we designed and synthesized novel steroidal derivatives bearing a chiral tertiary C-20 amino-moiety and we evaluated their effects on AR activity and PCa cell growth. We envisaged the introduction of an amino-group at the C-20 of the steroidal skeleton as an intriguing modification since close related positions of natural and synthetic ligands are involved in key interactions with amino acid residues of the receptor.[11] Particularly, the crystal structures of the ligand-binding domains (LBDs) of both the wtAR and the T877A mutant AR bound to DHT revealed that the 17β-hydroxy group is involved in key interactions with the protein.[11a] The ligand is located at similar position within the AR LBD or AR-T877A LBD and interacts similarly with the amino acid residues except that of threonine. The mutation T877A contributes to the broader ligand specificity of the AR mutant. For example, cyproterone acetate, a steroidal antiandrogen bearing a bulky substituent at C-17, induces such conformational changes to the protein in the presence of T877 that ligand-binding pocket is expanded and becomes susceptible to accommodate the bulky group. Consequently, cyproterone is tightly bound to the AR-T877A LBD and acts as an agonist. On the contrary, other compounds, bearing bulky side chains at the C-16, C-18 or C-20, reposition of the helix-12 and prevent the recruitment of coactivators leading to the AR inhibition.[12] Based on these observations, we intended to exploit the influence of an amino-group insertion at C-20 of pregnenolone in regard to the AR activity. Moreover, although steroidal skeleton has been exhaustively modified, to the best of our knowledge, C-20-lactame ring steroids have not been synthesized to date, thus providing a challenging synthetic target with potential activity against AR.

2. Chemistry

Nucleophilic addition of organometallic reagents to *N*-*tert*-butanesulfinyl imines is a well established method for the stereoselective synthesis of α-branched amines.[13] Utilizing this synthetic methodology we planned to transform initially the C-20 of pregnenolone into a new amino-substituted stereogenic center which was anticipated afterwards to provide us access to novel 20-amino-functionalized steroids.

To that purpose, condensation of 3β-TBS-protected-pregnenolone **1**[14] with the chiral auxiliary (*S*)-(−)-2-methyl-2-propanesulfinamide afforded under optimized conditions the corresponding (20S_S,*E*)-imine **2** as a single geometrical isomer whose structure established by X-ray analysis (Scheme 1).[15] Nucleophilic addition of allylmagnesium bromide to **2** completed in a stereoselective fashion providing the sulfinamide **3** as a single diastereomer in 80% yield. Assignment of stereochemistry to the newly created stereogenic center C-20 was enabled after removal of the chiral auxiliary from **3**. Thus, treatment with hydrogen chloride resulted in



Scheme 1. Synthesis of (20*R*)-aminosteroids. Reagent and conditions: (a) (*S*)-(−)-2-Methyl-2-propanesulfinamide, $Ti(OEt)_4$, 90 °C, 48 h, 63%; (b) **3**: allylMgBr, CH_2Cl_2 , −78 °C to rt, overnight, 80%; **5**: 1-methyl-2-propenylmagnesium chloride, CH_2Cl_2, −78 °C to rt, overnight, 63%; **7**: benzylMgCl, CH_2Cl_2, −78 °C to rt, overnight, 73%; (c) HCl, MeOH, rt, 30 min, (**4**: 91%, **6**: 80%, **8**: 93%); (d) acryloyl chloride, Et_3N, CH_2Cl_2, H_2O, 0 °C to rt, 21 h (**9**: 23%, **10**: 44%); (e) $NaOCH_3$, CH_3OH, rt, 6 h, 81%; (f) 2nd generation Grubbs catalyst (5%), CH_2Cl_2, reflux, 4.5 h, 92%; (g) Al(*i*-PrO)$_3$, cyclohexanone, benzene, 80 °C, 16 h, 58%; TBS, *tert*-butyldimethylsilyl.

the cleavage of the *tert*-butanesulfinyl moiety and concomitant removal of the 3-TBS-protective group, thereby affording compound **4**. The absolute configuration of **4** was determined by X-ray analysis proving the (20*R*)-stereochemistry.

Extensive trials to substitute the imine **2** with various Grignard reagents (pentyl-, hexyl-, isohexyl-, phenyl-, 4-methoxybenzyl-, vinyl- or prenyl-), *n*-butyl lithium or the Reformatsky reagent derived from ethyl bromoacetate, led either to decomposition or recovery of the starting material. However, addition of 1-methyl-2-propenylmagnesium chloride or benzylmagnesium chloride afforded the corresponding sulfinamides **5** and **7** as single diastereomers which upon acidic methanolysis furnished the hydrochloric salts **6** and **8**, respectively (Scheme 1).

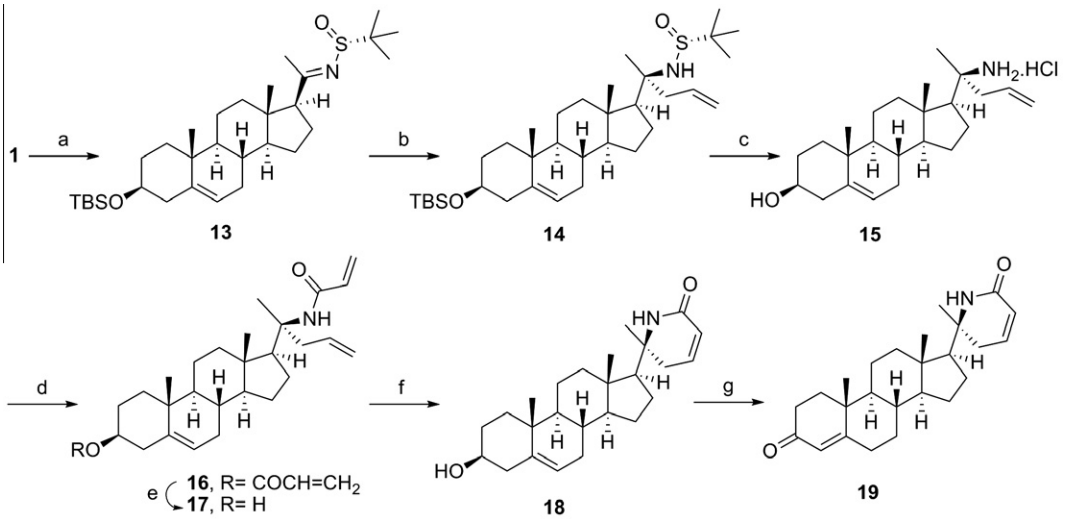
Further attempts to generate functionalized (20*R*)-aminosteroids were pursued using the steroidal amine **4**. Acylation with acryloyl chloride in the presence of triethyl amine under Schotten–Bauman conditions led to the biacylated **9** and the monoacylated product **10** (Scheme 1). Basic hydrolysis of the 3-acylgroup of **9** resulted in the recovery of the monoacylated product **10**. Subsequently, ring closing metathesis of **10** using 2nd generation Grubbs catalyst furnished the C-20 lactame ring steroidal derivative **11** in excellent yield. Eventually, the latter was easily converted to the corresponding testosterone derivative **12** after Oppenauer oxidation of the 3β-hydroxy-group and subsequent migration of the double bond to the more stable enone system.

To investigate the impact of the 20-amino moiety configuration on the biological activity, we turned our attention to the synthesis of the diastereomeric (20*S*)-aminosteroids. We postulated that the intermediate (20R_s)-imine with opposite chirality at sulfur would provide us access to these products. Condensation of **1** with the chiral auxiliary (*R*)-(+)-2-methyl-2-propanesulfinamide (Scheme 2) afforded exclusively the (20R_S,*E*)-imine **13** (structure verified by X-ray crystallography).[16] All trials to substitute **13** with ethylmagnesium bromide under various experimental conditions failed, confirming the previous findings obtained for the imine **2**. Nevertheless, addition of allylmagnesium bromide proceeded diastereoselectively providing the sulfinylamide **14** as a single diastereomer, whose structure was determined by X-ray analysis of the hydrochloric salt **15**.[17] To our delight, assignment of the stereochemistry revealed the (20*S*)-configuration confirming our expectations. Subsequently, following the same sequence of reactions as described above, **15** was transformed to the steroidal derivatives **18** and **19** via cyclization of **17**.

