Chiral Nonsteroidal Affinity Ligands for the Androgen Receptor. 1. Bicalutamide Analogues Bearing Electrophilic Groups in the B Aromatic Ring[1]

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Received January 19, 1999

A series of chiral analogues of bicalutamide bearing electrophilic groups (isothiocyanate, *N*-chloroacetyl, and *N*-bromoacetyl) on aromatic ring B of the parent molecule were synthesized. These compounds were designed as affinity ligands for the androgen receptor (AR). We prepared the *(R)*- and *(S)*-optical isomers of these compounds as pure enantiomers. The AR binding affinities of these compounds were measured in a competitive binding assay with the radiolabeled high-affinity AR ligand, [^{3}H]mibolerone. In accordance with our previous results for the enantiomers of bicalutamide, we found that all (*R*)-isomers demonstrated much higher binding affinity to the AR as compared to their corresponding (*S*)-isomers. The *para*-substituted affinity ligands in ring B bound the AR with higher affinities than the corresponding *meta*-substituted analogues. Oxidation of thioester affinity ligands to their sulfonyl analogues for the *para*-substituted compounds decreased AR binding affinities and similar modification increased binding affinities for corresponding *meta*-analogues. The least potent *para*-substituted sulfonyl compounds had higher AR binding affinities than the most potent *meta*-substituted sulfonyl compounds. Overall, the *para*-substituted unoxidized molecules demonstrated the highest AR binding affinity. Subsequent research using AR exchange assays and Scatchard analyses showed that the isothiocyanate affinity ligands (***R*)-7**, (***R*)-9**, and (***R*)-10** reported herein are the first specific chemoaffinity ligands for the AR.

Introduction

Affinity labeling is a powerful technique that can provide valuable information about the binding pocket of a protein, i.e., the substrate binding site of an enzyme or the ligand binding site of a receptor. This methodology is widely used for mapping the amino acids involved in ligand interaction. In addition to the basic application to protein characterization, affinity ligands can be utilized for practical purposes in cell biology, including irreversible tagging with a radioactive atom or fluorescent group for visualization of a particular protein (e.g., in flow cytometry or fluorescence microscopy).[2]

Steroid hormone receptors (SHR) are an important class of intracellular regulatory proteins that bind structurally diverse ligands and modulate estrogen, glucocorticoid, and androgen action. Chemoaffinity and photoaffinity ligands have played a critical role in structural characterization of many of these receptors. There are two major groups of affinity ligands for SHR: steroidal and nonsteroidal.

A number of steroidal analogues, including promegesterone and triamcinolone acetonide (TA), have been used to photoaffinity label the glucocorticoid receptor (GR). Promegesterone and TA labeled identical amino acids in the ligand binding domain of the GR.[3] Dexamethasone mesylate (Dex-mes) has been widely used as an electrophilic affinity labeling reagent and is known to bind with cysteine residues in the GR.[4,5] Chakraborti et al.[5] recently showed that tryptic digestion of the GR resulted in a 16-kDa fragment that retained its steroid binding specificity, justifying its designation as a steroid-binding core domain of the GR. [^{3}H]Dex-mes covalently labeled a single residue (Cys-656) of the rat GR. Using point mutations and Dex-mes affinity labeling, the authors were able to generate one of the first known models of the steroid binding "pocket" of the GR.

Katzenellenbogen and co-workers[6] developed steroidal compounds bearing electrophilic and photoreactive groups with the potential to affinity label the estrogen receptor (ER). However, electrophilic nonsteroidal estrogens proved superior in terms of efficiency and selectivity of covalent attachment to the ER.[7] Tamoxifen aziridine (TAZ), which acts as an antagonist for the ER, and ketononestrol aziridine (KNA), which acts as an estrogen agonist, were used to identify Cys-530 as the site of covalent attachment in the ligand binding domain of the ER.[8] These studies provided the necessary background for more detailed examination of the effect of specific amino acids on transcriptional activation and the discrimination of estrogens and antiestrogens by the ligand binding domain. Subsequent structural analysis of proteolytic cleavage products from the affinity-labeled wild-type and mutated ER allowed this group to propose a model for interaction of both agonists and antagonists with the ligand binding domain of the ER.[9] These studies demonstrate the unique advantages that affinity labels provide for steroid receptor characterization (i.e., stringent treatment of the protein without the risk of ligand dissociation during purification) and indicate that nonsteroidal affinity labels can serve as efficient, highly selective tools for steroid receptor characterization.

Affinity labeling studies of the androgen receptor (AR) have been limited. Chang et al.[10,11] used dihydrotestosterone 17β-bromoacetate (DHT-BA; Figure 1) for

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10.1021/jm990027x CCC: $19.00
Published on Web 02/01/2000

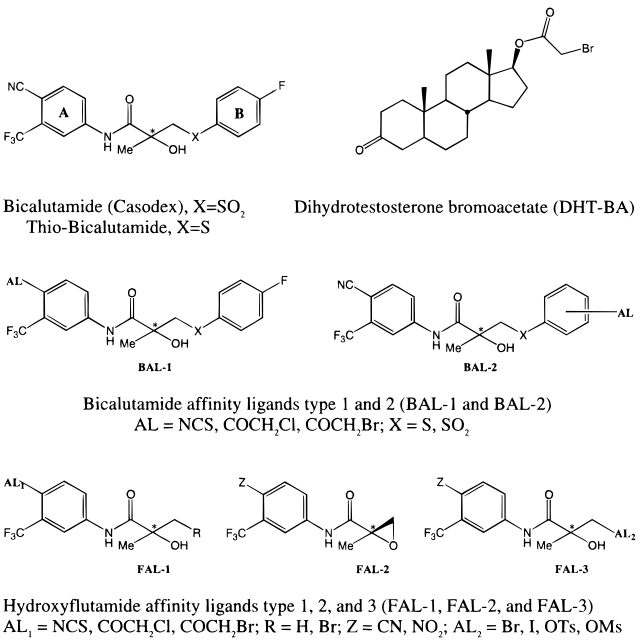


Figure 1. Structures of potential nonsteroidal affinity ligands for the AR.

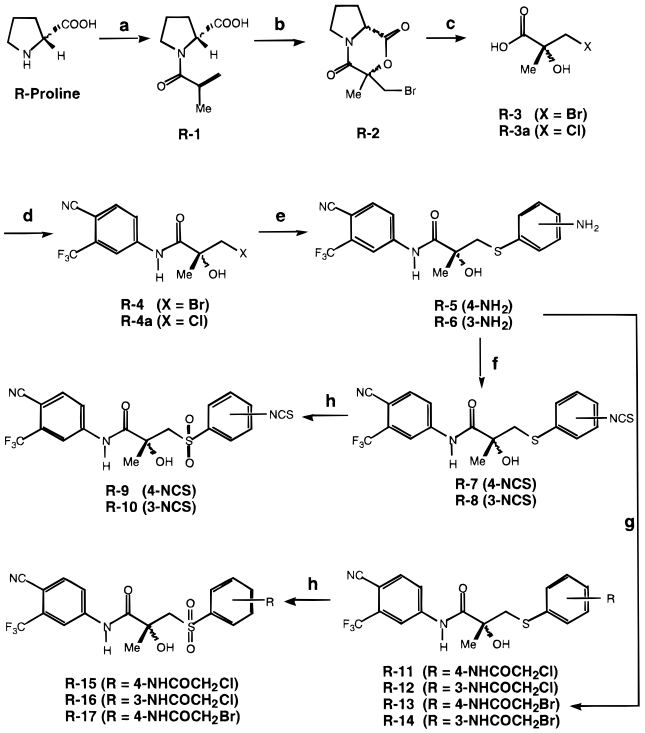
electrophilic labeling and methyltrienolone (R1881) for photoaffinity labeling of the AR. However, both of these steroidal compounds interact with a number of nonreceptor species, limiting their usefulness for AR characterization.[12,13] Initial studies suggested that DHT-BA covalently labeled a 56-kDa proteolytic fragment of the human AR.[14] McCammon et al.[15] recently reported that DHT-BA and R1881 in fact covalently bind to cytosolic aldehyde dehydrogenase, an enzyme expressed in greater abundance than the AR in genital skin fibroblasts, rather than the earlier reported binding to the AR. The lack of selective and efficient AR affinity labels has impeded more detailed analysis of the amino acids involved in ligand discrimination and contributed to the fact that the human AR remains one of the least characterized steroid receptors.

This manuscript describes our initial efforts toward development of novel nonsteroidal chemoaffinity ligands for the AR.[16] We developed several series of potential chemoaffinity and photoaffinity ligands based on structural modification of known clinically used AR antagonists. We report herein synthesis and AR binding affinities of bicalutamide analogues bearing electrophilic functional groups in aromatic ring B, hereafter referred to as BAL-2.[1] We also prepared bicalutamide affinity ligands with reactive groups in aromatic ring A, hereafter referred to as BAL-1,[17] and hydroxyflutamide affinity ligands, hereafter referred to as FAL-1, FAL-2,[18] and FAL-3,[19] as outlined in Figure 1. Detailed studies demonstrated the irreversible nature of some of these BAL-2 compounds.[20]

Chemistry

The (*R*)-isomers of compounds **5**−**17** were prepared according to the general synthetic scheme reported for the enantiomers of bicalutamide.[21−23] This synthetic route was utilized by Tucker[21,22] to prepare the (*S*)-isomer of bicalutamide. We successfully extended this approach to prepare the (*R*)-isomer of thiobicalutamide and bicalutamide.[23]

Scheme 1. Synthesis of Chiral AR Affinity Ligands **7**−**17** (with *(R)*-isomers as an example)[a]



[a] (a) Methacryloyl chloride, NaOH(aq), 5−10 °C, in aqueous acetone; (b) NBS, in anhydrous DMF; (c) HCl(concd), reflux; (d) **(*R*)-3**/**(*R*)-3a** with thionyl chloride, in anhydrous DMA, −5 to −10 °C, then 4-amino-2-trifluoromethylbenzonitrile, in anhydrous DMA; (e) **(*R*)-4**/**(*R*)-4a** with NaH, then 4- or 3-aminothiophenol, in anhydrous THF; (f) $CSCl_2$, CH_2Cl_2 + $NaHCO_3$; (g) $ClCH_2COCl$ or $BrCH_2COBr$ in CH_2Cl_2, $CaCO_3$, or iPr_2NEt; (h) *m*-chloroperbenzoic acid or peracetic acid, in CH_2Cl_2 or acetone.

