Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 6107–6111
Bioorganic & Medicinal Chemistry Letters

# The synthesis and evaluation of [2.2.1]-bicycloazahydantoins as androgen receptor antagonists

Aaron Balog,[a,*] Mark E. Salvati,[a] Weifang Shan,[a] Arvind Mathur,[d] Leslie W. Leith,[d] Donna D. Wei,[a] Ricardo M. Attar,[b] Jieping Geng,[b] Cheryl A. Rizzo,[b] Chihuei Wang,[b] Stanley R. Krystek,[c] John S. Tokarski,[c] John T. Hunt,[b] Marco Gottardis[b] and Roberto Weinmann[b]

[a]*Department of Oncology Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA*
[b]*Department of Discovery Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA*
[c]*Department of Computer Aided Drug Design, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA*
[d]*Department of Chemical Synthesis, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA*

Received 16 August 2004; revised 16 September 2004; accepted 18 September 2004

**Abstract**—A novel series of [2.2.1]-azahydantoins has been designed and synthesized in an enantiospecific manner. The ability of these compounds to act as antagonists to the androgen receptor was investigated and several were found to have potent activity in vitro.


Prostate cancer (CaP) is the 2nd leading cause of cancer related death in men with an estimated 182,000 new cases diagnosed each year in the United States.[1] The androgen receptor (AR) is a ligand binding transcription factor in the nuclear hormone receptor super-family and is a key molecular target in the growth and progression of prostate cancer. Binding of androgens, such as dihydro-testosterone (DHT), to the AR provides the mitogenic signal for growth of prostate cancer cells. For the past 50 years, androgen ablation via castration has been the most effective therapy for the treatment of advanced prostate cancer in the clinic. Complete androgen blockade is accomplished by treatment with an antiandrogen in addition to chemical castration with an luteinizing hormone releasing hormone (LHRH) agonist. Casodex (**1**) (bicalutamide) and Eulexin (**2**) (flutamide) are FDA approved AR antagonists that initially show a 80–90% response rate when used to treat advanced CaP.[2] However, when treatment is continued for 1–2 years, tumors become androgen independent and this therapy fails. Approximately 50% of patients undergoing complete androgen blockade progress to fatal androgen-independent disease within 5 years of starting therapy.[3] Clearly, there is an unmet medical need for the treatment of advanced CaP. Thus, we wanted to find a novel, non-steroidal, small-molecule antagonist of the AR that is more efficacious than Casodex and shows an increased time required to reach androgen independent disease.

Our initial screening efforts led to a series of [2.2.1]-bicyclicsuccinimides, exemplified by compound **3**, that bound tightly to the AR and functioned as antagonists to the wild-type (WT) and the mutant (MT) isoforms of the AR in vitro.[4,5] Closely related to the early succinimide leads was a series of bicyclic hydantoins represented by compound **4**.[6] These compounds showed very potent binding to the WT AR in addition to an antagonist profile in vitro.

*Keywords*: Androgen receptor; Hydantoin.
* Corresponding author. Tel.: +1 609 252 4632; fax: +1 609 252 6804; e-mail: aaron.balog@bms.com

0960-894X/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2004.09.049

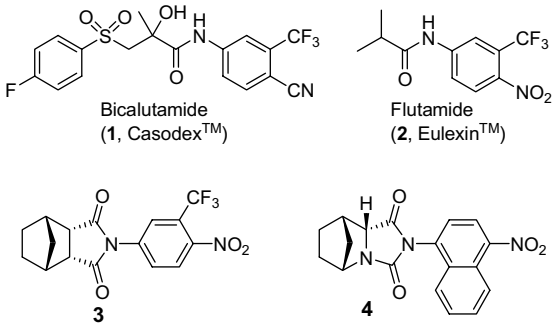



**Figure 1.** Clinically used antiandrogens.

Closely related to the early succinimide leads was a series of bicyclic hydantoins represented by compound **4**.[6] These series of compounds showed very potent binding to the AR in addition to an antagonist profile in vitro against the WT AR, comparing well to Casodex (**1**) and Eulexin (**2**), both clinically used antiandrogens (Fig. 1).

