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Nonsteroidal Androgen Receptor Ligands: Versatile Syntheses and Biological Data

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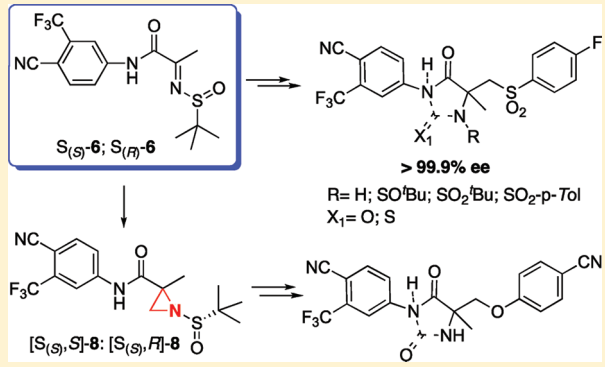
Supporting Information

ABSTRACT: We report herein a stereoselective and straightforward methodology for the synthesis of new androgen receptor ligands with (anti)-agonistic activities. Oxygen−nitrogen replacement in bicalutamide-like structures paves the way to the disclosure of a new class of analogues, including cyclized/nitrogen-substituted derivatives, with promising antiandrogen (or anabolic) activity.



KEYWORDS: *androgen receptor, enantiopure antiandrogens, hormone-refractory prostate cancer, sulfinylimino propanamides*

Nonsteroidal androgen receptor (AR) ligands represent a very important class of molecules acting as either antagonists or agonists of AR. In particular, nonsteroidal antiandrogens, such as (*R*,*S*)-bicalutamide (**1**) and nilutamide (**2**), have been shown to be effective in the treatment of prostate cancer (PCa) (Figure 1). These kinds of molecules

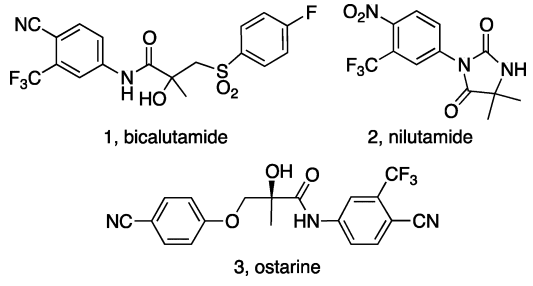


Figure 1. (*R*,*S*)-Bicalutamide (**1**), nilutamide (**2**), and ostarine (**3**).

present several advantages over steroidal antagonists such as oral bioavailability and lack of cross-reactivity with other steroid receptors.[1] In particular, because bicalutamide showed a superior pharmacokinetic profile along with minor side effects,[2] it has been chosen as the routine clinical treatment.

However, after its long-term use, a subset of patients had to discontinue bicalutamide treatment. This behavior has been termed "anti-androgen withdrawal syndrome" and indicates that these drugs serve as agonists under resistance circumstances.[3,4] Sawyers and co-workers showed that a 3−5-fold up-regulation of the AR is one of the most likely causes of resistance to antiandrogens.[5,6]

They further demonstrated that hormone-refractory prostate cancer (HRPC) was still dependent on the AR ligand binding domain for growth.[6] Therapeutic options for HRPC patients are limited, with lack of evidence for long-term survival. Besides PCa, it has been widely reported that little structural modifications of bicalutamide-like molecules provide dramatic changes on their biological activity, for example, from AR antagonist to agonist.[2−10] Nonsteroidal AR agonists are still in the early stages of drug discovery, and ostarine **3** (Figure 1) is one of the most promising for muscle wasting and osteoporosis treatment.

Indeed, there is an urgent need for novel compounds able to act as either antiandrogens in HRPC conditions or nonsteroidal anabolics. In view of these concerns, we developed an effective and inexpensive methodology, able to easily afford novel structural hybrids of bicalutamide and/or nilutamide and/or ostarine.