The reaction sequence began with coupling of a commercially available chiral auxiliary, (*S*)- or (*R*)-proline, with methacryloyl chloride in aqueous acetone at 5−10 °C under Schottenn−Baumann conditions according to the procedure proposed by Jew et al.[24] (*R*)- or (*S*)-prolineamide, (***R*)-1** or (***S*)-1** (Scheme 1), formed in the first step, was converted to its corresponding (*R*)- or (*S*)-bromolactone (***R*)-2** or (***S*)-2** using Terashima's method of asymmetric bromolactonization.[24,25] This step was critical for the reaction sequence due to creation of the asymmetric center which ultimately determined the chirality of the final affinity ligands. Unlike the previous procedure,[21] we used 2 equiv of *N*-bromosuccinimide (NBS) instead of 1 equiv to perform bromolactonization. This provided a higher yield of the product (64−80% in our preparations vs 49% obtained by Tucker).[21]

Hydrolysis of the bromolactone (***R*)-2** or (***S*)-2** in refluxing concentrated HCl for 8 h gave rise to (*R*)- or (*S*)-bromoacid (***R*)-3** or (***S*)-3**, critical intermediates for all further synthetic manipulations in our study. Although we followed the literature procedure and obtained a white crystalline compound (***R*)-3** with a melting point 112−113.5 °C (lit.[21] mp 109−113 °C), we observed by NMR that the product contained about 15% of another compound (see Experimental Section). This side product appeared to be the corresponding chloroacid **3a**, formed by displacement of bromine with chlorine. Indeed, short (1 h) incomplete hydrolysis of bromolactone (***S*)-2** showed exclusive formation of the product assigned as bromoacid (***S*)-3**. Increasing the

reaction time caused gradual accumulation of the second product (***S*)-3a** which reached a 50:50 ratio after 30 h. After 93 h of hydrolysis, the second product prevailed over the first one with a ratio of about 85:15.

We did not isolate and characterize the product assigned as chloroacid **3a**. Instead, we converted the mixture of the acids in situ to their acid chlorides and coupled them with commercially available 4-amino-2-trifluoromethylbenzonitrile in DMA at −10 to −15 °C which gave rise to (*R*)- or (*S*)-bromoanilide (***R*)-4** or (***S*)-4** along with another anilide (likely (***R*)-4a** or (***S*)-4a**). The ratio of bromo- and chloroanilides **4** and **4a** was the same as bromo- and chloroacids **3** and **3a** (approximately 85:15). It is interesting to note that bromoanilides **4** in our preparation had a much higher melting point than that reported by Tucker (134.5−135.5 °C vs 106−107 °C[21]). We did not separate these two compounds since they were converted to common anilides **5** or **6** when coupled with aminothiophenols.

The mixture of anilides **4** and **4a** was coupled with commercially available 4-amino- or 3-aminothiophenols. The reaction was performed in THF at room temperature using 1.1−1.2 equiv of sodium hydride. The final products of the reaction, aniline derivatives **(*R*)-5** and **(*S*)-5** or **(*R*)-6** and **(*S*)-6**, were used as the precursors for preparation of our final affinity ligands. Conversion of these aniline precursors **5** or **6** to the isothiocyanate affinity ligands **7** and **8** was achieved by reaction with thiophosgene in a heterogeneous aqueous−organic mixture according to the procedure of Leclerc.[26] The yields of these reactions were between 80% and 95%. Chloroacetyl and bromoacetyl compounds **11**−**14** were prepared by acylation of the corresponding aniline precursors **5** and **6** by commercially available chloroacetyl chloride or bromoacetyl bromide in dry ethyl acetate or methylene chloride solution under argon with solid $CaCO_3$ powder or *N*,*N*-diisopropylethylamine as proton scavengers. The reaction was completed within 15−30 min in the case of bromoacetyl bromide and required greater than 3 h in the case of chloroacetyl chloride. All thioester affinity ligands **7**, **8**, and **11**−**14** were oxidized to their corresponding sulfonyl derivatives **9**, **10**, and **15**−**17** by commercially available 3-chloroperbenzoic acid or peracetic acid in ethyl acetate or methylene chloride solutions.

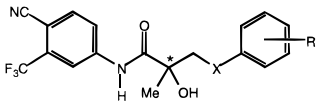
We noted some stability problems with the isothiocyanate affinity ligands when running ^{13}C NMR spectra of these compounds in DMSO-d_6 for 5−20 h at room temperature, as evidenced by the appearance of additional peaks in proton and carbon NMR spectra. The *p*-sulfonyl compound **9** was the least stable in DMSO-d_6, while the *p*-thiol analogue **7** was the most stable. The *m*-sulfonyl molecule **10** and the *m*-thiol molecule **8** demonstrated intermediate stability. First-order rate constants (k_{obs}) of solvolysis of isothiocyanate affinity labels were roughly determined by integration of peaks for the starting compounds and their products in proton NMR spectra. These rate constants demonstrated good correlation with the Hammett σ-constants calculated using the σ-values for methylthiol (MeS) and methylsulfonyl ($MeSO_2$) groups in *para*- and *meta*-positions of the phenyl ring.[27] Indeed, k_{obs} decreased in the order of 0.025, 0.022, 0.008, and 0.002 h^{-1} for **9** ($\sigma = 0.68$), **10** ($\sigma = 0.60$), **8** ($\sigma = 0.15$), and **7** ($\sigma = 0$), respectively. To obtain good quality ^{13}C spectra for isothiocyanate affinity ligands, we ran the spectra in DMSO-d_6 using high concentration of a compound and a relatively short scanning time (1−1.5 h). The NMR spectra for all our compounds were obtained in DMSO-d_6, allowing direct comparison of spectroscopic data.

Results and Discussion of AR Binding Affinity

AR binding affinities of the synthesized ligands were determined by competitive binding[28,29] in the presence of the high-affinity AR ligand, [^{3}H]mibolerone (MIB). AR binding studies were performed by incubating increasing concentrations (10^{-3}−10000 nM) of each ligand with cytosol and a saturating concentration of [^{3}H]MIB (1 nM) at 4 °C for 18 h. In preliminary experiments, the equilibrium dissociation constant (K_d) of MIB was determined under identical conditions by incubating increasing concentrations of [^{3}H]MIB (0.01−10 nM) with cytosol. We found that the minimum concentration of [^{3}H]MIB required to saturate AR sites in the cytosol preparation was 1 nM. Subsequent experiments used either 1 or 2 nM [^{3}H]MIB. The incubates also contained 1000 nM triamcinolone acetonide to block interaction of MIB with progesterone receptors.[30] For the determination of nonspecific binding, separate experiments were conducted by adding 1000 nM MIB to the incubate. Separation of bound and free radioactivity at the end of incubation was achieved by the hydroxyapatite (HAP) method, as described previously,[23] and 0.8 mL of the ethanolic supernatant was added to 5 mL of scintillation cocktail. Radioactivity was counted in a Beckman LS 6800 liquid scintillation counter (Beckman Instruments, Irvine, CA). Reversed-phase HPLC was used to determine that our ligands were stable under the experimental conditions used for AR competitive binding studies. It is important to note that binding affinity constants (K_i) represent "apparent" values because they characterized two steps which might be involved in the interaction of an affinity ligand with the AR. The first step was reversible binding of the affinity ligands to the AR, and the second step was irreversible interaction with formation of a covalent bond between the ligand and the receptor. Thus, depending on the extent of the AR labeling during the competitive binding assay, apparent K_i-values may reflect a combination of these two processes.

Synthesized ligands showed a wide range of binding affinities. In accordance with our previous results for enantiomers of bicalutamide,[23] we found that the (*R*)-isomers of our molecules demonstrated much higher binding affinity to the AR compared to the corresponding (*S*)-isomers. Thiol analogues of isothiocyanate affinity ligands **(*R*)-7** and **(*S*)-7** had the highest eudismic ratio (ratio of K_i-values for the (*R*)- and (*S*)-isomers) in our AR binding experiments. This ratio was close to 3 orders of magnitude for **(*R*)-7** and **(*S*)-7** (Table 1). The eudismic ratio was about 2 orders of magnitude less for thiochloroacetyl analogues **(*R*)-11** and **(*S*)-11**. This value decreased to about 1 order of magnitude for *meta*- and *para*-substituted anilines **(*R*)-6** and **(*S*)-6** and **(*R*)-5** and **(*S*)-5**. In fact, we found that the eudismic ratio was significantly correlated with the AR binding affinity of (*R*)-isomers within our series of compounds ($r^2 = 0.9809$, $P < 0.0001$). Compounds with lower K_i-values demon-

Table 1. Rat AR Binding Affinities (K_i) of (*R*)- and (*S*)-Optical Isomers of Synthesized Analogues **5**−**17** and Enantiomers of Bicalutamide (Casodex) **1**



R	X	compd	K_i (nM)	compd	K_i (nM)
4-F	SO_2	(*R*)-bicalutamide[23]	11.0 ± 1.5	(*S*)-bicalutamide[23]	365 ± 10
4-NH_2	S	***(R)*-5**	90 ± 15	***(S)*-5**	800
3-NH_2	S	***(R)*-6**	65 ± 25	***(S)*-6**	>1000
4-NCS	S	***(R)*-7**	0.6 ± 0.1	***(S)*-7**	430 ± 30
3-NCS	S	***(R)*-8**	230 ± 50	***(S)*-8**	130 ± 15
4-NCS	SO_2	***(R)*-9**	41 ± 2		
3-NCS	SO_2	***(R)*-10**	140 ± 10		
4-$NHCOCH_2Cl$	S	***(R)*-11**	1.65 ± 0.10	***(S)*-11**	200 ± 30
3-$NHCOCH_2Cl$	S	***(R)*-12**	>1000		
4-$NHCOCH_2Br$	S	***(R)*-13**	100 ± 10		
3-$NHCOCH_2Br$	S	***(R)*-14**	>1000		
4-$NHCOCH_2Cl$	SO_2	***(R)*-15**	10.7 ± 0.6		
3-$NHCOCH_2Cl$	SO_2	***(R)*-16**	130 ± 20		
4-$NHCOCH_2Br$	SO_2	***(R)*-17**	360 ± 30		

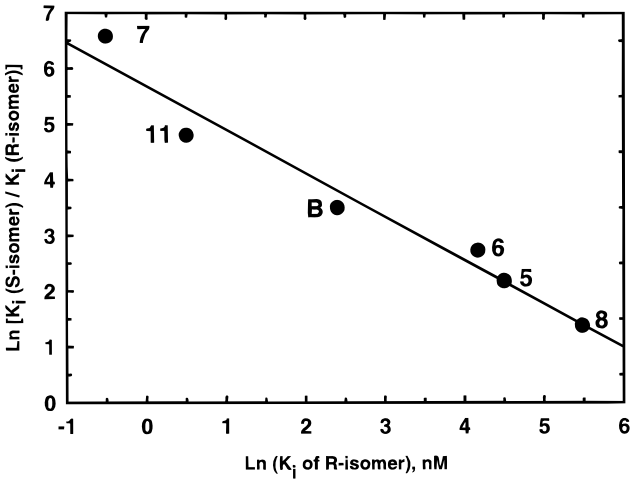


Figure 2. Correlation between logarithm of the eudismic ratios of the AR binding (K_i for (*S*)-isomers/K_i for (*R*)-isomers) for compounds **5**−**7**, **11**, and bicalutamide (B) with logarithm of binding affinities (K_i) of (*R*)-isomers.