3. Results and discussion

The novel 20-aminosteroids were first evaluated for agonistic or antiandrogenic activity of human AR (Fig. 1) performing transient transfection assays in CV1 cells expressing human AR with cotransfection of MMTV-luc, a well-known AR responsive promoter.[18] None of the tested compounds induced the AR-mediated transactivation (Fig. 1A) suggesting that none of these derivatives possess androgenic activity for the wtAR. The androgen-induced wtAR transactivation was potently inhibited by **11**, **12**, and **19** and weaker by **18** suggesting that these compounds act as complete AR antagonists. Also, we compared 0.1 to 10 micromolar range for both OH-Fl and Casodex and observed that higher doses of these AR antagonists activate the wtAR, strongly by OH-Fl and Casodex at a lower level (Fig. 1S, Supplementary data). Determination of IC_{50} values as inhibition of AR-mediated transactivation by 50% revealed for compounds **11** and **12** about 2 μM, for **18** about 3 μM, and for **19** about 0.5 μM. However, comparing the IC_{50} values of our new synthesized compounds to those of therapeutically used AR antagonists like flutamide (IC_{50} = 110 μM) or Casodex (IC_{50} = 0.3–0.9 μM)[19] we achieve similar or better potency. Coincidentally no inhibition of androgen-induced wtAR transactivation was observed by derivatives **4**, **6**, **8**, and **15** (Fig. 1A).

Using the AR-T877A mutant, which is known to be able to bypass the beneficiary effects of androgen ablation and hormone therapy by androgen antagonists, a different response of the 20-amino steroidal derivatives was provided (Fig. 1B). Interestingly, compounds **4**, **6**, and **15** exhibited potent agonistic activities. The compounds **8**, **12**, and **19** led to weak induction of the AR-T877A-mediated transactivation whereas **11** and **18** did not reveal agonistic activity for the mutant AR. Noteworthy, in the presence of R1881, a well known and highly potent AR agonist, the androgen-induced AR-mediated transactivation was inhibited by **18**. Compound **11** exhibited a partial inhibition of AR-T877A whereas the other compounds did not show an inhibition. These data, (Fig. 2S, Supplementary data) reveal that the tested compounds differently inhibit or activate the wtAR and the AR-T877A mutant acting as complete or mixed AR antagonists. Clearly, they present selectivity either for the wtAR (**12**, **19**) or the AR-T877A mutant



Scheme 2. Synthesis of (20*S*)-aminosteroids. Reagent and conditions: (*R*)-(+)-2-Methyl-2-propanesulfinamide, $Ti(OEt)_4$, 83 °C, 31.5 h, 85%; (b) allylMgBr, CH_2Cl_2, −78 °C to rt, overnight, 89%; (c) HCl, MeOH, rt, 2 h, 95%; (d) acryloyl chloride, Et_3N, CH_2Cl_2, H_2O, 0 °C to rt, 21 h (**16**: 23%, **17**: 46%); (e) $NaOCH_3$, CH_3OH, rt, 6 h, 99%; (f) 2nd generation Grubbs catalyst (5%), CH_2Cl_2, reflux, 4 h, 84%; (g) Al(*i*-PrO)$_3$, cyclohexanone, benzene, 80 °C, 15 h, 50%; TBS, *tert*-butyldimethylsilyl.

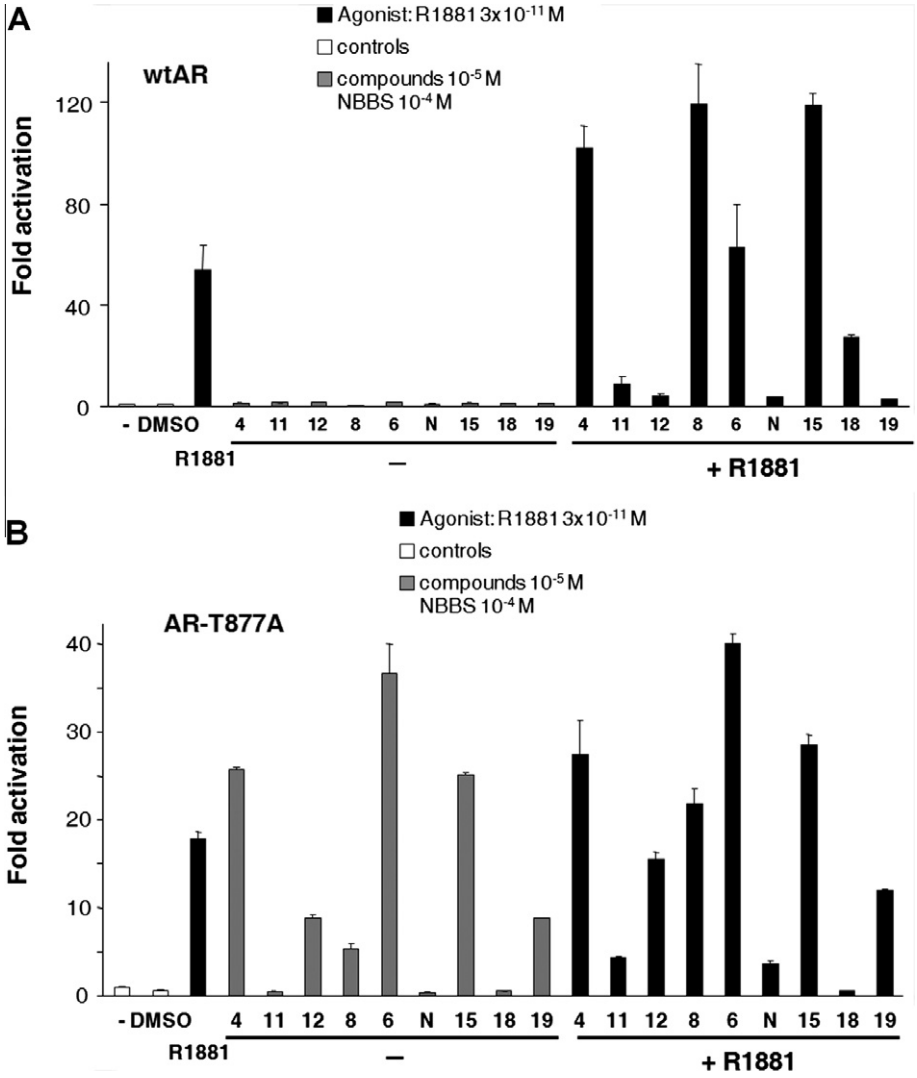


Figure 1. AR agonistic and antagonistic activities of 20-aminosteroids comparing wild-type AR (wtAR) and the AR-T877A mutant. (A) CV1 cells were transfected with the expression vector for human wild-type AR and the reporter MMTV-luciferase either untreated, treated with or without the compounds, or the potent AR-agonist methyltrienolone (R1881; K_D value for nuclear AR lies in between 5 and 12 nM[23]). Luciferase units obtained were normalized to the cotransfected pCMX-LacZ as internal control and values are indicated as relative light units (RLU). As control, cells were treated with the AR-antagonist NBBS (*N*-butylbenzene-sulfonamide; indicated as 'N'), or with the solvent DMSO alone. As further controls, cells were incubated without solvent. (B) The compounds were tested individually or combined with R1881 as described above, except the expression vector for the human AR-T877A mutant was used.

(**18**) indicating that only one amino acid exchange in the AR ligand-binding domain has an important impact on the action of these compounds. Nevertheless, derivative **18** serves as a promising anti-androgen inhibiting potently both the wtAR and the T877A mutant AR.

Concentration series showed that derivative **18** repressed wtAR and AR-T877A mediated transactivation at 1 μM (Fig. 3S, Supplementary data).