The promising in vitro profile seen with compounds such as **3** and **4** led us to design a series of modified [2.2.1]-bicyclic hydantoins. The X-ray crystal structure of dihydrotestosterone (DHT) bound to the wild-type (WT) AR ligand binding domain (LBD) was recently solved in our laboratories[7] and we utilized this information for computer-aided drug design. Figure 2a shows bicalutamide (purple) docked into the crystal structure of DHT (green) bound to the WT AR.[8] Bicalutamide occupies a much larger space than DHT in the LBD, thus Helix-12 (H-12) was repositioned to allow bicalutamide to dock into the LBD. The docking studies suggest that the aniline portion of bicalutamide has similar interactions with the AR as the A-ring of DHT. Further, the hydrogen bond to the hydroxyl group in bicalutamide from residue N705 appears to mimic the H-bond seen from the C-17 hydroxyl of DHT, and appears to be essential for high affinity binding to the AR.[10] More importantly, the 4-fluorophenyl ring of bicalutamide also appears to clash H-12, which would force it out of the conformation found in the depicted agonist conformation. It is commonly believed that the orientation of H-12 is, in part, responsible for agonist/antagonist function of nuclear hormone receptors.[11]

Based on our proposed model of binding of bicalutamide to the AR, we believe that compound **4** forms an analogous H-bond to residue N705 as bicalutamide through the tertiary nitrogen of the hydantoin ring system. In contrast to bicalutamide, compound **4** lacks any interactions with H-12, which we proposed to be a critical interaction for the generation of an antagonist phenotype. Based on our model of bicalutamide, we proposed that placing an appendage off the bicyclic portion of our ring system would result in a similar clash with H-12, thus generating a new series of analogs with improved antagonist activity against both the WT and MT AR. Modeling suggested that positioning an appendage at the C-5 methylene would give an optimal overlay with our model of bicalutamide. The most flexible way to accomplish this was to replace a methylene group with a nitrogen in the bicycle thereby providing a convenient point of attachment for parallel synthesis. This idea is represented in Figure 3 and in Figure 2b, where the proposed azahydantoin **5** is docked into the LBD of WT AR. As is shown, the 4-fluorophenylcarbamate appears to invade the space occupied by H-12 in the wild-type LBD. Based on these studies, we set out to make a series of modified hydantoins based on compound **5**.

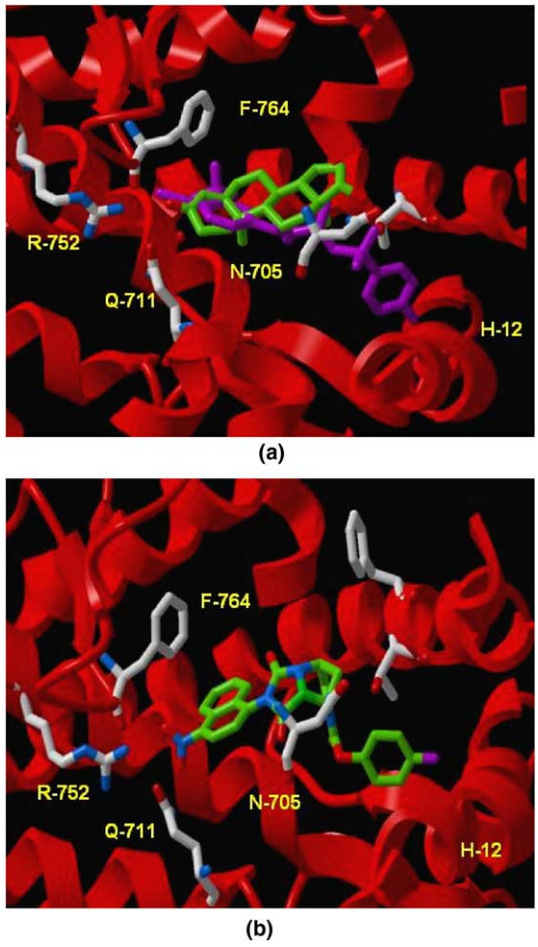



**Figure 2.** (a) Bicalutamide (**1**) (purple) and DHT (green) docked into a model of the AR derived from the crystal structure with DHT bound. Interaction with Helix-12 seen for bicalutamide. (b) Compound **5** docked into a model of the AR derived from the crystal structure with DHT bound. Shows similar interactions as bicalutamide with Helix-12.