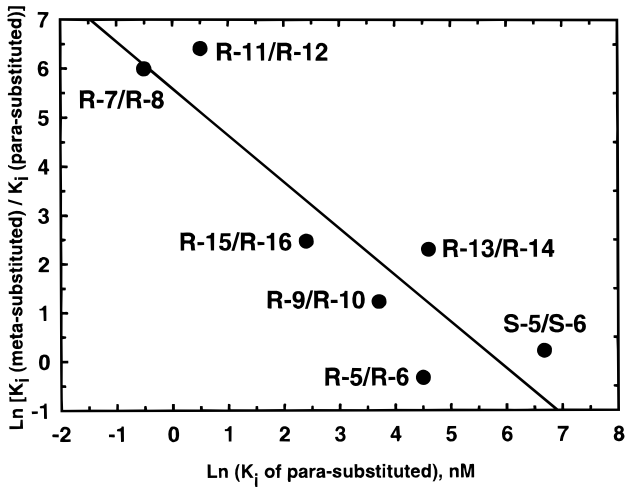


Figure 3. Correlation between logarithm of the *para* to *meta* AR binding regioselectivity (ratio of K_i-value for *meta*-substituted analogue to K_i-value for *para*-substituted analogue) with logarithm of binding affinity of the *para*-substituted counterpart.

strated higher eudismic ratios. This general trend for pairs of optical isomers to increase eudismic ratio (enantiomeric ratio) for more potent compounds is called Pfeiffer's rule[31] and could be quantitatively described as the correlation between enantiomeric specificity of the AR binding of compounds **5**−**7**, **11**, and bicalutamide (B) with the binding affinities of (*R*)-isomers (Figure 2).

In most cases, the *meta*-substituted affinity ligands bound the AR with affinities lower than that observed for the *para*-substituted analogues. This was true for pairs of compounds ***(R)*-8** and ***(R)*-7**, ***(R)*-9** and ***(R)*-10**, ***(R)*-11** and ***(R)*-12**, ***(R)*-13** and ***(R)*-14**, and ***(R)*-15** and ***(R)*-16**. The advantage in the AR binding for the *para*-substitution versus the *meta*-substitution (regioselectivity of the AR binding) increased with increasing binding potency of the *para*-substituted counterpart. For example, *para*-substituted molecules ***(R)*-7** and ***(R)*-11** bound the AR with high affinities (K_i-values were about 1 nM). Moving the isothiocyanate or chloroacetyl group to the *meta*-position resulted in a significant decrease (about 400 and 600, respectively). However, moving the bromoacetyl or chloroacetyl group from the *para*- to *meta*-position in compounds ***(R)*-13**/***(R)*-14** and ***(R)*-15**/***(R)*-16** resulted in only a 10-fold decrease in the AR binding. The *meta*/*para*-regioselectivity decreased further for compounds with less potent AR binding (e.g., ***(R)*-9**/***(R)*-10** and ***(R)*-5**/***(R)*-6**). The correlation between *para*/*meta*-regioselectivities and binding affinities of *para*-substituted analogues is shown in Figure 3 (r^2 = 0.8113, $P < 0.01$).

Oxidation of thioester affinity ligands to their sulfonyl analogues decreased binding affinities for the *para*-substituted compounds and increased it for the *meta*-substituted analogues. However, the most potent *meta*-substituted sulfonyl compounds had lower AR binding affinities than the least potent *para*-substituted sulfonyl compounds. Thus, the *para*-substituted unoxidized molecules provided the best binding AR affinity ligands in this series of synthesized compounds.

Conclusions

We found that nonsteroidal AR ligands bearing electrophilic functional groups in aromatic ring B maintained high-affinity binding to the AR. Moreover, these studies demonstrate for the first time that nonsteroidal AR ligands demonstrate stereoselective and regioselec-

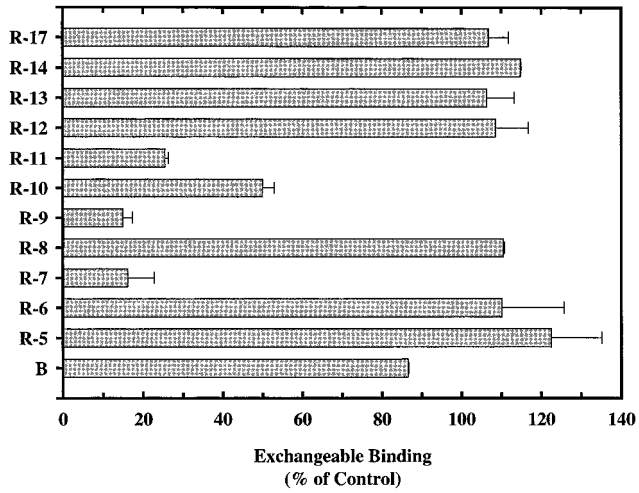


Figure 4. Exchangeable specific binding of [^{3}H]MIB after incubation with the indicated ligand. Values are expressed as mean (± SD) percent of specific [^{3}H]MIB binding to cytosol preincubated without ligand. Exchangeable specific binding of [^{3}H]MIB in the absence of any competitor was 2270 ± 267 dpm. Experiments were performed in triplicate for each ligand. Adapted from ref 20.

tive binding to the AR. (*R*)-Isomers demonstrated much higher binding affinity to the AR as compared to their corresponding (*S*)-isomers. The *para*-substituted affinity ligands in ring B bound the AR with higher affinities than the corresponding *meta*-substituted analogues. Oxidation of thioester affinity ligands to their sulfonyl analogues for the *para*-substituted compounds decreased AR binding affinities and similar modification increased binding affinities for corresponding *meta*-analogues. The least potent *para*-substituted sulfonyl compounds had higher AR binding affinities than the most potent *meta*-substituted sulfonyl compounds. Overall, the *para*-substituted unoxidized molecules demonstrated the highest AR binding affinity. In the synthesized series of bicalutamide affinity ligands BAL-2, compounds combining two features (an electrophilic group located at the *para*-position of the aromatic ring B and an unoxidized sulfur bridge) provided the best binding AR affinity.

Further, we recently reported the results of detailed studies to examine the potential of these ligands to covalently label the AR.[20] In exchange assays, the isothiocyanate affinity ligands **(*R*)-7**, **(*R*)-9**, and **(*R*)-10** reduced exchangeable specific binding to an average of 16%, 15%, and 50%, respectively (Figure 4). (*R*)-Bicalutamide (B) and the aniline derivatives **(*R*)-5** and **(*R*)-6** were used as reversible controls and did not demonstrate significant decreases in exchangeable binding. The thiochloroacetyl affinity ligand **(*R*)-11** also decreased exchangeable binding to 26% of control. However, Scatchard analyses with this ligand showed that **(*R*)-11** did not irreversibly bind the AR (data not shown).[20] The results of AR exchange assay and Scatchard analysis indicated that isothiocyanate affinity ligands **(*R*)-7**, **(*R*)-9**, and **(*R*)-10** are the first specific chemoaffinity ligands for the AR.[20]

As a whole, these studies demonstrate that chiral nonsteroidal ligands demonstrate potent, stereoselective, regioselective, and irreversible binding to the AR. These compounds will provide valuable tools for the molecular characterization of the ligand binding domain of the AR.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer system 2000 FT-IR. Proton and carbon-13 magnetic resonance spectra were obtained on a Bruker AX 300 spectrometer (300 and 75 MHz for ^{1}H and ^{13}C, respectively). Chemical shift values are reported as parts per million (δ) relative to DMSO-d_6 peak (2.49 and 39.5 for ^{1}H and ^{13}C, respectively). Spectral data were consistent with assigned structures. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and found values were within 0.4% of the theoretical values. Specific rotations were recorded on Autopol III automatic polarimeter (Rudolph Research, Fairfield, NJ) in 1-dm sample tube with use of sodium D-line at ambient temperature. Routine thin-layer chromatography (TLC) was performed on silica gel aluminum plates (Whatman Ltd., Maidstone, Kent, England). Flash chromatography was performed on silica gel (Merck; grade 60, 230−400 mesh, 60 Å).