To confirm AR antagonism we used the human origin LNCaP PCa cells expressing endogenously the AR-T877A mutant. The LNCaP cell line is known to grow androgen-dependently and thus also useful for androgen-regulated growth studies. To determine AR antagonism on an endogenous gene we analyzed the expression of the prostate specific antigen (PSA) known as a diagnostic marker for PCa. Quantitative real-time reverse transcription PCR (qRT-PCR) was used to determine the PSA mRNA levels of LNCaP cells (Fig. 2A). Compounds **11** and **18** showed potent inhibition of PSA-expression. Compound **6** increased PSA-expression while compound **19** exhibited weak AR antagonism, which is in line with the reporter assays (Fig. 1B). Thus, **11** and **18** emerged as AR antagonists displaying a potency comparable to that of the well-known AR antagonists flutamide and Casodex.

Further we analyzed the growth of LNCaP cells treating them with **11** and **18** for the indicated days (Fig. 2B). Atraric acid (AA) was used as positive control known to inhibit LNCaP cell growth.[20] At 10 μM **18** potently inhibited LNCaP cell growth whereas at 1 μM the inhibition was only slight. Also **11** inhibited LNCaP cell growth albeit a bit weaker compared to **18**. Therefore, we conclude that **18** is a potent growth inhibitor of LNCaP cells. To exclude, however, a general growth inhibition by **18** we used the human PCa cell line PC3, that lacks functional AR. Treatment of PC3 cells with **18** did not affect cell growth (Fig. 2C) indicating that **18** is not a general inhibitor of cell growth.

Taken together, the data suggest that the derivatives **11** and **18** are potent AR antagonists, **18** being a more potent inhibitor of cell growth.

To get insights into the underlying molecular pathway of the growth inhibition we focused on compound **18** being the most potent inhibitor of LNCaP cell growth. Treating cells with **18** did not result in enhanced apoptosis. A further possibility for reducing

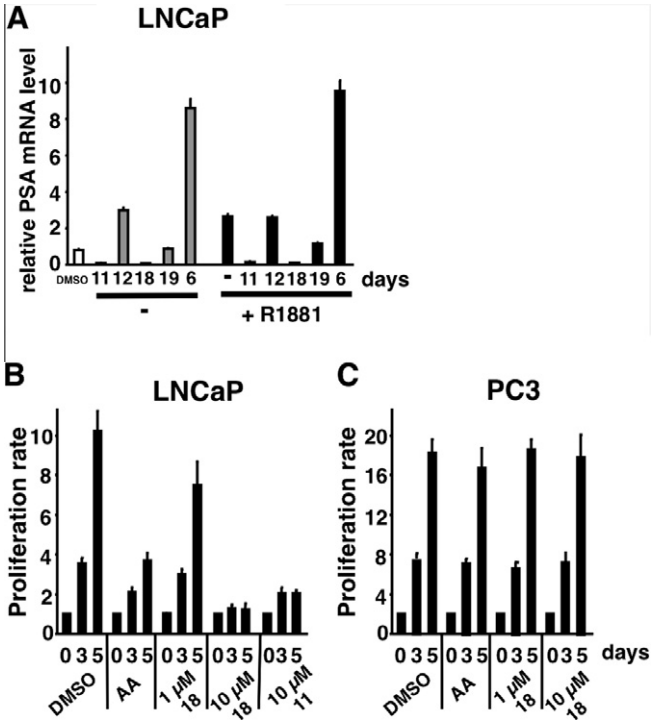


Figure 2. (A) Compounds **11** and **18** potently inhibit the endogenous androgen-induced PSA gene expression. Endogenously expressed mRNA levels of the PSA gene, a direct AR target gene, were detected using qRT-PCR. LNCaP cells were treated with or without androgens plus the indicated compounds (10 μM). DMSO treated cells were used as controls. Values were normalized to the expression of beta-actin. (B) Compounds **18** and **11** inhibit growth of the LNCaP cells. Indicated concentrations of **18** and **11** were tested for the ability to influence growth of the model human PCa cell line LNCaP. Equal amounts of cells were seeded out and the number of cells was counted at the indicated days and normalized to the cells of day 0. The known AR antagonist atraric acid (AA) served as positive control. (C) Compound **18** does not affect the growth of the PC3 cells lacking AR expression. Growth analysis was performed as described in (B).

growth is the phenomenon of cellular senescence leading to an irreversible cell cycle arrest of metabolic active cells. A very specific marker for cellular senescence is the activity of the senescence-associated β-galactosidase (SA-β-Gal).[21] Cells undergoing cellular senescence remain metabolically active but exhibit an irreversible growth arrest. Interestingly, treating LNCaP cells with **18** induced the number of SA-β-gal expressing cells (Fig. 4S, Supplementary data). This suggests that **18** enhances cellular senescence that might explain in part the growth inhibition mediated by **18**.

Interestingly, whereas the 3-keto derivative **19** activated the AR-T877A mutant, the corresponding 3β-hydroxy-one **18** inhibited it, suggesting that the 3β-hydroxy-group is crucial for the AR-mediated transactivation. Moreover, while the 3-keto diastereomers **12** and **19** acted almost similarly, only **19** proved significantly able to inhibit the ligand-activated AR-T877A. On the other hand, the 3β-hydroxy diastereomers **11** and **18**, acted very similarly against both the wtAR and the AR-T877A mutant, indicating that the configuration of the lactamic ring is not critical for the antiandrogenic activity. Albeit, the C-20 lactame moiety represents a unique structural feature of the new compounds that contributes substantially to the biological activity as the simple steroidal amines **4**, **6**, **8** and **15** exhibited no antiandrogenic effects. Also, these findings confirm in part our thoughts that a bulky substituent at C-20 might disturb the right folding of the protein inducing antagonistic effects, although further studies are necessary to confirm this hypothesis.

Importantly, compounds **11** and **18** showed the highest specificity for AR without inducing transactivation of glucocorticoid receptor or progesterone receptor and only weakly inhibiting the hormone-induced transactivation of these receptors (data not shown). Unlike the AR natural ligand DHT which bears a 3-keto-group both of these derivatives bear a 3β-hydroxy-group. Thus, the 3β-hydroxy-group emerges as a preferable substituent of these novel antiandrogens contributing to the maintenance of the AR specificity.

To our knowledge **11** and **18** are the first steroids that act as complete AR antagonists and exhibit AR specificity.

Interestingly, **11** and **18** inhibited the growth of the PCa cell line LNCaP. These cells express the AR-T877A mutant which is unexpectedly activated by the non-steroidal antiandrogen OH-F.[10] Since **11** and **18** inhibit the growth of LNCaP cells they may serve as advantageous tumor inhibitors with minimal risk of therapy resistance occurrence in contrast to the currently used antiandrogens.

The obtained results offered evidence that the underlying inhibition mechanism of AR transactivation by derivatives **11** and **18** could be partly based on the induction of cellular senescence, a phenomenon that is known to induce irreversibly cell cycle arrest.[22]

Comparing the efficacy on cell growth inhibition by **18** with that of Casodex, the compound **18** exhibits a similar inhibition at one micromolar concentration and a higher inhibition at 10 μM of LNCaP cell growth (Fig. 3). On the other hand comparing the effects on inhibition of AR-mediated transactivation, Casodex exhibits a more potent antagonism of AR-mediated transactivation at one micromolar concentration or lower (see Fig. 1S, Supplementary data) as compared to **18**. Therefore, the data suggest that different molecular mechanisms of **18** and Casodex are used to induce growth inhibition or to inhibit AR-mediated transactivation. Taken together, compound **18** emerged as an AR antagonist with a higher potency of growth inhibition compared to the well-known AR antagonist Casodex.