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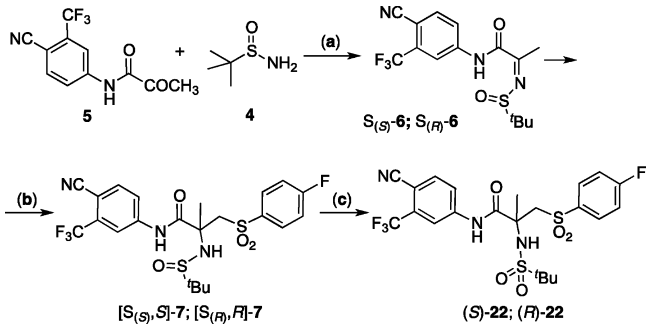
tert-Butane sulfinamide (**4**), introduced by Ellman's group[11−13] (Scheme 1), represents a very attractive precursor

Scheme 1. *tert*-Butane Sulfinylimino-*N*-(4-cyano-3-trifluoromethylphenyl)propanamides $S_{(S)}$- and $S_{(R)}$-6[a]



[a]Reagents and conditions: (a) $Ti(OEt)_4$; THF, 40 °C. (b) *p*-F-$(CH_3SO_2)C_6H_4$, ^{n}BuLi, THF, −45 °C. (c) MCPBA, CH_2Cl_2, rt, 2 h.

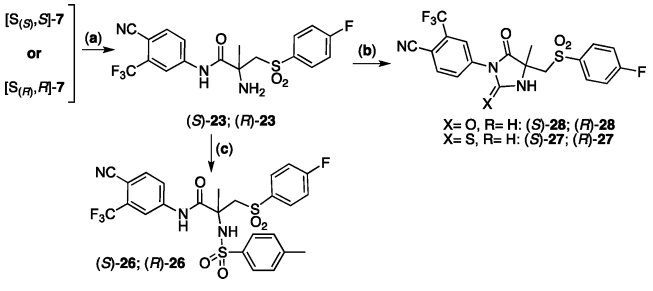
to chiral amines. Thus, we exploited this chemistry to provide a new synthetic and straightforward route to novel and enantiopure AR modulators. Their synthesis should also allow us to study the relationship between stereochemistry and pharmacological activity, the importance of stereochemistry already having been demonstrated for bicalutamide.[14]

To the best of our knowledge, no examples of highly derivatizated *N*-*tert*-butane-sulfinyl ketimines bearing an electron poor aryl propanamide skeleton have been reported so far. Sulfinylimino propanamides $S_{(S)}$- and $S_{(R)}$-**6** were obtained by reaction of oxopropanamide (**5**)[15,16] with (*S*)/(*R*)-**4** with acceptable isolated yield (56%), and they represent the key starting materials for the preparation of the new AR ligands. In fact, their reactivity allows the reaction with different carbanions. In particular, an alkylation reaction with *p*-fluorophenyl methyl sulfone occurs in good yield (78%) and with 76% diastereomeric excess, which was evaluated by NMR analysis of the crude material.[17] During the purification step, only one diastereoisomer could be isolated.

Thus, ketimine $S_{(S)}$-**6** affords compounds (*S*)-**22**, (*S*)-**23**, (*S*)-**26**, (*S*)-**27**, and (*S*)-**28**, while their enantiomers are provided by ketimine $S_{(R)}$-**6** (Schemes 1 and 2). The absolute configuration of compound (*S*)-**23** was determined by electronic circular dichroism (ECD) (see the Supporting Information for details).

For in vitro experiments, we selected LNCaP and LNCaP-AR human PCa cell lines, considering them the most

Scheme 2. Synthesis of Amino Bicalutamide Analogues[a]



[a]Reagents and conditions: (a) HCl, MeOH, −5 °C to rt, 3 h. (b) CDI, DPEA, toluene, 100 °C, 3 h or DPTC, DPEA, toluene, 100 °C, 5 h. (c) *p*-CH_3−$C_6H_4SO_2Cl$, pyridine.

representative for our preliminary studies. LNCaP cells express a point-mutated AR, which leads to a decrease in steroid-binding specificity.[18,19] Nonetheless, these cells have largely been used as a model of androgen-responsive growth.[20] On the other hand, human PCa cells LNCaP-AR have been engineered by Jung et al. with the aim to evaluate the potency of emerging nonsteroidal antiandrogens (MDV3100).[5,20] These cells, in fact, express 3−5-fold higher levels of the AR, thus mimicking the clinical scenario of HRPC.[5,21,22] Because PCa progression most frequently correlates with a rise in PSA concentrations,[23] we first measured the effect of our compounds on PSA levels in culture medium of LNCaP and LNCaP-AR cells. Figure 2A