(2*R*)-1-Methacryloylpyrrolidine-2-carboxylic Acid (-*(R)*-1). Prolineamide **(*R*)-1** was prepared according to the general procedure described by Jew et al.[24] D-Proline (10.33 g, 89.7 mmol) was dissolved in 2 N NaOH (53 mL) and cooled in an ice bath, and the resulting alkaline solution was diluted with acetone (53 mL). An acetone solution (53 mL) of metacryloyl chloride (14.08 g, 134.7 mmol) and the 2 N NaOH solution (70 mL) were simultaneously added over 40 min to the aqueous solution of L-proline with stirring in an ice bath. The pH of the mixture was kept at 10−11 (measured by pH meter with glass electrode) during the addition of the metacryloyl chloride. After the stirring was continued for 3 h at room temperature, the mixture was evaporated in vacuo on a rotary evaporator at a slightly elevated temperature (35−45 °C) to remove acetone. The residual solution was washed with ether and acidified to pH 2 with concentrated HCl. The acidic mixture was saturated with NaCl and extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were dried over $MgSO_4$, reduced in volume by evaporation in vacuo, mixed with hexanes (with about 1:1 ratio), and further concentrated until the beginning of crystallization to give 12.50 g (76%) of the title compound as white crystals: mp 102.5−103.5 °C (lit.[32] mp 103−104.5 °C for the (*S*)-prolineamide). NMR spectrum of this compound demonstrated existence of two rotamers of the title compound: ^{1}H NMR (DMSO-d_6) δ 5.28 (s) and 5.15 (s) (first rotamer), 5.15 (s) and 5.03 (s) (second rotamer) (totally 2H for both rotamers, vinyl CH_2), 4.24−4.20 (m) (first rotamer), 4.48−4.44 (m) (second rotamer) (totally 1H for both rotamers, CH at the chiral center), 3.57−3.38 (m, 2H, aliphatic CH_2), 2.27−2.12 (m, 1H, aliphatic CH), 1.97−1.72 (m, 6H, aliphatic CH_2, CH and Me); ^{13}C NMR (DMSO-d_6) δ for the first (major) rotamer 173.26, 169.12, 140.87, 116.38, 116.38, 58.26, 48.73, 28.89, 24.70, 19.51; for the second (minor) rotamer 174.04, 170.03, 141.61, 115.24, 60.26, 45.94, 30.95, 22.34, 19.66. Mass spectra data were obtained on Bruker Daltonics Esquire LC multipole ion trap mass spectrometer with ESI: $[\alpha]_D^{25}$ +80.8° (c = 1, MeOH). Anal. ($C_9H_{13}NO_3$) C, H, N.

(2*S*)-1-Methacryloylpyrrolidine-2-carboxylic Acid (*(S)*-1). Prolineamide **(*S*)-1** was prepared using L-proline ($[\alpha]^{20}_D$ −84°, c = 4, H_2O) as a chiral auxiliary according to the procedure described for preparation of **(*R*)-1**. The yield was 70%: mp 102−103 °C (lit.[32] mp 103−104.5 °C); $[\alpha]_D^{25}$ −81.2° (c = 1, MeOH) (lit.[32] $[\alpha]_D$ −79.2° (c = 1, MeOH)).

***N*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-bromo-2-hydroxy-2-methylpropanamide (*(R)*-2)**. Bromolactone **(*R*)-2** was prepared from prolineamide **(*R*)-1** according to the slightly modified procedure described by Tucker.[21] We used 2 equiv of NBS instead of 1 equiv as in the original procedure and obtained the title compound with a better yield (80% vs 49%): mp 152−154 °C (lit.[21] mp 157−159 °C for the (*S*)-bromolactone); ^{1}H NMR (DMSO-d_6) δ 4.69 (dd, J = 9.6 Hz, J = 6.7 Hz, 1H, CH at the chiral center), 4.02 (d, J = 11.4 Hz,

1H, CH(1)), 3.86 (d, J = 11.4 Hz, 1H, CH(2)), 2.30−2.20 (m, 1H, aliphatic CH), 2.04−1.72 (m, 3H, aliphatic CH_2 and CH), 1.56 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 167.26, 163.08, 83.89, 57.23, 45.42, 37.82, 29.03, 22.93, 21.58; $[\alpha]_D^{26}$ +124.5° (c = 1.3, chloroform) (for **(*S*)-2** lit.[21] $[\alpha]_D$ −126.5° (c = 1.19, chloroform)). Anal. ($C_9H_{12}BrNO_3$) C, H, N.

***N*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-bromo-2-hydroxy-2-methylpropanamide ((*S*)-2)**. Bromolactone **(*S*)-2** was prepared from prolineamide **(S)-1** by the same method used for preparation of **(*R*)-2**. The title compound was obtained with 64% yield: mp 157−159 °C (lit.[21] mp 157−159 °C); $[\alpha]_D^{25}$ −123.6° (c = 1.3, chloroform) (lit.[21] $[\alpha]_D$ −126.5° (c = 1.19, chloroform)).

(2*R*)-3-Bromo-2-hydroxy-2-methylpropanoic Acid ((*R*)-3). Bromoacid **(*R*)-3** was prepared by acid-catalyzed hydrolysis of bromolactone **(*R*)-2** in refluxing concentrated HCl according to the procedure described by Tucker et al.[21] The title compound was obtained as the mixture of **(*R*)-3** and another compound (corresponding chloroacid **(2*R*)-3-chloro-2-hydroxy-2-methylpropanoic acid, (*R*)-3a**) with the ratio of 85:15 and 79% yield: mp 106.5−109 °C (lit.[21] mp 109−113 °C for the (*S*)-bromoacid **(*S*)-3**); $[\alpha]_D^{25}$ +10.5° (c = 2.6, MeOH) (lit.[21] $[\alpha]_D$ −11.78° (c = 1.16, MeOH) for **(*S*)-3**); for **(*R*)-3** ^{1}H NMR (DMSO-d_6) δ 3.63 (d, J = 10.1 Hz, 1H, CH(1)), 3.52 (d, J = 10.1 Hz, 1H, CH(2)), 1.35 (s, 3H, Me).

(2*R*)-3-Chloro-2-hydroxy-2-methylpropanoic Acid ((*R*)-3a). Chloroacid **(*R*)-3a** was not isolated from the mixture with corresponding bromoacid **(*R*)-3** (see Chemistry section): ^{1}H NMR (DMSO-d_6) δ 3.73 (d, J = 10.9 Hz, 1H, CH(1)), 3.61 (d, J = 10.9 Hz, 1H, CH(2)), 1.30 (s, 3H, Me).

(2*S*)-3-Bromo-2-hydroxy-2-methylpropanoic Acid ((*S*)-3). Bromoacid **(*S*)-3** was prepared by acid-catalyzed hydrolysis of bromolactone **(*S*)-2** in refluxing concentrated HCl according to the procedure described by Tucker.[21] The title compound was obtained as the mixture of **(*S*)-3** and another compound (the most likely, corresponding chloroacid **(2*S*)-3-bromo-2-hydroxy-2-methylpropanoic acid ((*S*)-3a)**) with the ratio of 85:15 and 64% yield: mp 112−113.5 °C (lit.[21] mp 109−113 °C for the (*S*)-bromoacid **(*S*)-3**); $[\alpha]_D^{27}$ −11.3° (c = 2.6, MeOH) (lit.[21] $[\alpha]_D$ −11.78° (c = 1.16, MeOH)); for **(*S*)-3** ^{1}H NMR (DMSO-d_6) δ 3.63 (d, J = 10.1 Hz, 1H, CH(1)), 3.52 (d, J = 10.1 Hz, 1H, CH(2)), 1.35 (s, 3H, Me).

(2*S*)-3-Chloro-2-hydroxy-2-methylpropanoic Acid ((*S*)-3a). Chloroacid **(*S*)-3a** was not isolated form the mixture with corresponding bromoacid **(*S*)-3** (see Chemistry section): ^{1}H NMR (DMSO-d_6) δ 3.73 (d, J = 10.9 Hz, 1H, CH(1)), 3.61 (d, J = 10.9 Hz, 1H, CH(2)), 1.31 (s, 3H, Me).

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-bromo-2-hydroxy-2-methylpropanamide ((*R*)-4).** Bromoanilide **(*R*)-4** was prepared from the mixture of the acids **(*R*)-3** and **(*R*)-3a** according to the procedure described by Tucker.[21] The title compound was obtained as the mixture of **(*R*)-4** and another compound (the most likely, corresponding chloroanilide ***N1*-[4-cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-chloro-2-hydroxy-2-methyl propanamide, (*R*)-4a)** with the ratio of 85:15 and 59% yield: mp 132−134 °C (lit.[21] mp 106−107 °C for the (*S*)-isomer); $[\alpha]_D^{27}$ −48.5° (c = 1.0, MeOH) (lit.[21] $[\alpha]_D$ +50.73° (c = 1.1, MeOH) for **(*S*)-4**); for **(*R*)-4** ^{1}H NMR (DMSO-d_6) δ 10.51 (s, 1H, NH), 8.53 (d, J = 1.8 Hz, 1H, ArH), 8.28 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H, ArH), 8.09 (d, J = 8.7 Hz, 1H, ArH), 6.39 (s, 1H, OH), 3.81 (d, J = 10.4 Hz, 1H, CH(1)), 3.57 (d, J = 10.4 Hz, 1H, CH(2)), 1.47 (s, 3H, Me).

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-chloro-2-hydroxy-2-methylpropanamide ((*R*)-4a).** Chloroanilide **(*R*)-4a** was not isolated from the mixture with corresponding bromoanilide **(*R*)-4** (see Chemistry section): ^{1}H NMR (DMSO-d_6) δ 10.52 (s, 1H, NH), 8.53 (d, J = 1.8 Hz, 1H, ArH), 8.28 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H, ArH), 8.09 (d, J = 8.7 Hz, 1H, ArH), 6.37 (s, 1H, OH), 3.90 (d, J = 11.1 Hz, 1H, CH(1)), 3.67 (d, J = 11.2 Hz, 1H, CH(2)), 1.42 (s, 3H, Me).

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-bromo-2-hydroxy-2-methylpropanamide ((*S*)-4).** Bromoanilide **(*S*)-4** was prepared from the mixture of the acids **(*S*)-3** and **(*S*)-3a** according to the procedure described by Tucker.[21] The title compound was obtained as the mixture of **(*S*)-4** and another compound (the most likely, corresponding chloroanilide ***N1*-[4-cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-chloro-2-hydroxy-2-methyl propanamide ((*S*)-4a)**) with the ratio of 85:15 and 80% yield: mp 134.5−135.5 °C (lit.[21] mp 106−107 °C for the (*S*)-isomer); $[\alpha]_D^{27}$ +47.0° (c = 1.0, MeOH) (lit.[21] $[\alpha]_D$ +50.73° (c = 1.1, MeOH)); for **(*S*)-4** ^{1}H NMR (DMSO-d_6) δ 10.51 (s, 1H, NH), 8.53 (d, J = 1.5 Hz, 1H, ArH), 8.29 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, ArH), 8.10 (d, J = 8.6 Hz, 1H, ArH), 6.39 (s, 1H, OH), 3.81 (d, J = 10.4 Hz, 1H, CH(1)), 3.57 (d, J = 10.4 Hz, 1H, CH(2)), 1.47 (s, 3H, Me).