4. Conclusion

Newly synthesized 20-aminosteroids were evaluated for their activity against AR. Among them, derivatives **11** and **18**, which bear a C-20 lactame ring moiety, acted as complete AR antagonists and exhibited AR specificity. Moreover, **11** and **18** inhibited the cell growth of androgen-dependent prostate cancer cells and the

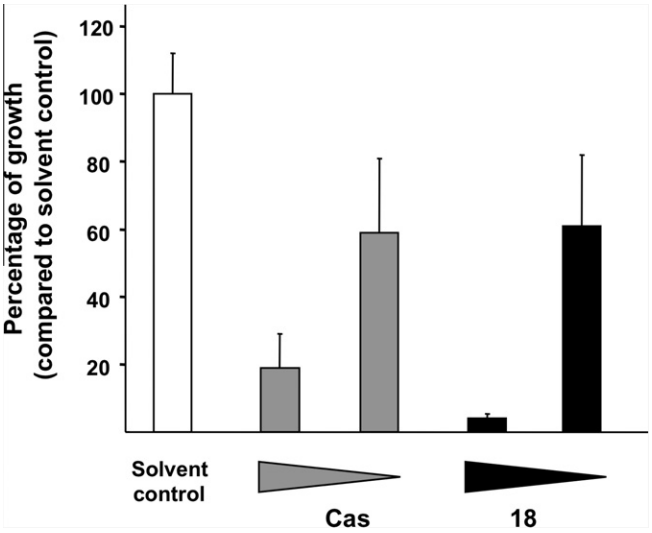


Figure 3. Compound **18** inhibits more potently the growth of human androgen-dependent PCa cells compared to Casodex. LNCaP cells were treated with Casodex (Cas) or compound **18** at concentrations of 10^{-5} or 10^{-6} M for 8 days. The number of cells treated with the solvent as control was set arbitrarily to 100%. The deviation of three independent experiments is shown.

expression of the prostate specific antigen. Remarkably, enhancement of the cellular senescence, that might explain in part the mediated growth inhibition, was induced by compound **18**.

In summary, the newly synthesized 20-aminosteroids may serve not only as a novel class of AR antagonists but also as valuable chemical tools for the investigation of novel inhibitory mechanisms of the AR transactivation and prostate cell growth. Specifically, the 20-aminosteroidal derivative **18** figures as a promising lead compound for the development of more potent AR antagonists with pure antagonistic activities against AR variants and as an effective inhibitor of PCa growth.

5. Experimental section

5.1. Chemistry

All reagents were obtained commercially from Acros, Alfa Aesar, Sigma-Aldrich or Merck and used without further purification. Reactions involving moisture-sensitive reactants were run in flame-dried glassware under an atmosphere of argon. Reagents and anhydrous solvents were transferred via syringe. Dichloromethane was distilled under argon from SICAPENT (phosphorus pentoxide on solid support with indicator). Benzene and methanol were purchased in anhydrous form and used without further purification. Analytical TLC was performed on Merck Silica gel 60 F_{254} on precoated silica gel plates, with visualization under UV light (254 and 365 nm) or/and by use of Seebach staining solution. Flash column chromatography was performed on silica gel (Acros 60A, 0.035–0.070 mm). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-300BB (300 MHz for ^{1}H; 75 MHz for ^{13}C), Varian Mercury-300BB (300 MHz for ^{1}H; 75 MHz for ^{13}C) and Varian Mercury-400BB spectrometer (400 MHz for ^{1}H; 100 MHz for ^{13}C). The chemical shifts (d) are reported in parts per million (ppm) and the residual solvent signal is used as an internal standard. The following abbreviations are used for the proton spectra multiplicities: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad signal (br). Coupling constants (*J*) are given in Hertz (Hz). High resolution mass spectra were obtained on a Bruker Daltonics APEX II (for ESI). Infrared spectra were obtained on an ATI/MATTSON Genesis FT-IR in KBr disks. Absorbance frequencies are reported in reciprocal centimeters (cm^{-1}). Melting points were measured with a Büchi Melting Point B-540 and are uncorrected. Optical rotations were determined with a half-automatic Schmidt+Haensch Polartronic D MHZ-8 at the sodium-D line (589 nm) using a 50 mm path-length cell and are reported as follows $[\alpha]_D^{23}$, concentration (g/100 mL), and solvent. Preparative HPLC was performed using Varian ProStar 210 solvent delivery module with a Jasco-Lichrosorb column (Si 60, 10 μm, 250 × 20 mm; eluent MeOH 2% in $CHCl_3$; flow rate of 10 ml min^{-1}) and a Varian ProStar 325 UV–vis detector (λ 254, 364 nm). Purity of the synthesized compounds was determined by a combination of ^{1}H, ^{13}C NMR and HLPC techniques and was found to be >95%.

5.1.1. (20*E*)-*N*-[*t*-Butyl-(*S*)-sulfinyl]-3β-(*t*-butyldimethylsilyloxy)-pregn-5-en-20-imine (2)

To a mixture of protected-pregnenolone **1** (289 mg, 0.67 mmol) and (*S*)-(−)-2-methyl-2-propanesulfinamide (68 mg, 0.559 mmol) titanium(IV) ethoxide (1.17 mL, 5.59 mmol) was added at rt under argon atmosphere. The reaction mixture was stirred at 90 °C for 48 h. After completion, it was allowed to cool to rt, cooled to 0 °C and quenched with an equal volume of saturated NaCl aqueous solution while vigorously stirring. The resulting suspension was filtered through a Celite pad and the filtered cake rinsed thoroughly with ethyl acetate. The two layers were separated and the aqueous layer was washed with ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–20% EtOAc in *n*-hexane) to afford **2** (187 mg, 63%) as a white crystalline solid; mp 182–184 °C; $[\alpha]_D^{23}$ = +89.9 (*c* 0.623 in $CHCl_3$); IR (KBr): ν_{max} 2933, 1608, 1094, 1068, 838 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.68 (s, 3H), 0.89 (s, 9H), 1.00 (s, 3H), 1.25 (s, 9H), 2.32 (s, 3H), 3.44–3.52 (m, 1H), 5.31 (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.59, 13.43, 18.24, 19.42, 21.09, 22.38, 23.70, 24.29, 25.06, 25.92, 31.78, 32.02, 32.03, 36.60, 37.37, 38.91, 42.77, 44.64, 50.10, 56.28, 56.99, 63.40, 72.53, 120.81, 141.62, 185.63; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{31}H_{56}NO_2SSi$: 534.37955, found: 534.37988.

5.1.2. (20*R*)-*N*-[*t*-Butyl-(*S*)-sulfinyl]-20-allyl-3β-(*t*-butyldimethylsilyloxy)-pregn-5-en-20-amine (3)

To a solution of **2** (258.8 mg, 0.4847 mmol) in dry CH_2Cl_2 (3.1 mL) a solution of allylmagnesium bromide 1.0 M in diethyl ether (0.97 mL, 0.97 mmol) was added dropwise at −78 °C under argon atmosphere. The reaction mixture was allowed to warm up slowly to rt with stirring overnight. Then it was quenched with saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–20% EtOAc in *n*-hexane) to afford **3** (222 mg, 80%) as an oil; $[\alpha]_D^{23}$ = −25.1 (*c* 0.303, $CHCl_3$); IR (KBr): ν_{max} 2931, 1472, 1462, 1436, 1252, 1093, 1071, 835 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.85 (s, 3H), 0.88 (s, 9H), 0.99 (s, 3H), 1.22 (s, 9H), 1.33 (s, 3H), 2.39 (dd, *J* = 13.9, 8.3 Hz, 1H), 2.53 (dd, *J* = 14.1, 6.2 Hz, 1H), 3.34 (s, 1H), 3.44–3.51 (m, 1H), 5.12–5.17 (m, 2H), 5.31 (d, *J* = 5.2 Hz, 1H), 5.90–6.00 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.59, 15.06, 18.24, 19.39, 20.87, 22.88, 23.01, 23.74, 24.76, 25.92, 31.34, 31.71, 32.04, 36.56, 37.34, 40.60, 42.77, 43.14, 47.56, 49.96, 55.93, 56.55, 57.90, 61.51, 72.52, 118.88, 120.92, 134.03, 141.54. HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{34}H_{62}NO_2SSi$: 576.42650, found: 576.42610.