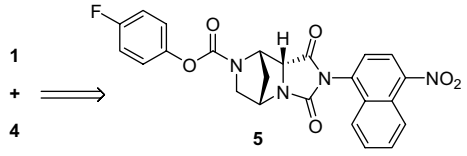
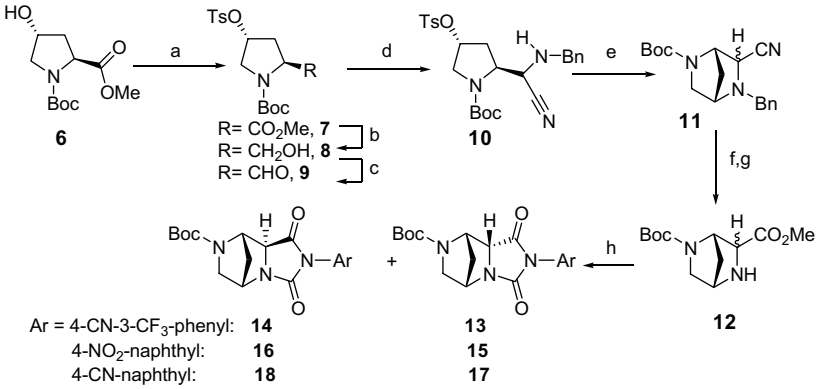



**Figure 3.** Proposed modified hydantoin **5**.

We began the synthesis by treatment of commercially available *N*-Boc-*trans*-hydroxy-L-proline methyl ester (**6**)[12] with tosyl chloride and pyridine to give the corre-



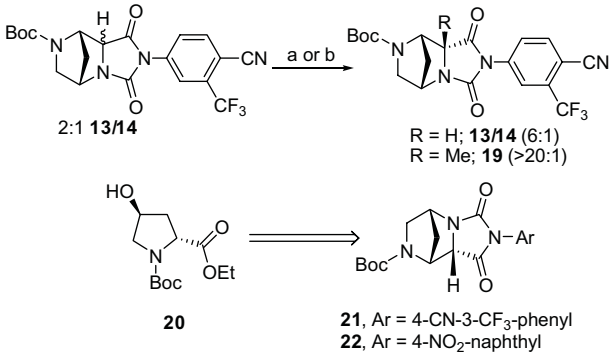

**Scheme 1.** Reagents and conditions: (a) tosylchloride, pyridine, $CH_2Cl_2$, 99%; (b) $LiBH_4$, THF, 22 °C, quant.; (c) $(COCl)_2$, DMSO, TEA, $CH_2Cl_2$, −78 to 0 °C; (d) $(EtO)_2P(O)CN$, $BnNH_2$, 4 Å MS, $CH_2Cl_2$, 95%, two steps; (e) DBU, $(CH_2Cl)_2$, 80 °C, 99%, 2:1 *endo*/*exo*; (f) 3 N NaOH then HCl/MeOH; (g) $H_2$, 10% Pd/C, EtOAc, 78%, two steps; (h) arylisocyanate,[16] 4 Å MS, $(CH_2Cl)_2$, 22 °C then DBU 60 °C, 2 h, 58%, 2:1 *endo*/*exo* (>98% ee).

sponding tosylate **7**, which was taken on in crude form (Scheme 1). The ester was reduced with lithium borohydride to give the primary alcohol **8** in excellent yield. The aldehyde **9**, obtained by a Swern oxidation of **8**,[13] was then treated with cyanodiethylphosphonate and benzylamine to give the aminonitrile cyclization precursor **10** in 95% yield as a 1:1 mixture of diastereomers.[14]

Precedent for the desired transannular cyclization was found in Jordis' synthesis of diazabicyclo-[2.2.1]heptanes.[15] Accordingly, the amine **10** was treated with DBU in dichloroethane at 60 °C in hopes of displacing the *trans*-oriented tosylate. Gratifyingly, the cyclization occurred, giving the desired bicycle **11** in 99% yield as a separable 2:1 mixture of diastereomers. Basic hydrolysis of the mixture of diastereomeric nitriles, followed by treatment with acidic methanol, gave the corresponding methyl ester intermediate. Subsequent removal of the *N*-benzyl group by catalytic hydrogenation gave the secondary amine **12** in 78% yield as a 2:1 mixture of diastereomers.