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-chloro-2-hydroxy-2-methyl propanamide ((*R*)-4).** Chloroanilide **(*S*)-4a** was not isolated from the mixture with corresponding bromoanilide **(*S*)-4** (see Chemistry section): ^{1}H NMR (DMSO-d_6) δ 10.53 (s, 1H, NH), 8.53 (d, J = 1.5 Hz, 1H, ArH), 8.29 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, ArH), 8.10 (d, J = 8.6 Hz, 1H, ArH), 6.38 (s, 1H, OH), 3.90 (d, J = 11.1 Hz, 1H, CH(1)), 3.67 (d, J = 11.2 Hz, 1H, CH(2)), 1.42 (s, 3H, Me).

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-[(4-aminophenyl)sulfanyl]-2-hydroxy-2-methylpropanamide ((*R*)-5).** A solution of freshly distilled 4-aminothiophenol (short-path distillation on Kugelrohr at approximately 150 °C) (1.38 g, 11.0 mmol) in anhydrous THF (10 mL) was added under argon atmosphere to preliminary washed 60% oil dispersion of NaH (0.45 g, 11.2 mmol) in 10 mL of anhydrous THF. The reaction mixture was stirred over 36 h until forming a white suspension. A solution of anilides **(*R*)-4** and **(*R*)-4a** (85:15 ratio) (3.00 g, 8.7 mmol) in 15 mL of anhydrous THF was injected dropwise. The reaction mixture was stirred for 26 h. The final suspension was filtered out, and the filtrate was completely evaporated to give an oil. The oil was solidified after addition of small amount of methanol. This solid was recrystallized from methanol to give 2.53 g (73%) of the title compound as white crystals: mp 166.5−167 °C; ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H, NH), 8.48 (d, J = 1.9 Hz, 1H, ArH), 8.23 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.06 (d, J = 8.6 Hz, 1H, ArH), 7.05−7.01 (m, 2H, ArH), 6.43−6.48 (m, 2H, ArH), 6.08 (s, 1H, OH), 5.14 (br s, 2H, NH_2), 3.17 (d, J = 13.0 Hz, 1H, CH(1)), 3.07 (d, J = 13.0 Hz, 1H, CH(2)), 1.40 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.97, 148.18, 143.18, 136.13, 133.36, 131.42 (q, $^2J_{C-F}$ = 31.7 Hz), 122.49 (q, $^1J_{C-F}$ = 273.6 Hz), 122.64, 119.83, 117.36 (q, $^3J_{C-F}$ = 5.0 Hz), 115.83, 114.22, 101.74 (q, $^3J_{C-F}$ = 2 Hz), 75.32, 47.26, 25.64; IR (KBr) 3486, 3447, 3386, 3363 (NH, NH_2, OH), 2234 (CN), 1681 (CO), 1623, 1596, 1583, 1513, 1331 cm^{-1}; $[\alpha]_D^{27}$ +33.0° (c = 2, acetone). Anal. ($C_{18}H_{16}F_3N_3O_2S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-[(4-aminophenyl)sulfanyl]-2-hydroxy-2-methylpropanamide ((*S*)-5).** Title compound **(*S*)-5** was obtained as white crystals (71% yield) from the mixture of anilides **(*S*)-4** and **(*S*)-4a** according to the procedure used for preparation of **(*R*)-5**: mp 166.5−167 °C; ^{1}H NMR (DMSO-d_6) δ 10.41 (s, 1H, NH), 8.47 (d, J = 1.9 Hz, 1H, ArH), 8.22 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H, ArH), 8.05 (d, J = 8.6 Hz, 1H, ArH), 7.06−7.01 (m, 2H, ArH), 6.44−6.48 (m, 2H, ArH), 6.05 (s, 1H, OH), 5.22 (br s, 2H, NH_2), 3.17 (d, J = 13.0 Hz, 1H, CH(1)), 3.07 (d, J = 13.0 Hz, 1H, CH(2)), 1.39 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.95, 147.80, 143.15, 136.11, 133.27, 131.42 (q, $^2J_{C-F}$ = 31.7 Hz), 122.63, 122.48 (q, $^1J_{C-F}$ = 273.5 Hz), 120.17, 117.36 (q, $^3J_{C-F}$ = 5.0 Hz), 115.80, 114.38, 101.74 (q, $^3J_{C-F}$ = 2 Hz), 75.30, 47.17, 25.62; IR (KBr) 3485, 3447, 3385, 3365 (NH, NH_2, OH), 2234 (CN), 1681 (CO), 1623, 1596, 1583, 1517, 1330 cm^{-1}; $[\alpha]_D^{27}$ −33.7° (c = 2, acetone). Anal. ($C_{18}H_{16}F_3N_3O_2S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-[(3-aminophenyl)sulfanyl]-2-hydroxy-2-methylpropanamide ((*R*)-6).** Neat 3-aminothiophenol (1.30 mL, d 1.179, 12.2 mmol) was added dropwise under argon atmosphere to preliminary washed 60% oil dispersion of NaH (0.46 g, 11.50 mmol) in 10 mL of anhydrous THF. The reaction mixture was stirred for 30 min until bubbles stopped to eliminate and a white suspension formed. The solution of anilides **(*R*)-4** and **(*R*)-4a** (85:15 ratio) (3.00 g, 8.7 mmol) in 20 mL of anhydrous THF was injected dropwise. The reaction mixture was stirred