5.1.3. (20*R*)-*N*-[*t*-Butyl-(*S*)-sulfinyl]-3β-(*t*-butyldimethylsilyloxy)-20-(1-methyl-2-propenyl)-pregn-5-en-20-amine (5)

To a solution of **2** (100 mg, 0.187 mmol) in dry CH_2Cl_2 (1.2 mL) a solution of 1-methyl-2-propenylmagnesium chloride 0.5 M in THF (0.756 mL, 0.378 mmol) was added dropwise at −78 °C under argon atmosphere. The reaction mixture was allowed to warm up slowly to rt with stirring overnight. Then it was quenched with saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–30% EtOAc in *n*-hexane) to afford **5** (67.5 mg, 63%) as an oil; $[\alpha]_D^{23}$ +13.6 (*c* 1.0, $CHCl_3$); IR (KBr): ν_{max} 2957, 2931, 1471, 1463, 1090, 1067, 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.06 (s, 6H), 0.83 (s, 3H), 0.89 (s, 9H), 0.99 (s, 3H), 1.12 (s, 1.5H), 1.13 (s, 1.5H), 1.23 (s, 9H), 1.35 (s, 3H), 2.53–2.63 (m, 1H), 3.29 (s, 1H), 3.44–3.52 (m, 1H), 5.02–5.09 (m, 2H), 5.31 (d, *J* = 5.2 Hz, 1H), 5.97 (ddd, *J* = 16.9, 10.3, 8.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.61, 14.69, 15.54, 18.26, 19.36, 20.90, 22.97, 24.01, 24.11, 25.92, 29.69, 31.21, 31.74, 32.00, 36.52, 37.30, 40.74, 42.72, 43.43, 48.65, 49.88, 56.12, 56.42, 56.52, 64.00, 72.50, 115.72, 120.98, 141.39, 141.46; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{35}H_{64}NO_2SSi$: 590.44215; found: 590.44161.

5.1.4. (20*R*)-*N*-[*t*-Butyl-(*S*)-sulfinyl]-20-benzyl-3β-(*t*-butyldimethylsilyloxy)-pregn-5-en-20-amine (7)

To a solution of **2** (100 mg, 0.187 mmol) in dry CH_2Cl_2 (1.2 mL) a solution of benzylmagnesium chloride 20% wt in THF (1.5 mL, 1.988 mmol) was added dropwise at −78 °C under argon atmosphere. The reaction mixture was allowed to warm up slowly to rt with stirring overnight. Then it was quenched with saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–30% EtOAc in *n*-hexane) to afford **7** (85.8 mg, 73%) as an oil; $[\alpha]_D^{23}$ = +10.8 (*c* 0.89, $CHCl_3$); IR (KBr): ν_{max} 2931, 2855, 1496, 1472, 1462, 1252, 1093, 1067, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.88 (s, 9H), 0.93 (s, 3H), 0.98 (s, 3H), 1.19 (s, 9H), 1.33 (s, 3H), 2.94 (dd, *J* = 78.3, 13.0 Hz, 2H), 3.42–3.50 (m, 1H), 3.56 (s, 1H), 5.30 (d, *J* = 5.1 Hz, 1H), 7.16–7.29 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.61, 14.75, 18.23, 19.35, 20.75, 22.86, 23.14, 23.85, 25.91, 26.82, 31.27, 31.66, 31.97, 36.49, 37.23, 39.97, 42.73, 43.03, 47.33, 49.73, 56.00, 56.49, 56.64, 61.56, 72.49, 120.86, 126.38, 127.88, 131.06, 137.39, 141.52; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{38}H_{64}NO_2SSi$: 626.44215, found: 626.44212, $[M+Na]^+$ calcd for $C_{38}H_{63}NNaO_2SSi$: 648.42410, found: 648.42352.

5.1.5. (20*E*)-*N*-[*t*-Butyl-(*R*)-sulfinyl]-3β-(*t*-butyldimethylsilyloxy)-pregn-5-en-20-imine (13)

To a mixture of **1** (517 mg, 1.2 mmol) and (*R*)-(+)-2-methyl-2-propanesulfinamide (242.4 mg, 2 mmol) titanium(IV) ethoxide (2.26 mL, 10.8 mmol) was added at rt under argon atmosphere. The reaction mixture was stirred at 83 °C for 31.5 h. Then, it was allowed to cool to rt, cooled to 0 °C and quenched with an equal volume of saturated NaCl aqueous solution while vigorously stirring. The resulting suspension was filtered through a Celite pad and the filtered cake rinsed thoroughly with ethyl acetate. The two layers were separated and the aqueous layer was washed with a mixture of ethyl acetate–tetrahydrofuran (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–20% EtOAc in *n*-hexane) to afford **13** (542 mg, 85%) as a white crystalline solid; mp 184.5–186.5 °C; $[\alpha]_D^{23}$ = −34.0 (*c* 1.0, $CHCl_3$); IR (KBr): ν_{max} 2934, 1618, 1081, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.65 (s, 3H), 0.88 (s, 9H), 0.99 (s, 3H), 1.24 (s, 9H), 2.32 (s, 3H), 3.44–3.52 (m, 1H), 5.32 (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.60, 13.50, 18.26, 19.41, 21.11, 22.20, 24.21, 24.23, 24.81, 25.92, 31.73, 32.02, 32.03, 36.58, 37.35, 39.09, 42.75, 44.78, 50.03, 56.09, 57.06, 63.12, 72.51, 120.87, 141.52, 186.23; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{31}H_{55}NNaO_2SSi$: 556.36150, found: 556.36200.

5.1.6. (20*S*)-*N*-[*t*-Butyl-(*R*)-sulfinyl]-20-allyl-3β-(*t*-butyldimethylsilyloxy)-pregn-5-en-20-amine (14)

To a solution of **13** (300 mg, 0.562 mmol) in dry CH_2Cl_2 (3.6 mL) a solution of allylmagnesium bromide 1.0 M in diethyl ether (1.12 mL, 1.12 mmol) was added dropwise at −78 °C under argon atmosphere. The reaction mixture was allowed to warm up slowly to rt with stirring overnight. Then it was quenched with saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (10–20% EtOAc in *n*-hexane) to afford **14** (287 mg, 89%) as an oil; $[\alpha]_D^{23}$ = −56.2 (*c* 0.705, $CHCl_3$); IR (KBr): ν_{max} 2933, 1472, 1462, 1252, 1093, 1071, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.82 (s, 3H), 0.88 (s, 9H), 0.98 (s, 3H), 1.20 (s, 9H), 1.39 (s, 3H), 2.41 (dd, *J* = 13.8, 8.4 Hz, 1H), 2.53 (dd, *J* = 13.9, 6.3 Hz, 1H), 3.31 (s, 1H), 3.43–3.52 (m, 1H), 5.06–5.13 (m, 2H), 5.30 (d, *J* = 5.3 Hz, 1H), 5.81 (dddd, *J* = 16.6, 10.1, 8.4, 6.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.60, 15.02, 18.26, 19.36, 20.83, 22.35, 22.79, 23.63, 24.88, 25.93, 31.31, 31.68, 32.02, 36.53, 37.32, 40.08, 42.75, 42.84, 48.75, 49.94, 55.80, 56.45, 57.75, 60.91, 72.53, 118.30, 120.98, 134.26, 141.51; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{34}H_{62}NO_2SSi$: 576.42650, found: 576.42588.

5.1.7. General procedure for the synthesis of the 20-aminosteroids (4), (6), (8) and (15)

To a solution of the appropriate sulfinamide (1 equiv) in MeOH a 5–6 N solution of HCl in *iso*-propanol (∼2.0 equiv) was added. The mixture was stirred for the indicated time at rt and then concentrated to near dryness. Diethyl ether was added and the product precipitated as solid. The precipitate was filtered off, washed with diethyl ether to provide the corresponding amine as hydrochloride salt which was used without further purification.