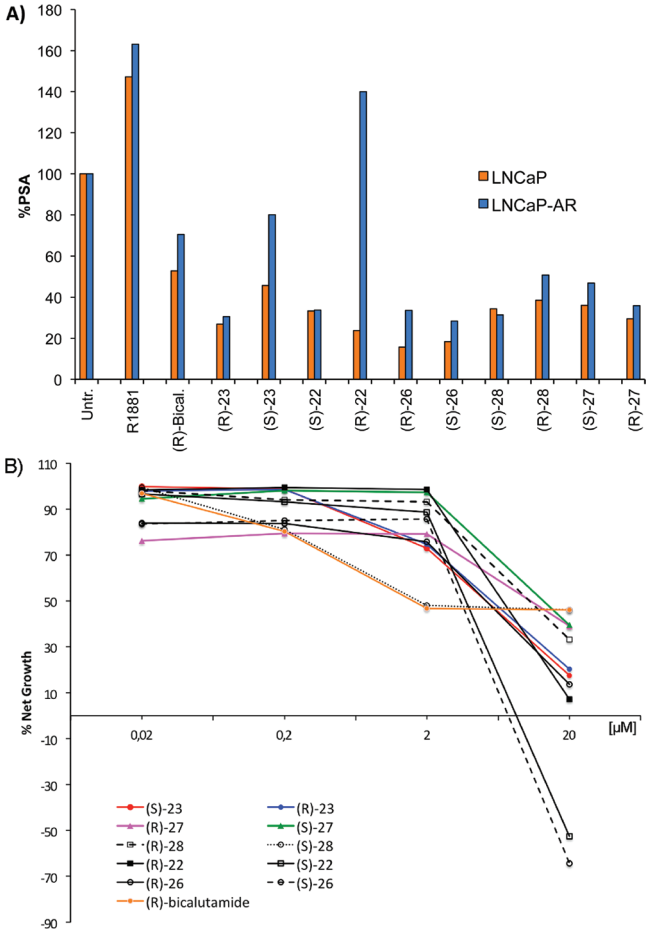


Figure 2. (A) PSA levels induced by antiandrogens (20 μM). A significant increment ($p < 0.01$) of PSA levels in LNCaP (47%) and LNCaP-AR (63%) was observed upon 48 h of exposure to the synthetic androgen R1881, as expected. (B) Cytotoxic activity of (*R*)-bicalutamide and new AR ligands in LNCaP cell lines (average of three independent experiments).

shows that some of the antiandrogens synthesized by us inhibit PSA secretion in culture medium of both cell lines, causing a PSA level reduction significantly higher ($p < 0.01$) than that produced by (*R*)-bicalutamide, the most active enantiomer. In particular, compound (*S*)-**26** showed the highest PSA reduction effect (82% on LNCaP and 72% on LNCaP-AR). Chemosensitivity analysis was performed by SRB assay on LNCaP cells,[24] which were incubated for 144 h to scalar drug concentrations ranging from 0.02 to 20 μM. As shown in Figure 2B, all compounds showed higher cell growth inhibition, as compared to (*R*)-bicalutamide (orange line), in the range of

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2−20 μM, with (*S*)-**26** and (*S*)-**22** being the most potent of the series. These preliminary data prompted us to further explore the AR antagonistic capability of derivatives (*S*)-**26** and (*S*)-**22**. A chemosentitivity test on LNCaP-AR cells exhibited a stronger cytotoxic effect of (*S*)-**22** and (*S*)-**26** with respect to the control drug, especially between 2 and 20 μM. In fact, even at these concentrations, (*R*)-bicalutamide was ineffective in inhibiting cells proliferation (Figure 3A). Moreover, a high cytocidal effect

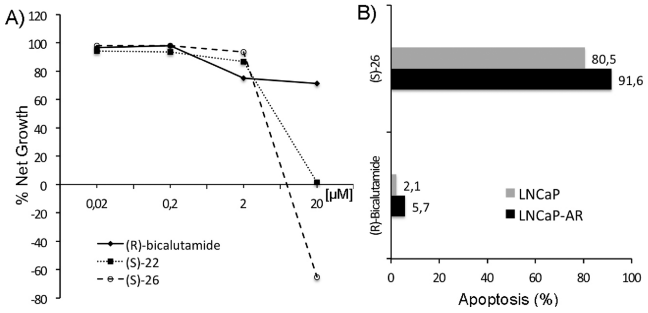


Figure 3. (A) Cytotoxic activity of (*R*)-bicalutamide and (*S*)-**22**/**26** in LNCaP-AR cell lines (average of three independent experiments). (B) Apoptosis analysis by flow cytometry TUNEL assay.

at 20 mM was observed for (*S*)-**26** in both LNCaP and LNCaP-AR cell lines (LC_{50} = 18.3 μM in both cell lines). Overall, (*R*)-bicalutamide never reached LC_{50} and IG_{50} (1.8 μM) only in LNCaP cells.