for 15 h. Final suspension was diluted with 50 mL of water and extracted with EtOAc (2 × 150 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered, and evaporated to give an oil. The oil was applied to a flush column (stationary phase silica gel, chloroform as mobile phase). Title compound was obtained as a white solid (1.95 g, 58%): mp 123−125 °C; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.49 (d, J = 1.7 Hz, 1H, ArH), 8.25 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H, ArH), 8.06 (d, J = 8.6 Hz, 1H, ArH), 6.86 (t, J = 7.8 Hz, 1H, ArH), 6.52 (t, J = 1.9 Hz, 1H, ArH), 6.44 (dm, J = 8 Hz, 1H, ArH), 6.32 (dm, J = 8 Hz, 1H, ArH), 6.22 (s, 1H, OH), 5.06 (br s, 2H, NH_2), 3.33 (d, J = 12.5 Hz, 1H, CH(1), overlapped with water peak), 3.22 (d, J = 12.6 Hz, 1H, CH(2)), 1.46 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.91, 149.00, 143.12, 136.80, 136.16, 131.47 (q, $^2J_{C-F}$ = 31.7 Hz), 129.17, 122.71, 122.48 (q, $^1J_{C-F}$ = 273.7 Hz), 117.42 (q, $^3J_{C-F}$ = 5.1 Hz), 115.80, 115.71, 113.44, 111.55, 101.86 (q, $^3J_{C-F}$ = 2 Hz), 75.12, 43.44, 25.65; IR (KBr) 3440, 3340, 3300 (NH, NH_2, OH), 2248 (CN), 1683 (CO), 1612, 1594, 1513, 1487, 1428, 1325, 1240, 1182, 1136 cm^{-1}; $[\alpha]_D^{27}$ −2.8° (c = 2, acetone). Anal. ($C_{18}H_{16}F_3N_3O_2S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-[(3-aminophenyl)sulfanyl]-2-hydroxy-2-methylpropanamide (*(S)*-6).** Title compound **(*S*)-6** was obtained as a white solid (75% yield) from the mixture of the anilides **(*S*)-4** and **(*S*)-4a** according to the procedure used for preparation of **(*R*)-6**: mp 123−124.5 °C; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.49 (d, J = 1.6 Hz, 1H, ArH), 8.25 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H, ArH), 8.06 (d, J = 8.6 Hz, 1H, ArH), 6.86 (t, J = 7.8 Hz, 1H, ArH), 6.52 (t, J = 1.8 Hz, 1H, ArH), 6.43 (dm, J = 7.7 Hz, 1H, ArH), 6.31 (dm, J = 7.9 Hz, 1H, ArH), 6.21 (s, 1H, OH), 5.06 (br s, 2H, NH_2), 3.33 (d, J = 12.3 Hz, 1H, CH(1), overlapped with water peak), 3.22 (d, J = 12.6 Hz, 1H, CH-(2)), 1.46 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.95, 149.03, 143.15, 136.82, 136.20, 131.48 (q, $^2J_{C-F}$ = 31.8 Hz), 129.20, 122.72, 122.50 (q, $^1J_{C-F}$ = 273.9 Hz), 117.43 (q, $^3J_{C-F}$ = 4.9 Hz), 115.84, 115.67, 113.40, 111.55, 101.87 (q, $^3J_{C-F}$ = 1.8 Hz), 75.13, 43.43, 25.70; IR (KBr) 3440, 3340, 3301 (NH, NH_2, OH), 2248 (CN), 1683 (CO), 1614, 1594, 1515, 1487, 1429, 1326, 1240, 1183, 1135 cm^{-1}; $[\alpha]_D^{27}$ +3.2° (c = 2, acetone). Anal. ($C_{18}H_{16}F_3N_3O_2S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-2-hydroxy-3-[(4-isothiocyanatophenyl)sulfanyl]-2-methylpropanamide (*(R)*-7).** Title compound was prepared by general method proposed by Leclerc.[26] Aqueous solutions of $NaHCO_3$ (200 mg, 2.4 mmol) in water (20 mL) were added to a vigorously stirred solution of **(*R*)-5** (460 mg, 1.16 mmol) in a mixture of 50 mL of chloroform, 20 mL of CH_2Cl_2, and 10 mL of EtOAc, followed by thiophosgene (0.15 mL, d 1.508, 1.91 mmol). The reaction mixture with a yellow precipitate was stirred for 3 h until the organic layer become transparent. The organic layer was separated, and the aqueous portion of the reaction mixture was extracted with CH_2Cl_2 (10 mL). Both organic fractions were combined together, dried over Na_2SO_4, and evaporated to give a yellow oil which crystallized when standing. This mixture was triturated with toluene (20 mL) to give the product (460 mg, 91%) as a light yellow solid: mp 129−130 °C; ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H, NH), 8.41 (d, J = 1.8 Hz, 1H, ArH), 8.19 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.05 (d, J = 8.6 Hz, 1H, ArH), 7.39−7.35 (m, 2H, ArH), 7.26−7.22 (m, 2H, ArH), 6.29 (s, 1H, OH), 3.47 (d, J = 13.3 Hz, 1H, CH(1)), ·3.28 (d, J = 14 Hz, 1H, CH(2), overlapped with water peak), 1.47 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.63, 142.96, 136.95, 136.09, 133.45, 131.35 (q, $^2J_{C-F}$ = 31.7 Hz), 129.26, 127.08, 126.10, 122.68, 122.44 (q, $^1J_{C-F}$ = 273.6 Hz), 117.29 (q, $^3J_{C-F}$ = 5.0 Hz), 115.73, 101.87 (q, $^3J_{C-F}$ = 2 Hz), 75.04, 43.05, 25.72; IR (KBr) 3428, 3377 (NH, OH), 2243 (CN), 2080 (NCS), 1710 (CO), 1582, 1522, 1430, 1337, 1187, 1139 cm^{-1}; $[\alpha]_D^{27}$ + 54.9° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_2S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-2-hydroxy-3-[(4-isothiocyanatophenyl)sulfanyl]-2-methylpropanamide (*(S)*-7).** Title compound was obtained with 87% yield from **(*S*)-5** according to the same method used for preparation of **(*R*)-7**: mp 131.5−132 °C; ^{1}H NMR (DMSO-d_6) was the same as for compound **(*R*)-7** ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.41 (d, J = 1.7 Hz, 1H, ArH), 8.19 (dd, J = 8.6 Hz, J = 1.7 Hz, 1H, ArH), 8.06 (d, J = 8.6 Hz, 1H, ArH), 7.38−7.34 (m, 2H, ArH), 7.26−7.23 (m, 2H, ArH), 6.30 (s, 1H, OH), 3.47 (d, J = 13.3 Hz, 1H, CH(1)), ·3.28 (d, J = 13.4 Hz, 1H, CH(2), overlapped with water peak), 1.46 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.67, 143.00, 136.98, 136.13, 133.43, 131.38 (q, $^2J_{C-F}$ = 31.3 Hz), 129.27, 127.09, 126.14, 122.71, 122.46 (q, $^1J_{C-F}$ = 273.7 Hz), 117.31 (q, $^3J_{C-F}$ = 5.1 Hz), 115.77, 101.90 (q, $^3J_{C-F}$ = 2 Hz), 75.07, 43.05, 25.76; IR (KBr) 3415, 3376 (NH, OH), 2240 (CN), 2097 (NCS), 1709 (CO), 1582, 1521, 1431, 1328, 1181, 1135 cm^{-1}; $[\alpha]_D^{27}$ −54.8° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_2S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-2-hydroxy-3-[(3-isothiocyanatophenyl)sulfanyl]-2-methylpropanamide (*(R)*-8).** Title compound was obtained from **(*R*)-6** with 99% yield by the same method used for preparation of **(*R*)-7**: mp 100−102 °C; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.41 (d, J = 1.8 Hz, 1H, ArH), 8.18 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H, ArH), 8.05 (d, J = 8.6 Hz, 1H, ArH), 7.36 (t, J = 1.8 Hz, 1H, ArH), 7.31 (dt, J = 7.9 Hz, J = 1.5 Hz, 1H, ArH), 7.25 (t, J = 7.8 Hz, 1H, ArH), 7.11 (dt, J = 7.6 Hz, J = 1.6 Hz, 1H, ArH), 6.28 (s, 1H, OH), 3.49 (d, J = 13.4 Hz, 1H, CH(1)), 3.28 (d, J = 13.5 Hz, 1H, CH(2), overlapped with water peak), 1.47 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.61, 142.96, 138.80, 136.09, 133.62, 131.40 (q, $^2J_{C-F}$ = 31.7 Hz), 130.37, 129.88, 127.81, 125.10, 122.94, 122.63, 122.44 (q, $^1J_{C-F}$ = 274.0 Hz), 117.24 (q, $^3J_{C-F}$ = 5.3 Hz), 115.76, 101.94 (q, $^3J_{C-F}$ = 2 Hz), 75.05, 42.96, 25.75; IR (KBr) 3448, 3316 (NH, OH), 2243 (CN), 2209, 2124 (NCS), 1683 (CO), 1581, 1511, 1491, 1429, 1330, 1237, 1174, 1135 cm^{-1}; $[\alpha]_D^{27}$ −18.3° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_2S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-2-hydroxy-3-[(3-isothiocyanatophenyl)sulfanyl]-2-methylpropanamide (*(S)*-8).** Title compound was obtained from **(*S*)-6** with 60% yield by the same method used for preparation of **(*R*)-7**: mp 100−102 °C; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 8.40 (d, J = 1.9 Hz, 1H, ArH), 8.18 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.04 (d, J = 8.6 Hz, 1H, ArH), 7.35 (t, J = 1.7 Hz, 1H, ArH), 7.31 (dt, J = 8.1 Hz, J = 1.5 Hz, 1H, ArH), 7.24 (t, J = 7.8 Hz, 1H, ArH), 7.10 (dt, J = 7.7 Hz, J = 1.6 Hz, 1H, ArH), 6.29 (s, 1H, OH), 3.49 (d, J = 13.4 Hz, 1H, CH(1)), 3.28 (d, J = 13.5 Hz, 1H, CH(2), overlapped with water peak), 1.47 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.61, 142.96, 138.80, 136.09, 133.67, 131.42 (q, $^2J_{C-F}$ = 31.7 Hz), 130.39, 129.89, 127.83, 125.11, 122.95, 122.64, 122.46 (q, $^1J_{C-F}$ = 273.7 Hz), 117.25 (q, $^3J_{C-F}$ = 5.1 Hz), 115.77, 101.96 (q, $^3J_{C-F}$ = 2 Hz), 75.06, 42.98, 25.76; IR (KBr) 3448, 3316 (NH, OH), 2243 (CN), 2209, 2125 (NCS), 1683 (CO), 1581, 1511, 1491, 1429, 1330, 1237, 1174, 1135 cm^{-1}; $[\alpha]_D^{27}$ +20.4° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_2S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-2-hydroxy-3-[(4-isothiocyanatophenyl)sulfonyl]-2-methylpropanamide (*(R)*-9).** A solution of **(*R*)-7** (260 mg, 0.59 mmol) in CH_2Cl_2 (30 mL) was mixed with solution of *m*-chloroperbenzoic acid (620 mg, 3.6 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 80 min and washed with saturated aqueous solution of Na_2SO_3 (2 × 50 mL) and saturated aqueous solution of $NaHCO_3$ (3 × 50 mL). The organic layer was separated, dried over Na_2SO_4, reduced in volume in vacuo, and applied to a flash column (stationary phase silica gel, mobile phase chloroform/MeOH = 195:5). Final compound was crystallized from ether/hexane as a white solid: mp 162.5−163 °C; ^{1}H NMR (DMSO-d_6) δ 10.31 (s, 1H, NH), 8.40 (d, J = 1.6 Hz, 1H, ArH), 8.18 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H, ArH), 8.08 (d, J = 8.6 Hz, 1H, ArH), 7.90−7.86 (m, 2H, ArH), 7.52−7.48 (m, 2H, ArH), 6.44 (s, 1H, OH), 3.97 (d, J = 14.8 Hz, 1H, CH(1)), 3.72 (d, J = 14.8 Hz, 1H, CH(2)), 1.39 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 173.54, 143.03, 138.97, 136.42, 136.12, 134.82, 131.38 (q, $^2J_{C-F}$ = 31.9 Hz), 130.15, 126.29, 122.77, 122.49 (q, $^1J_{C-F}$ = 273.5 Hz), 117.40 (q, $^3J_{C-F}$ = 5.5 Hz), 115.79, 101.97 (d, $^3J_{C-F}$ = 2.5 Hz), 72.99, 63.34, 27.26; IR (KBr) 3431, 3350 (NH, OH), 2232 (CN), 2193, 2042 (NCS), 1702 (CO), 1588, 1526, 1431, 1327 (SO_2 asym), 1179,