5.1.7.1. (20*R*)-20-Allyl-20-amino-pregn-5-ene-3β-ol hydrochloride (4). Compound **4** was synthesized according to the general procedure from compound **3** (322 mg, 0.559 mmol), using a 5–6 N solution of HCl in *iso*-propanol (∼2.0 equiv, 0.22 mL) in MeOH (1.78 mL). The reaction mixture was stirred for 30 min at rt. Yield: 201.2 mg (91%), white solid; mp 322–324 °C (dec.); $[\alpha]_D^{23}$ = −52.8 (*c* 0.394, CH_3OH); IR (KBr): ν_{max} 3076, 2930, 1461, 1437, 1056, 933 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 0.92 (s, 3H), 1.03 (s, 3H), 1.41 (s, 3H) 2.43 (dd, *J* = 14.5, 7.0 Hz, 1H), 2.52 (dd, *J* = 14.5, 7.9 Hz, 1H), 3.37–3.43 (m, 1H), 5.26–5.31 (m, 2H), 5.35 (d, *J* = 5.3 Hz, 1H), 5.84–5.94 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.62, 19.86, 21.96, 23.87, 24.00, 24.54, 32.30, 32.72, 32.77, 37.70, 38.57, 40.50, 43.02, 44.07, 44.28, 51.44, 58.01, 58.31, 61.08, 72.41, 121.90, 122.21, 132.13, 142.33; HRMS-ESI (*m*/*z*): $[M]^+$ calcd for $C_{24}H_{40}NO$: 358.31044, found: 358.31032.

5.1.7.2. (20*R*)-20-Amino-20-(1-methyl-2-propenyl)-pregn-5-ene-3β-ol hydrochloride (6). Compound **6** was synthesized according to the general procedure from compound **5** (59.7 mg, 0.101 mmol), using a 5–6 N solution of HCl in *iso*-propanol (∼2.0 equiv, 0.04 ml) in MeOH (0.32 mL). The reaction mixture was stirred for 30 min at rt. Yield: 33.1 mg (80%), brownish solid; mp 279–280 °C (dec.); $[\alpha]_D^{23}$ = −9.0 (*c* 0.621, CH_3OH); IR (KBr): ν_{max} 3379, 2932, 1638, 1504, 1071, 937 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 0.94 (s, 3H), 1.04 (s, 3H), 1.13 (s, 1.5H), 1.15 (s, 1.5H), 1.29 (s, 3H), 2.70 (qd, *J* = 13.9, 6.9 Hz, 1H), 3.36–3.44 (m, 1H), 5.24–5.31 (m, 2H), 5.36 (d, *J* = 5.2 Hz, 1H), 5.87 (ddd, *J* = 17.0, 10.1, 9.1 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.17, 15.88, 19.86, 20.57, 22.00, 24.39, 24.48, 32.30, 32.71, 32.79, 37.69, 38.55, 40.45, 43.01, 44.31, 46.70, 51.36, 55.26, 57.83, 63.82, 72.41, 119.52, 122.22, 138.50, 142.31; HRMS-ESI (*m*/*z*): $[M-Cl]^+$ calcd for $C_{25}H_{42}NO$: 372.32609, found: 372.32602.

5.1.7.3. (20*R*)-20-Amino-20-benzyl-pregn-5-ene-3β-ol hydrochloride (8). Compound **8** was synthesized according to the general procedure from compound **7** (68.2 mg, 0.109 mmol), using a 5–6 N solution of HCl in *iso*-propanol (∼2.0 equiv, 0.044 ml) in MeOH (0.34 mL). The reaction mixture was stirred for 30 min at rt. Yield: 44.9 mg (93%), yellowish solid; mp 263–265 °C (dec.); $[\alpha]_D^{23}$ = −47.6 (*c* 0.807, CH_3OH); IR (KBr): ν_{max} 3397, 2930, 1605, 1498, 1060, 750, 705 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 0.97 (s, 3H), 1.04 (s, 3H), 1.37 (s, 3H), 3.04 (dd, *J* = 67.9, 13.8 Hz, 2H), 3.34–3.45 (m, 1H), 5.36 (d, *J* = 5.1 Hz, 1H), 7.28–7.42 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.43, 19.86, 22.01, 22.61, 24.08, 24.64,

32.30, 32.73, 32.82, 37.69, 38.54, 40.90, 43.01, 44.40, 46.13, 51.40, 58.03, 58.62, 61.86, 72.41, 122.19, 128.80, 129.92, 132.06, 135.67, 142.35; HRMS-ESI (m/z): $[M-Cl]^+$ calcd for $C_{28}H_{42}NO$: 408.32609, found: 408.32590.

5.1.7.4. (20*S*)-20-Allyl-20-amino-pregn-5-ene-3β-ol hydrochloride (15). Compound **15** was synthesized according to the general procedure from compound **14** (263 mg, 0.457 mmol), using a 5–6 N solution of HCl in *iso*-propanol (~2.0 eq, 0.18 mL) in MeOH (1.45 mL). The reaction mixture was stirred for 2 h at rt. Yield: 171.5 mg (95%), white solid; mp 282–284 °C (dec.); $[\alpha]_D^{23} = -66.4$ (c 0.506, CH_3OH); IR (KBr): ν_{max} 3283, 2926, 1613, 1593, 1530,1519, 1450, 1056, 951; ^1H NMR (400 MHz, CD_3OD): δ 0.86 (s, 3H), 1.00 (s, 3H), 1.37 (s, 3H), 2.49 (d, J = 7.2 Hz, 2H), 3.31–3.41 (m, 1H), 5.25 (d, J = 7.5 Hz, 1H), 5.28 (s, 1H), 5.32 (d, J = 4.8 Hz, 1H), 5.84–5.94 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.48, 19.86, 21.99, 22.91, 23.69, 24.60, 32.30, 32.75, 32.80, 37.69, 38.55, 40.77, 43.01, 44.33, 44.96, 51.42, 56.99, 57.97, 61.10, 72.40, 121.98, 122.20, 132.29, 142.33; HRMS-ESI (m/z): $[M]^+$ calcd for $C_{24}H_{40}NO$: 358.31044, found: 358.31010.

5.1.8. (20*R*)-20-Acrylamido-20-allyl-pregn-5-en-3β-ol acrylate (9) and (20*R*)-20-acrylamido-20-allyl-pregn-5-en-3β-ol (10)

To a solution of **4** (201.2 mg, 0.51 mmol) in H_2O (5.24 mL) and triethylamine (1.279 mL, 9.19 mmol) dichloromethane (5.24 mL) was added. The two-phase mixture was cooled at 0 °C and acryloyl chloride (0.74 mL, 9.19 mmol) was added dropwise. The reaction mixture was allowed to warm up and stirred at rt for 21 h. Then, it was diluted with chloroform and the layers were separated. The aqueous layer was washed with chloroform (4×) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (20–40% EtOAc in *n*-hexane) to afford **9** (53.9 mg, 23%) and **10** (91.6 mg, 44%) both as white solids; **9**: mp 123–125 °C; $[\alpha]_D^{23} = -55.4$ (c 0.808, $CHCl_3$); IR (KBr): ν_{max} 3351, 2944, 1725, 1696, 1656, 1541, 1413, 1215 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H), 1.02 (s, 3H), 1.40 (s, 3H), 2.41 (dd, J = 13.9, 7.4 Hz 1H), 2.73 (dd, J = 13.8, 7.3 Hz, 1H), 4.64–4.72 (m, 1H), 5.04–5.09 (m, 2H), 5.39 (br s, 2H), 5.57 (dd, J = 10.2, 1.4 Hz 1H), 5.66–5.76 (m, 1H), 5.80 (dd, J = 10.4, 1.5 Hz, 1H), 5.93–6.19 (m, 2H), 6.21 (dd, J = 16.9, 1.4 Hz, 1H), 6.44 (ddd, J = 44.9, 17.3, 1.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.99, 19.30, 20.80, 22.26, 23.15, 23.53, 27.71, 31.28, 31.60, 36.53, 36.91, 38.04, 39.31, 42.90, 43.05, 49.75, 55.69, 56.12, 59.76, 74.01, 118.35, 122.53, 125.56, 128.98, 130.30, 132.18, 134.01, 139.54, 164.71, 165.64; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{30}H_{44}NO_3$: 466.33157, found: 466.33166; **10**: mp 188–190 °C; $[\alpha]_D^{23} = -88.8$ (c 0.608, $CHCl_3$); IR (KBr): ν_{max} 3314, 2905, 1666, 1624, 1557, 1461, 1438, 1058 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H), 0.99 (s, 3H), 1.38 (s, 3H), 2.37 (dd, J = 13.8, 7.4 Hz, 1H), 2.76 (dd, J = 13.8, 7.2 Hz, 1H), 3.48–3.56 (m, 1H), 5.03–5.08 (m, 2H), 5.34 (dd, J = 5.3, 1.7 Hz, 1H), 5.39 (s, 1H), 5.56 (dd, J = 10.2, 1.5 Hz, 1H), 5.72 (tdd, J = 17.8, 10.5, 7.3 Hz, 1H), 6.01 (dd, J = 16.9, 10.2 Hz, 1H), 6.20 (dd, J = 16.9, 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.00, 19.36, 20.82, 22.24, 23.19, 23.54, 31.31, 31.58, 31.59, 36.42, 37.17, 39.29, 42.20, 42.86, 43.04, 49.84, 55.58, 56.16, 59.71, 71.61, 118.33, 121.47, 125.54, 132.16, 134.01, 140.72, 164.68; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{27}H_{42}NO_2$: 412.32101, found: 412.32119.