After 144 h of exposure to 20 μM (*R*)-bicalutamide, a moderate fraction of apoptotic cells, 2.1−5.7%, respectively, was observed in the two cell lines. Conversely, apoptosis analysis showed that (*S*)-**26** was capable of inducing a strong LNCaP cell death (80.5%), and this cell-killing capability was even higher on LNCaP-AR (91.6%) (Figure 3B).

The assay of the human androgen receptor (hAR) transcriptional activity was conducted to evaluate (anti-) agonistic activities of our compounds. For androgen-stimulated transcriptional analysis, subconfluent (60−70%) LNCaP or Cos-7 cells were plated in phenol red-free DMEM containing 5% charcoal-stripped serum. After 48 h, the cells were transfected with 0.3 μg of 3416-pTK-TATA-Luc construct, as reported.[25] AR-negative Cos-7 cells were also cotransfected with 1.5 μg of pSG5-empty plasmid (pSG5) or pSG5-hAR (hAR) expressing plasmid. After 24 h, LNCaP and Cos-7 transfected cells were left unstimulated or stimulated for 24 h with a 10 nM concentration of the synthetic androgen, R1881, in the absence or presence of the indicated concentrations of synthetic compounds. AR was detected by Western blot analysis, and the luciferase activity in cell lysates was measured and expressed as fold induction.[25] Figure 4 shows that 10 nM R1881 increases by 4.2-fold the AR-mediated transcriptional activity in LNCaP cells. Expectedly, the antiandrogen Casodex (at 10 μM) inhibits such an activation. Notably, inhibition of the androgen-induced transcriptional activation is observed in cells challenged with (*S*)-**26**, and this effect is similar to that observed by using Casodex. Similar findings were observed in ARE-luc reporter assay established in Cos-7 cells ectopically expressing hAR (see the Supporting Information), indicating that (*S*)-**26** exerts its antagonistic effect independently of the cell type. Figure 4 also shows that in the range of concentration used (from 10 nM to 10 μM), (*S*)-**26** did not exhibit agonistic activity. Overall, these data confirm that the activity of (*S*)-**26** is

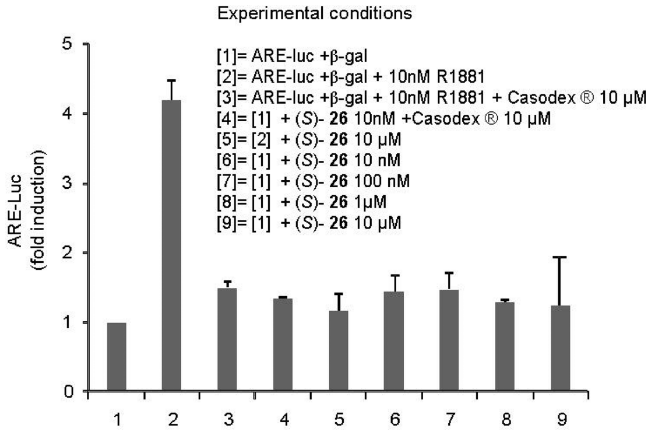
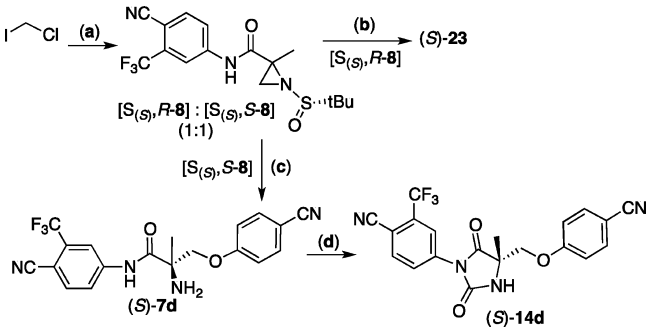


Figure 4. Effect of Casodex and (*S*)-**26** on the androgen activated transcription analyzed by ARE-luc reporter assay established in LNCaP cells (average of three independent experiments).

due, at least in part, to its antagonistic behavior toward mutated AR.