1141 (SO_2 sym) cm^{-1}; $[\alpha]_D^{27}$ −59.1° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_4S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-2-hydroxy-3-[(3-isothiocyanatophenyl)sulfonyl]-2-methylpropanamide ((*R*)-10).** Title compound was obtained from **(*R*)-8** with 56% yield by the same method used for preparation **(*R*)-9**: mp 97−100 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.41 (s, 1H, NH), 8.42 (d, J = 1.5 Hz, 1H, ArH), 8.20 (dd, J = 8.6 Hz, J = 1.5 Hz, 1H, ArH), 8.08 (d, J = 8.6 Hz, 1H, ArH), 7.85−7.75 (m, 2H, ArH), 7.63−7.61 (m, 2H, ArH), 6.40 (s, 1H, OH), 4.00 (d, J = 14.9 Hz, 1H, CH(1)), 3.80 (d, J = 14.9 Hz, 1H, CH(2)), 1.41 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 173.56, 143.03, 132.33, 136.12, 135.51, 131.39 (q, $^2J_{C-F}$ = 31.6 Hz), 130.77, 130.61, 130.56, 127.03, 125.48, 122.81, 122.46 (q, $^1J_{C-F}$ = 273.5 Hz), 117.48 (q, $^3J_{C-F}$ = 5.3 Hz), 115.77, 102.04 (q, $^3J_{C-F}$ = 2 Hz), 73.03, 63.07, 27.09; IR (KBr) 3455, 3355 (NH, OH), 2232 (CN), 2019 (NCS), 1690 (CO), 1588, 1522, 1432, 1331 (SO_2 asym), 1295, 1275, 1182, 1137 (SO_2 sym) cm^{-1}; $[\alpha]_D^{27}$ −98.1° (c = 2, acetone). Anal. ($C_{19}H_{14}F_3N_3O_4S_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({4-[(2-chloroacetyl)amino]phenyl}sulfanyl)-2-hydroxy-2-methylpropanamide ((*R*)-11).** Neat chloroacetyl chloride (0.1 mL, d 1.418, 1.2 mmol) was added dropwise under argon over period of 1−2 min to the vigorously stirred suspension, containing solution of **(*R*)-5** (410 mg, 1.04 mmol) in anhydrous CH_2Cl_2 (50 mL) and dry powder of $CaCO_3$ (0.56 g, 5.6 mmol). The reaction mixture was stirred overnight and filtered out, and the filtrate was completely evaporated in vacuo to give a white solid. The solid was recrystallized from EtOAc/hexane to give 377 mg (77%) of the title compound in ball-shape crystals: mp 134.5−135 °C; ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H, NH), 10.24 (s, 1H, NH), 8.43 (d, J = 1.8 Hz, 1H, ArH), 8.20 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.03 (d, J = 8.6 Hz, 1H, ArH), 7.45−7.43 (m, 2H, ArH), 7.31−7.28 (m, 2H, ArH), 6.20 (s, 1H, OH), 4.20 (s, 2H, CH_2Cl), 3.38 (d, J = 13.1 Hz, 1H, CH(1)), 3.23 (d, J = 13.1 Hz, 1H, CH(2)), 1.45 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.76, 164.43, 143.05, 136.69, 136.09, 131.39 (q, $^2J_{C-F}$ = 31.7 Hz), 130.85, 130.06, 122.64, 122.46 (q, $^1J_{C-F}$ = 273.7 Hz), 119.67, 117.33 (q, $^3J_{C-F}$ = 5.0 Hz), 115.79, 101.85 (q, $^3J_{C-F}$ = 2 Hz), 75.17, 44.46, 43.44, 25.69. IR (KBr) 3464, 3321, ·3265 (NH, OH), 2238 (CN), 1679 (CO), 1609, 1597, 1522, 1429, 1327, 1174, 1135 cm^{-1}; $[\alpha]_D^{27}$ +23.4° (c = 2, acetone). Anal. ($C_{20}H_{17}ClF_3N_3O_3S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*S*)-3-({4-[(2-chloroacetyl)amino]phenyl}sulfanyl)-2-hydroxy-2-methylpropanamide ((*S*)-11).** Title compound was obtained with 56% yield from ***(S)*-5** by the same method used for preparation of **(*R*)-11**: mp 132−134 °C; ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H, NH), 10.23 (s, 1H, NH), 8.43 (d, J = 1.7 Hz, 1H, ArH), 8.20 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.04 (d, J = 8.6 Hz, 1H, ArH), 7.45−7.42 (m, 2H, ArH), 7.31−7.28 (m, 2H, ArH), 6.20 (s, 1H, OH), 4.20 (s, 2H, CH_2Cl), 3.38 (d, J = 13.2 Hz, 1H, CH(1), overlapped with water peak), 3.23 (d, J = 13.1 Hz, 1H, CH(2)), 1.44 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.81, 164.46, 143.09, 136.71, 136.14, 131.41 (q, $^2J_{C-F}$ = 31.7 Hz), 130.86, 130.07, 122.67, 122.49 (q, $^1J_{C-F}$ = 273.8 Hz), 119.67, 117.34 (q, $^3J_{C-F}$ = 5.3 Hz), 115.84, 101.86 (q, $^3J_{C-F}$ = 1.8 Hz), 75.19, 44.46, 43.49, 25.75; IR (KBr) 3464, 3320, 3268 (NH, OH), 2239 (CN), 1679 (CO), 1609, 1598, 1521, 1429, 1327, 1174, 1135 cm^{-1}; $[\alpha]_D^{27}$ −23.4° (c = 2, acetone). Anal. ($C_{20}H_{17}ClF_3N_3O_3S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({3-[(2-chloroacetyl)amino]phenyl}sulfanyl)-2-hydroxy-2-methylpropanamide ((*R*)-12).** Neat chloroacetyl chloride (0.12 mL, d 1.418, 1.5 mmol) and neat *N*,*N*-diisopropylethylamine (0.26 mL, d 0.742, 1.5 mmol) were simultaneously added dropwise over a period of 5 min to a solution of **(*R*)-6** (500 mg, 1.26 mmol) in anhydrous CH_2Cl_2 (50 mL) in the ice bath under argon. The reaction mixture was stirred for 4 h in the ice bath, warmed to room temperature, diluted with CH_2Cl_2 (50 mL), and washed with water (50 mL), 0.1 N HCl (50 mL), aqueous saturated $NaHCO_3$ solution (50 mL), and brine (50 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated to dryness to give a white solid. The solid was triturated under ether/hexane mixture to give 560 mg (94%) of the title compound: mp 67 °C started melting; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 10.22 (s, 1H, NH), 8.45 (d, J = 1.8 Hz, 1H, ArH), 8.21 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.05 (d, J = 8.6 Hz, 1H, ArH), 7.60 (t, J = 1.8 Hz, 1H, ArH), 7.29 (dm, J = 7 Hz, 1H, ArH), 7.18 (t, J = 7.9 Hz, 1H, ArH), 7.06 (dt, J = 7.9 Hz, J = 1.4 Hz, 1H, ArH), 6.27 (s, 1H, OH), 4.21 (s, 2H, CH_2Cl), 3.41 (d, J = 12.9 Hz, 1H, CH(1)), 3.27 (d, J = 12.9 Hz, 1H, CH(2)), 1.47 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.80, 164.68, 143.08, 138.86, 137.29, 136.19, 131.46 (q, $^2J_{C-F}$ = 31.7 Hz), 129.28, 123.82, 122.73, 122.51 (q, $^1J_{C-F}$ = 273.7 Hz), 118.87, 117.40 (q, $^3J_{C-F}$ = 5.1 Hz), 116.60, 115.87, 101.92 (q, $^3J_{C-F}$ = 2 Hz), 75.09, 43.52, 43.47, 25.75; IR (KBr) 3330 (broad, NH, OH), 2232 (CN), 1683 (CO), 1588, 1523, 1430, 1327, 1181, 1136 cm^{-1}; $[\alpha]_D^{27}$ −10.1° (c = 2, acetone); MS (ESI) m/z 472. Anal. ($C_{20}H_{17}ClF_3N_3O_3S\cdot 0.1H_2O$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({4-[(2-bromoacetyl)amino]phenyl}sulfanyl)-2-hydroxy-2-methylpropanamide ((*R*)-13).** Title compound was obtained with 95% yield by acylation of **(*R*)-5** with bromoacetyl bromide according to the same procedure used for preparation of **(*R*)-11**: mp 158−159.5 °C; ^{1}H NMR (DMSO-d_6) δ 10.45 (s, 1H, NH), 10.33 (s, 1H, NH), 8.43 (d, J = 1.8 Hz, 1H, ArH), 8.20 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H, ArH), 8.04 (d, J = 8.6 Hz, 1H, ArH), 7.45−7.38 (m, 2H, ArH), 7.31−7.28 (m, 2H, ArH), 6.21 (s, 1H, OH), 3.99 (s, 2H, CH_2Br), 3.38 (d, J = 13.1 Hz, 1H, CH(1)), 3.22 (d, J = 13.1 Hz, 1H, CH(2)), 1.44 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.81, 164.62, 143.08, 136.84, 136.14, 131.41 (q, $^2J_{C-F}$ = 31.7 Hz), 130.86, 130.08, 122.67, 122.48 (q, $^1J_{C-F}$ = 273.5 Hz), 119.54, 117.35 (q, $^3J_{C-F}$ = 5.1 Hz), 115.84, 101.85 (q, $^3J_{C-F}$ = 1.8 Hz), 75.19, 44.47, 30.28, 25.75; IR (KBr) 3466, 3317, 3263 (NH, OH), 2238 (CN), 1679 (CO), 1667, 1608, 1597, 1510, 1429, 1327, 1173, 1135 cm^{-1}; $[\alpha]_D^{27}$ +24.0° (c = 2, acetone). Anal. ($C_{20}H_{17}BrF_3N_3O_3S\cdot 0.09CH_2Cl_2$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({3-[(2-bromoacetyl)amino]phenyl}sulfanyl)-2-hydroxy-2-methylpropanamide ((*R*)-14).** Title compound was obtained with 94% yield by acylation of **(*R*)-6** with bromoacetyl bromide according to the same procedure used for preparation of **(*R*)-11**: mp 60−65 °C started melting; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, NH), 10.36 (s, 1H, NH), 8.45 (d, J = 1.8 Hz, 1H, ArH), 8.21 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.04 (d, J = 8.6 Hz, 1H, ArH), 7.59 (t, J = 1.7 Hz, 1H, ArH), 7.29 (dm, J = 8.8 Hz, 1H, ArH), 7.18 (t, J = 7.9 Hz, 1H, ArH), 7.05 (dt, J = 7.9 Hz, J = 1.3 Hz, 1H, ArH), 6.27 (s, 1H, OH), 3.99 (s, 2H, CH_2-Br), 3.41 (d, J = 12.9 Hz, 1H, CH(1)), 3.27 (d, J = 12.9 Hz, 1H, CH(2)), 1.47 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 174.76, 164.78, 143.05, 138.96, 137.28, 136.15, 131.41 (q, $^2J_{C-F}$ = 31.7 Hz), 129.23, 123.75, 122.68, 122.47 (q, $^1J_{C-F}$ = 273.7 Hz), 118.68, 117.37 (q, $^3J_{C-F}$ = 4.9 Hz), 116.43, 115.82, 101.88 (q, $^3J_{C-F}$ = 2 Hz), 75.06, 43.42, 30.27, 25.70; IR (KBr) 3331 (broad, NH, OH), 2232 (CN), 1678 (CO), 1587, 1523, 1430, 1327, 1181, 1137 cm^{-1}; MS (ESI) m/z 516, 518; $[\alpha]_D^{27}$ −14.7° (c = 2, acetone). Anal. ($C_{20}H_{17}BrF_3N_3O_3S\cdot 0.65H_2O$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({4-[(2-chloroacetyl)amino]phenyl}sulfonyl)-2-hydroxy-2-methylpropanamide ((*R*)-15).** Aqueous solution of peracetic acid (4 mL, 32%, d 1.13, 19 mmol) was added to a solution of **(*R*)-11** (220 mg, 0.47 mmol) in CH_2Cl_2 (10 mL). After 2.5 h of stirring at room temperature the reaction mixture was diluted with 200 mL of EtOAc and washed with saturated aqueous solution of Na_2SO_3 (50 mL) and brine (30 mL). The organic layer was separated, dried over Na_2SO_4, and completely evaporated to dryness to give a white solid. Recrystallization of the solid from EtOAc/hexane gave the title compound in quantitative yield: mp 174−175 °C; ^{1}H NMR (DMSO-d_6) δ 10.61 (s, 1H, NH), 10.33 (s, 1H, NH), 8.40 (d, J = 1.8 Hz, 1H, ArH), 8.17 (dd, J = 8.6 Hz, J = 1.9 Hz, 1H, ArH), 8.05 (d, J = 8.6 Hz, 1H, ArH), 7.81−7.78 (m, 2H, ArH), 7.70−7.67 (m, 2H, ArH), 6.38 (s, 1H, OH), 4.25 (s, 2H, CH_2Cl), 3.89 (d, J = 14.7 Hz, 1H, CH(1)), 3.62 (d, J = 14.7 Hz, 1H, CH(2)), 1.39 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 173.67, 165.23, 143.13, 142.93, 136.12, 143.85, 131.38 (q, $^2J_{C-F}$ = 31.6 Hz), 129.53, 122.78, 122.51 (q, $^1J_{C-F}$ = 273.6 Hz), 118.65, 117.43 (q, $^3J_{C-F}$ = 5 Hz),