Treatment of amine **12** with 3-trifluoromethyl-4-cyanophenylisocyanate[16] in the presence of 4 Å molecular sieves at 23 °C for 4 h gave the corresponding urea intermediate, which was only isolated in the initial experiments. Once urea formation was complete, DBU was added followed by heating to 60 °C to promote cyclization of the urea onto the methyl ester. After acidic workup, the diastereomeric azahydantoins **13** (*endo*) and **14** (*exo*) were produced in a separable 2:1 ratio, respectively (95% yield). When the reaction was simply concentrated in vacuo avoiding the acidic workup, and loaded directly onto silica for purification, only the *exo*-isomer was formed in 87% yield. This result suggests that the *exo*-isomer **14** is the thermodynamically more stable, and predominates when the reaction is concentrated without neutralizing the organic base. The *endo*/*exo* ratio did not depend on which diastereomer of the cyclization precursor **12** or which arylisocyanate was used in the reaction.

When the *endo*-isomer was desired, the 2:1 *endo*/*exo* ratio of diastereomers could be improved by enolization




**Scheme 2.** Reagents and conditions: (a) LDA, THF, −78 °C, then aq satd $NH_4Cl$, 98%, 6:1 *endo*/*exo*; (b) LDA, THF, −78 °C, then MeI, 92%, >20:1 *endo*/*exo*.

of the mixture with LDA, followed by quenching with a proton source (Scheme 2). In this way, the 2:1 ratio is transformed to 6:1 ratio favoring the *endo*-isomer in 96% yield. When the enolate is instead quenched with methyl iodide, the methylated analog **19** is produced in a >20:1 ratio by alkylation from the less hindered β-face.

The opposite antipode of this series (**21** and **22**) was made by starting from *trans*-hydroxy-L-proline utilizing the same synthetic sequence. A small number of examples were made to assess, which enantiomer had better potency in vitro.

To evaluate the activity of this new series, we investigated the ability of compounds to bind to ($K_i$) and functionally antagonize ($IC_{50}$) the WT AR found in the MDA-453 cell line as well as antagonize the MT AR (T877A) found in the LNCap cell line.[6] The Boc-protected analogs incorporating the 4-cyano-3-trifluoromethyl aniline, 4-nitronaphthylamine, and 4-cyanonaphthylamine (Table 1) were tested first in hopes of narrowing the series to one diastereomer. The *endo*-isomers **13** and **15** demonstrated increased binding ($K_i$) to the WT AR compared to the corresponding *exo*-isomers. In the case of compounds **17** and **18**, there was minimal difference in terms of binding. Overall, the $K_i$

**Table 1.** Biological data for initial azahydantoin analogs

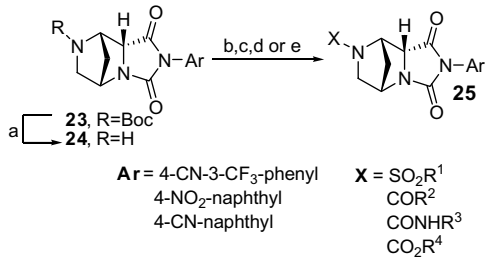
| Compound no. | $K_i$[a](μM) | MDA-453 $IC_{50}$[b] (μM) | LNCaP $IC_{50}$[b] (μM) |
|---|---|---|---|
| **13** | 0.040 | 0.774 | 4.38 |
| **14** | 0.189 | 0.644 | >5.00 |
| **21** | >5.00 | >5.00 | >5.00 |
| **15** | 0.062 | 3.32 | 4.43 |
| **16** | 1.03 | 1.33 | 5.86 |
| **22** | 0.237 | 0.252 | 2.39 |
| **17** | 0.248 | 2.54 | 4.97 |
| **18** | 0.197 | >5.00 | 3.51 |
| **19** | 1.40 | 0.78 | 10.4 |
| **4** | 0.001 | 1.30 | >5.00 |
| **3** | 0.005 | 0.012 | >5.00 |
| **1** | 0.065 | 0.17 | 0.40 |