Besides alkylation reaction, we report herein the unprecedented, straightforward formation of aziridines **8**, which smoothly occurs in 90% yield (1/1 diastereomeric ratio) by adding $S_{(S)}$-**6** to a (chloromethyl)lithium solution in the presence of LiBr.[26,27] Aziridine ring opening with thiolates or phenolates takes place with good yield and complete regioselectivity (Scheme 3). In the ARE-luc assay established

Scheme 3. Aziridine Formation and Reaction[a]



[a]Reagents and conditions: (a) (i) LiBr/MeLi, −78 °C; (ii) $S_{(S)}$-**6**, THF. (b) (i)*p*-F-C_6H_4SH, DBU; (ii) HCl/MeOH, −5 °C to rt; (iii) MCPBA/CH_2Cl_2, rt. (c) (i) *p*-CN-C_6H_4OH, toluene, DBU; 80 °C, 36 h; (ii) HCl/MeOH, −5 °C to rt. (d) CDI/DPEA, 100 °C, 3 h.

in Cos-7 cells (Figure 5), compound (*S*)-**7d** slightly decreases the androgen-stimulated ARE-luc activity. These findings suggest that (*S*)-**7d** exerts both agonistic and antagonistic effects. This behavior might depend on AR levels. Thus, in cells expressing high levels of hAR, such as transfected Cos-7 cells (and most likely LNCaP-AR cells), (*S*)-**7d** used at 10 μM is unable to fully antagonize the androgen-induced transcriptional effect.

On the other hand, at that concentration, (*S*)-**7d** does not behave as a full agonist. Therefore, this compound represents a promising tool because of its pronounced flexibility, likely due to its molecular scaffold, which, besides, is susceptible of targeted synthetic modifications. The possibility that (*S*)-**7d** differently acts on AR-mediated actions (i.e., nongenomic vs genomic actions) or different types of AR (i.e., membrane or cytoplasm or nuclear AR) cannot be excluded at moment.

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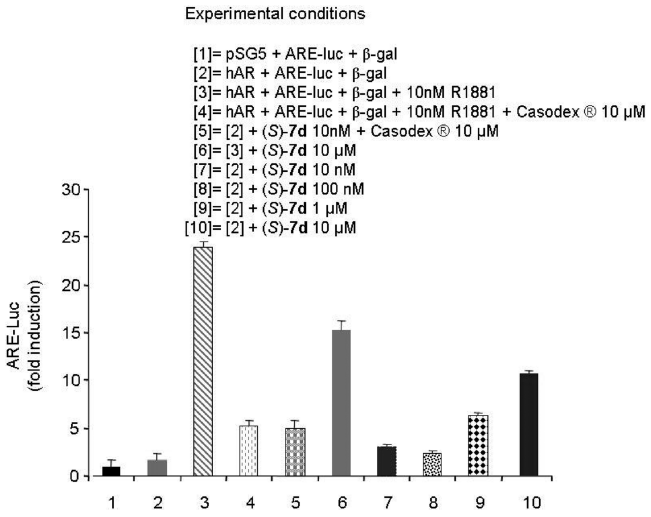

Figure 5. Effect of Casodex and (*S*)-**7d** on AR-mediated transcription analyzed by ARE-luc reporter assay in Cos-7 cells ectopically expressing hAR (average of three different experiments). Similar amounts of hAR were detected by Western blot analysis of cell lysates (not shown).

Further investigation in several cell types expressing a wide range of AR levels (i.e., nonreproductive vs reproductive cell types), differently localized in specific subcellular compartments (i.e., membrane or cytoplasm or nuclei) could improve our understanding of the (*S*)-**7d** mode of action.

Figures S3 and S10 in the Supporting Information show that (*S*)-**14d** (Scheme 3) neither significantly antagonizes the androgen-induced transcriptional effect nor behaves as an agonist when used in the ARE-luc gene reporter assay established in Cos-7 cells ectopically expressing hAR.

In conclusion, we reported herein a straightforward and versatile methodology for the synthesis of new AR ligands with (anti)-agonistic activities. From a chemical viewpoint, the great reactivity of the chiral ketimine **6** toward nucleophiles allows the formation of the corresponding aziridine under easily achievable reaction conditions. Although numerous methods are known for the stereoselective synthesis and ring opening of sulfynil aziridines, to the best of our knowledge, compound **8** represents the first example of highly functionalized, electron-poor amidic aziridine.[13,28] Moreover, the ring-opening reaction with phenolates is completely regioselective, affording the very interesting ostarine nitrogen isostere **7d**. Oxygen−nitrogen replacement in bicalutamide-like structures paves the way to the synthesis of a new class of analogues, including cyclized/nitrogen-substituted derivatives with promising antiandrogen (or anabolic) activity. Among the described compounds, derivative (*S*)-**26** showed the best biological activity as an antiandrogen, especially toward HRPC cell lines.

■ ASSOCIATED CONTENT

Supporting Information

General informations, general procedures, comparative analysis, selected NMR spectra, general biological assay procedures, and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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