115.85, 101.93 (q, $^3J_{C-F}$ = 2 Hz), 73.09, 63.56, 43.46, 27.26; IR (KBr) 3477, 3357, 3325 (NH, OH), 2235 (CN), 1692 (CO), 1592, 1527, 1422, 1403, 1329 (SO_2 asym), 1301, 1186, 1144 (SO_2 sym) cm^{-1}; MS (ESI) *m/z* 516, 518; $[\alpha]_D^{27}$ −49.4° (*c* = 2, acetone). Anal. ($C_{20}H_{17}ClF_3N_3O_5S$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({3-[(2-chloroacetyl)amino]phenyl}sulfonyl)-2-hydroxy-2-methylpropanamide (*(R)*-16).** Aqueous solution of peracetic acid (1 mL, 32%, *d* 1.13, 5 mmol) was added to a solution of **(*R*)-12** (208 mg, 0.44 mmol) in acetone (3 mL). After 10 min of stirring at room temperature the reaction mixture was diluted with 200 mL of EtOAc, and washed with saturated aqueous solution of Na_2SO_3 (50 mL). The organic layer was separated, dried over Na_2SO_4, and completely evaporated to dryness to give a colorless oil which crystallized when triturated under hexane to give quantitative yield of a creamy solid: mp 130 °C dec, started melting; ^{1}H NMR (DMSO-d_6) δ 10.58 (s, 1H, NH), 10.37 (s, 1H, NH), 8.42 (d, *J* = 1.8 Hz, 1H, ArH), 8.20 (dd, *J* = 8.6 Hz, *J* = 1.9 Hz, 1H, ArH), 8.11 (t, *J* = 1.7 Hz, 1H, ArH), 8.07 (d, *J* = 8.6 Hz, 1H, ArH), 7.79 (dt, *J* = 7.7 Hz, *J* = 1.7 Hz, 1H, ArH), 7.59−7.50 (m, 2H, ArH), 6.40 (s, 1H, OH), 4.24 (s, 2H, CH_2Cl), 3.91 (d, *J* = 14.7 Hz, 1H, CH(1)), 3.67 (d, *J* = 14.7 Hz, 1H, CH(2)), 1.41 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 173.65, 165.01, 143.13, 141.45, 138.90, 136.15, 131.38 (q, $^2J_{C-F}$ = 31.7 Hz), 129.75, 123.68, 123.08, 122.79, 122.51 (q, $^1J_{C-F}$ = 273.8 Hz), 118.12, 117.48 (q, $^3J_{C-F}$ = 5.1 Hz), 115.86, 101.93 (q, $^3J_{C-F}$ = 2 Hz), 73.17, 63.29, 43.39, 27.16; IR (KBr) 3336 (broad, NH, OH), 2232 (CN), 1696 (CO), 1598, 1527, 1431, 1328 (SO_2 asym), 1304, 1181, 1137 (SO_2 sym) cm^{-1}; MS (ESI) *m/z* 504; $[\alpha]_D^{27}$ −54.2° (*c* = 2, acetone). Anal. ($C_{20}H_{17}ClF_3N_3O_5S\cdot 0.25H_2O$) C, H, N.

***N1*-[4-Cyano-3-(trifluoromethyl)phenyl]-(2*R*)-3-({4-[(2-bromoacetyl)amino]phenyl}sulfonyl)-2-hydroxy-2-methylpropanamide (*(R)*-17).** Title compound was obtained with 83% yield by oxidation of ***(R)*-13** with *m*-chloroperbenzoic acid according to the procedure used for preparation of **(*R*)-9**: mp 112−114 °C dec, started melting; ^{1}H NMR (DMSO-d_6) δ 10.68 (s, 1H, NH), 10.31 (s, 1H, NH), 8.40 (d, *J* = 1.7 Hz, 1H, ArH), 8.17 (dd, *J* = 8.6 Hz, *J* = 1.9 Hz, 1H, ArH), 8.05 (d, *J* = 8.6 Hz, 1H, ArH), 7.84−7.75 (m, 2H, ArH), 7.72−7.63 (m, 2H, ArH), 6.35 (s, 1H, OH), 4.03 (s, 2H, CH_2Br), 3.89 (d, *J* = 14.7 Hz, 1H, CH(1)), 3.62 (d, *J* = 14.7 Hz, 1H, CH(2)), 1.39 (s, 3H, Me); ^{13}C NMR (DMSO-d_6) δ 173.68, 165.42, 143.13, 142.93, 136.12, 143.88, 131.38 (q, $^2J_{C-F}$ = 32.0 Hz), 129.53, 122.78, 122.51 (q, $^1J_{C-F}$ = 273.7 Hz), 118.57, 117.44 (q, $^3J_{C-F}$ = 5.4 Hz), 115.86, 101.92 (q, $^3J_{C-F}$ = 2 Hz), 73.09, 63.56, 30.06, 27.24; IR (KBr) 3442, 3350, 3327 (NH, OH), 2234 (CN), 1704 (CO), 1593, 1527, 1432, 1404, 1329 (SO_2 asym), 1181, 1139 (SO_2 sym) cm^{-1}; $[\alpha]_D^{27}$ −45.4° (*c* = 2, acetone). Anal. ($C_{20}H_{17}BrF_3N_3O_5S$) C, H, N.

Materials for Binding Studies. Chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI (L-proline, thionyl chloride, 3-aminothiophenol, 4-aminothiophenol, peracetic acid, chloroacetyl chloride, bromoacetyl bromide, *N*,*N*-diisopropylethylamine, and anhydrous dichloromethane and THF in SureSeal bottles), Lancaster Synthesis, Wyndham, NH (D-proline), Lancaster Synthesis, England (4-amino-2-trifluoromethylbenzonitrile), and Janssen Chimica, Geel, Belgium (methacryloyl chloride and *m*-chloroperbenzoic acid). [*17α-methyl*-^{3}H]Mibolerone ([^{3}H]MIB, 83.5 Ci/mmol) and unlabeled MIB were purchased from DuPont Research NEN Products, Boston, MA. Triamcinolone acetonide, phenylmethanesulfonyl fluoride (PMSF), Tris base, sodium molybdate, and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, MO. EcoLite (+) scintillation cocktail was purchased from ICN Research Products Division, Costa Mesa, CA.

Preparation of Cytosolic AR. Male Sprague−Dawley rats (Harlan Sprague−Dawley, Indianapolis, IN), weighing 248−290 g, were castrated 24 h prior to the removal of prostates. Ventral prostates were surgically removed, weighed, and immersed immediately in ice-cold homogenization buffer. Homogenization buffer consisted of 10 mM Tris, 1.5 mM disodium EDTA, 0.25 M sucrose, 10 mM sodium molybdate, and 1 mM PMSF and was adjusted to pH 7.4.[28] The prostate tissue (about 0.4 g/rat) was minced, weighed, and homogenized (PRO 200 homogenizer, PRO Scientific, Monroe, CT) with 1 mL of the homogenization buffer per 500 mg of prostate tissue. The homogenate was then centrifuged at 105000*g* for 1 h at 0 °C in a Beckman L8-M ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA).[29] The supernatant (cytosol) containing AR protein was removed and stored at −80 °C until use. Total protein was determined by Peterson's modification of the micro-Lowry method, using a protein assay kit from Sigma Diagnostics, St. Louis, MO.

Data Analysis. Specific binding for each experiment was calculated by subtracting the binding of [^{3}H]MIB observed in the presence of excess unlabeled MIB (nonspecific binding) from the binding of [^{3}H]MIB observed in the absence of unlabeled MIB (total binding). Competitive radioligand displacement curves were then constructed with percent specific binding (specific binding of [^{3}H]MIB at a particular ligand concentration expressed as a percentage of the specific binding of [^{3}H]MIB in the absence of ligand) on the vertical axis and ligand concentration on the horizontal axis. The ligand concentration that reduces the percentage of specific binding by 50% (IC_{50}) was determined by computer fitting data for the competitive binding of each AR ligand to the following equation, using a FORTRAN subroutine written for WIN-NONLIN (SCI Software, Lexington, KY):

$$B = B_o[1 - C/(IC_{50} + C)]$$

where *B* is the specific binding of [^{3}H]MIB in the presence of a particular concentration of ligand, B_o is the specific binding of [^{3}H]MIB in the absence of ligand, and *C* is the ligand concentration. Binding affinity of the ligand was then compared using the equilibrium dissociation constant (K_i). The K_i of each ligand was calculated using the equation:

$$K_i = (IC_{50} \times K_d)/(L + K_d)$$

where K_d is the equilibrium dissociation constant of [^{3}H]MIB and *L* is the concentration of [^{3}H]MIB (1 nM).

Acknowledgment. We thank Dr. Howard Tucker (Zeneca Pharmaceuticals, U.K.) for assistance with synthetic procedure and providing samples of racemic bicalutamide and thiobicalutamide. We also thank Dr. Nina Stourman and graduate student Craig Marhefka for preparation of some starting materials. This work was supported in part by a grant from the National Cancer Institute (1 R29 CA68096-01 to J.D.), The American Cancer Society (Grant IN.176-C), Assisi Foundation (Memphis, TN), and Van Vleet Foundation (Memphis, TN).

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JM990027X