5.1.8.1. Hydrolysis of compound (9). To a solution of **9** (43.9 mg, 0.094 mmol) in dry MeOH (2.06 ml) sodium methylate (22.4 mg, 0.415 mmol) was added and the reaction mixture was stirred for 6 h at rt under argon atmosphere. The solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (35–40% EtOAc in *n*-hexane) to afford **10** (31.6 mg, 81%) as a white solid.

5.1.9. (20*S*)-20-Acrylamido-20-allyl-pregn-5-en-3β-ol acrylate (16) and (20*S*)-20-acrylamido-20-allyl-pregn-5-en-3β-ol (17)

To a solution of **15** (130 mg, 0.33 mmol) in H_2O (3.39 ml) and triethylamine (0.827 mL, 5.939 mmol) dichloromethane (3.39 mL) was added. The two-phase mixture was cooled at 0 °C and acryloyl chloride (0.48 mL, 5.939 mmol) was added dropwise. The reaction mixture was allowed to warm up and stirred at rt for 21 h. Then, it was diluted with chloroform and the layers were separated. The aqueous layer was washed with chloroform (4×) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (20–40% EtOAc in *n*-hexane) to afford **16** (36 mg, 23%) as a yellow solid and **17** (61.9 mg, 46%) as a white solid; **16**: mp 158–160 °C; $[\alpha]_D^{23} = -71.4$ (c 0.639, $CHCl_3$); IR (KBr): ν_{max} 3362, 2936, 1724, 1665, 1530, 1405, 1196 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.83 (s, 3H), 1.02 (s, 3H), 1.42 (s, 3H), 3.10 (dd, J = 13.6, 6.5 Hz, 1H), 4.64–4.72 (m, 1H), 5.05–5.10 (m, 2H), 5.37–5.38 (m, 2H), 5.55 (dd, J = 10.2, 1.5 Hz, 1H), 5.71–5.81 (m, 2H), 5.97–6.12 (m, 2H), 6.18 (dd, J = 16.9, 1.5 Hz, 1H), 6.37 (dd, J = 17.3, 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.44, 19.27, 20.75, 22.85, 23.52, 23.65, 27.69, 31.32, 31.63, 36.54, 36.90, 38.04, 39.59, 42.70, 43.12, 49.76, 56.12, 56.25, 59.62, 74.01, 117.98, 122.48, 125.38, 128.98, 130.24, 132.27, 134.41, 139.60, 164.46, 165.60; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{30}H_{43}NNaO_3$: 488.31352, found: 488.31390; **17**: mp 194.5–196.5 °C; $[\alpha]_D^{23} = -73.9$ (c 0.460, $CHCl_3$); IR (KBr): ν_{max} 3272, 3075, 2926, 1666, 1625, 1556, 1433, 1251, 1063 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (s, 3H), 0.99 (s, 3H), 1.40 (s, 3H), 3.10 (dd, J = 13.6, 6.5 Hz, 1H), 3.46–3.56 (m, 1H), 5.04–5.10 (m, 2H), 5.33–5.35 (m, 2H), 5.55 (d, J = 10.3 Hz, 1H), 5.70–5.81 (m, 1H), 6.00 (dd, J = 16.9, 10.2 Hz, 1H), 6.18 (d, J = 16.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.49, 19.34, 20.79, 22.87, 23.51, 23.57, 31.36, 31.56, 31.62, 36.44, 37.17, 39.62, 42.15, 42.67, 43.04, 49.85, 55.99, 56.32, 59.67, 71.61, 118.01, 121.42, 125.51, 132.17, 134.40, 140.80, 164.51; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{27}H_{41}NNaO_2$: 434.30295, found: 434.30275.

5.1.9.1. Hydrolysis of compound (16). To a solution of **16** (29.6 mg, 63.6 μmol) in dry MeOH (1.39 ml) sodium methylate (15.1 mg, 279.5 μmol) was added and the reaction mixture was stirred for 6 h at rt under argon atmosphere. The solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (35–40% EtOAc in *n*-hexane) to afford **17** (26 mg, 99%) as white solid.

5.1.10. General procedure for the synthesis of the 20-aminosteroids (11) and (18)

To a solution of the appropriate 20-acrylamido derivative (1 equiv) in dry and previously degassed dichloromethane, 2nd generation Grubbs catalyst (5 mol %) was added at rt under an argon atmosphere. After being refluxed for the indicated time, the reaction mixture was exposed to air with stirring for 1 h and the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (2% MeOH in dichloromethane) to afford the corresponding C-20 lactame ring steroidal derivative.

5.1.10.1. 17-[(6*R*)-6-Methyl-5,6-dihydropyridin-2(1*H*)-one-6-yl]-androst-5-ene-3β-ol (11). Compound **11** was synthesized according to the general procedure from compound **10** (90.4 mg, 219.6 μmol), using 2nd generation Grubbs catalyst (9.33 mg, 11 μmol, 5 mol %) in dichloromethane (7 mL). The reaction mixture was refluxed for 4.5 h. Yield: 77.5 mg (92%), white solid; mp 207–

209 °C; $[\alpha]_D^{23} = -53.5$ (*c* 0.680, $CHCl_3$); IR (KBr): ν_{max} 3388, 2927, 1729, 1667, 1605, 1427 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.83 (s, 3H), 1.00 (s, 3H), 1.34 (s, 3H), 2.60 (ddd, *J* = 18.0, 3.6, 2.3 Hz, 1H), 3.48–3.55 (m, 1H), 5.34 (d, *J* = 5.2 Hz, 1H), 5.48 (s, 1H), 5.89 (dd, *J* = 9.9, 1.6 Hz, 1H), 6.51 (td, *J* = 9.7, 4.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.36, 19.36, 20.78, 23.62, 23.64, 26.92, 31.26, 31.59, 35.72, 36.43, 37.16, 40.13, 42.20, 42.94, 49.85, 56.59, 57.58, 58.57, 71.65, 121.30, 123.54, 140.01, 140.83, 165.06; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{38}NO_2$: 384.28971, found: 384.29008, $[M+Na]^+$ calcd for $C_{25}H_{37}NNaO_2$: 406.27165, found: 406.27203.

5.1.10.2. 17-[(6*S*)-6-Methyl-5,6-dihydropyridin-2(1*H*)-one-6-yl]-androst-5-ene-3β-ol (18). Compound **18** was synthesized according to the general procedure from compound **17** (55.9 mg, 135.8 μmol), using 2nd generation Grubbs catalyst (5.76 mg, 6.79 μmol, 5 mol %) in dichloromethane (4.9 mL). The reaction mixture was refluxed for 4 h. Yield: 44 mg (84%), white solid; mp 213–215 °C; $[\alpha]_D^{23} = -42.9$ (*c* 0.504, CH_3OH); IR (KBr): ν_{max} 3429, 2935, 1673, 1613, 1428 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H), 0.98 (s, 3H), 1.34 (s, 3H), 2.58 (td, *J* = 18.2, 2.8 Hz, 1H), 3.44–3.52 (m, 1H), 5.31 (d, *J* = 5.2 Hz, 1H), 5.61 (s, 1H), 5.86 (d, *J* = 10.0 Hz, 1H), 6.47 (ddd, *J* = 9.8, 5.2, 3.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.78, 19.31, 20.66, 23.33, 23.55, 27.13, 31.15, 31.46, 31.53, 35.91, 36.36, 37.13, 39.79, 42.12, 43.06, 49.77, 56.47, 57.64, 59.23, 71.44, 121.14, 123.08, 139.90, 140.81, 165.45; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{25}H_{37}NNaO_2$: 406.27165, found: 406.27160.

5.1.11. General procedure for the synthesis of the 20-aminosteroids (12) and (19)

To a solution of the appropriate 3β-hydroxy C-20 lactame ring steroidal derivative (20.1 mg, 52.4 μmol) in dry benzene (5 mL), cyclohexanone (135.7 μL, 1.31 mmol) was added and the mixture was refluxed with a Dean–Stark apparatus until ~2 mL of solvents were distilled under an argon atmosphere. Then, a suspension of $Al(i\text{-}PrO)_3$ (32.1 mg, 157.2 μmol) in dry benzene (2 mL) was added and the reaction mixture was refluxed again until ~2 mL of solvents were distilled. Then, the reaction mixture was stirred at 80 °C for the indicated time. After the end of the reaction, water was added and the mixture was extracted with chloroform. The aqueous phase was washed (4×) with chloroform and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (1–3% MeOH in dichloromethane) to afford the corresponding 3-keto-4,5-en-steroidal derivative. Analytical samples of the final products were provided after HPLC purification (Preparative HPLC, $CHCl_3$–MeOH 2%, 10 mL/min, 30 min, 254–364 nm).

5.1.11.1. 17-[(6*R*)-6-Methyl-5,6-dihydropyridin-2(1*H*)-one-6-yl]-androst-4-ene-3-one (12). Compound **12** was synthesized according to the general procedure from compound **11**. The reaction mixture was stirred at 80 °C for 16 h. Yield 11.6 mg (58%), white solid; mp 248–250 °C; $[\alpha]_D^{23} = +101.2$ (*c* 0.253, $CHCl_3$); IR (KBr): ν_{max} 3431, 2927, 1682, 1619, 1427, 877, 824 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H), 1.18 (s, 3H), 1.34 (s, 3H), 2.60 (ddd, *J* = 18.0, 3.7, 2.2 Hz, 1H), 5.39 (s, 1H), 5.72 (s, 1H), 5.90 (ddd, *J* = 9.9, 3.7, 1.8 Hz, 1H), 6.51 (td, *J* = 9.9, 4.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.40, 17.36, 20.75, 23.53, 27.07, 31.73, 32.75, 33.92, 34.93, 35.64, 35.77, 38.49, 39.99, 43.02, 53.56, 55.77, 57.47, 58.42, 123.54, 123.93, 139.92, 165.01, 170.90, 199.43; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{36}NO_2$: 382.27406, found: 382.27431.

5.1.11.2. 17-[(6*S*)-6-Methyl-5,6-dihydropyridin-2(1*H*)-one-6-yl]-androst-4-ene-3-one (19). Compound **19** was synthesized according to the general procedure from compound **18**. The reaction mixture was stirred at 80 °C for 15 h. Yield 10 mg (50%), white solid; mp 226–228 °C; $[\alpha]_D^{23} = +72.90$ (*c* 0.417, $CHCl_3$); IR (KBr): ν_{max} 3431, 2927, 1676, 1612, 1414, 892, 825 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H), 1.18 (s, 3H), 1.37 (s, 3H), 2.60 (td, *J* = 18.2, 2.8 Hz, 1H), 5.54 (s, 1H), 5.72 (s, 1H), 5.89 (d, *J* = 10.1 Hz, 1H), 6.49 (ddd, *J* = 9.9, 5.3, 3.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.95, 17.34, 20.70, 23.42, 23.54, 27.26, 31.74, 32.73, 33.92, 34.88, 35.66, 36.08, 38.49, 39.79, 43.24, 53.55, 55.73, 57.71, 59.36, 123.17, 123.91, 139.90, 165.46, 170.87, 199.41; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{25}H_{35}NNaO_2$: 404.25600, found: 404.25584.

5.2. Biological assays

5.2.1. Chemicals and hormones

Methyltrienolone (R1881) was obtained from Perkin Elmer and AA from Merck, Darmstadt. All test compounds were dissolved in ethanol or/and dimethylsulfoxide (DMSO). These compounds were added to the culturing medium such that the final concentration of ethanol and/or DMSO did not exceed 0.1%.

5.2.2. Plasmids

The plasmid pMMTV-luc, which contains a luciferase reporter gene driven by the mouse mammary tumor virus long terminal repeats responsive to androgens, is described in Gast et al.[18] The expression vector for the human AR or AR T877A, pSG-hAR or pSG-hAR T877A are described in the literature.[24] The plasmid for expression of human GR is described also in the literature.[25] Human PR-B expression vector was kindly provided by P. Chambon (Strasbourg, France).

5.2.3. Reporter assays

CV1 cells were seeded onto 6-well tissue culture plates (Nunc, Roskilde, Denmark) at 1.2×10^5 cells per well and grown in DMEM (Invitrogen) supplemented with 5% (v/v) dextran-coated charcoal stripped serum and 1% (v/v) penicillin and streptomycin.[26] 18 hours later cells were transfected by using the $CaPO_4$ method.[24] The DNA mixture for transfections consisted of 1 μg of the appropriated luciferase reporter constructs, 0.2 μg of the appropriated mammalian steroid receptor expression vector and 0.2 μg of the cytomegalovirus (CMV)-driven β-galactosidase expressions vector, as internal control for transfection efficiency. After 24 h media were replaced either with or without the addition of the appropriate hormones together with the indicated compounds. After additional 96 h cells were harvested and assayed for luciferase and β-galactosidase activity. All transfection assays shown were performed in duplicate and were repeated at least twice.

5.2.4. Cell growth assays

Human prostate carcinoma LNCaP cells[27] were cultured in RPMI-1640 medium (Invitrogen), supplemented with 10% (v/v) fetal calf serum (FCS) (Invitrogen), 1% (v/v) penicillin and streptomycin (Invitrogen), 1% (v/v) L-glutamin (Invitrogen), 1% (v/v) sodium pyruvate (Sigma). PC3 were kindly provided by Dr. A. Cato, Karlsruhe, Germany[28] and were cultured in DMEM supplemented with 10% (v/v) FCS, 1% (v/v) penicillin and streptomycin and 1% (v/v) L-glutamin. For cell growth assays, cells were seeded onto six-well tissue culture plates (Nunc, Roskilde, Denmark) at 5×10^3 (PC3) or 25×10^3 (LNCaP) cells per well in appropriated medium in triplicates containing 5% FCS. After 2 days, cells were fed with fresh medium and treated with DMSO or with the indicated compounds. Every second/third day the media was replaced with fresh media together with freshly added compounds. The cells of the same two 8 μm^2 areas were counted per well at the indicated times.

5.2.5. Real time RT-PCR

The real time RT-PCR (qRT-PCR) was performed essentially as described previously[20] with specific primers for detection of PSA mRNA and beta-actin mRNA for normalization. As modifications 1.28×10^6 cells were seeded out directly in media containing 10% charcoal stripped FCS. After two days cells were treated with the indicated compounds or solvent (DMSO) or androgen (R1881) was added to a final concentration of 3×10^{-11} M.

5.2.6. Detection of SA-β galactosidase

Senescence-associated β-Gal (SA-β-Gal) staining was carried out as described previously[21,29] using 40,000 cells per well in a 6-well plate. The treatment was performed for 3 days.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.029.

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15. During optimization trials, we noticed unexpectedly the formation of the (20*E*)-sulfonylimine derivative **2a** when (*S*)-(−)-2-methyl-2-propanesulfinamide was used in excess to the steroidal ketone. The structure of **2a** was verified by X-ray analysis. For spectroscopic data of **2a** see Supplementary data.
16. In the case of imine **13** excess of the chiral auxiliary was necessary for the complete consumption of the steroidal ketone **2**.
17. CCDC-753934, 753933, 753936, 753935 and 753937 (**2**, **2a**, **4**, **13** and **15**, respectively) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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