[a] Binding determined through direct displacement of ligand with [$^3$H]-DHT in the MDA-453 cell line ($K_i$).[5]

[b] Functional antagonist activity determined through a transiently transfected reporter system utilizing a secreted alkaline phosphatase (SEAP) reporter construct and a PSA AR promoter domain.[5]

values for these compounds compared very well to bicalutamide ($K_i$ = 65 nM). In terms of functional activity, it was less clear which diastereomer was more potent overall. The *exo*-isomers **14** and **16** were slightly more potent than the *endo*-isomers but neither compared well to bicalutamide. We chose to focus our efforts on the *endo*-isomers because the initial examples demonstrated tighter binding to the AR and past results with the hydantoin series suggested that tight binding was essential for potent functional activity.

Compounds **21** and **22** were tested to determine which antipode would have superior binding and functional activity. Compound **21** is completely devoid of any AR activity but, compound **22** does have potent binding to the AR and reasonable functional activity. Modeling was performed in an attempt to explain the significant difference in activity seen between compounds **21** and **22**. Docking of each compound into the AR model (not shown) demonstrated that compound **22** was better able to form the critical H-bond with N705 as compared to compound **21**. Based on initial SAR as well as the results of our modeling exercise, we chose to pursue additional analogs in this series from the antipode depicted in compound **5**. The methylated analog **19** showed a drop in binding and functional antagonist activity to the MT AR as compared to **13**. This result is consistent with the methylation of hydantoins such as **4** (data not shown).[6]

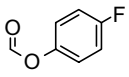
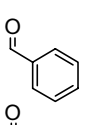
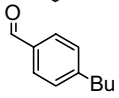



**Scheme 3.** Reagents and conditions:[17] (a) TFA, $CH_2Cl_2$, 22 °C, 99%; (b) $R^1SO_2Cl$, DIEA, $CH_2Cl_2$, 22 °C; (c) $R^2OCOCl$, DIEA, 4-DMAP, $CH_2Cl_2$, 22 °C; (d) $R^3COCl$, DIEA, 4-DMAP, $CH_2Cl_2$, 22 °C; (e) $R^4NCO$, DIEA, $CH_2Cl_2$, 22 °C.

These initial results were promising enough for us to embark on the preparation of a small solution-phase library of azahydantoins, focusing on the *endo*-isomer. We prepared a small library of 96 compounds where the aromatic domain and the N-substituent were varied (Scheme 3).[17] The library was made from the amine depicted by the general structure **24**, formed by removal of the *N*-Boc group in **23** by treatment with TFA. The amine was then converted into a series of sulfonamides, amides, carbamates, and ureas by conventional chemistry as shown in Scheme 3. When the amine **24** was alkylated rather than acylated, the resulting products were found to be unstable to storage and purification.

Table 2 contains the biological data for the library compounds with the highest affinity for WT AR. Compounds not presented either had $K_i$ > 1.5 μM or had MDA-453 $IC_{50}$ > 1.0 μM. The urea and sulfonamide series had no active members by these criteria and are not presented. The most active carbamate, **26**, contains the same two aromatic domains as bicalutamide, and

**Table 2.** Results from solution library synthesis with compound **25**

| Compound no. | X | Ar[a] | $K_i$[b] (μM) | MDA-453 $IC_{50}$[c] (μM) | LNCaP $IC_{50}$[c] (μM) |
|---|---|---|---|---|---|
| **26** |  | A | 0.018 | 0.200 | 3.92 |
| **27** | | B | 0.011 | 0.349 | 30.0 |
| **28** | | C | 0.041 | 0.588 | 1.87 |
| **29** |  | A | 0.124 | 0.300 | 2.60 |
| **30** | | B | 0.012 | >5.000 | 2.54 |
| **31** |  | A | 0.693 | 17.9 | 33.7 |
| **32** | | B | 0.073 | 0.060 | 4.75 |
| **33** | | C | 0.106 | 0.580 | 6.80 |

[a] A = 4-Cyano-3-trifluoromethylphenyl; B = 4-nitronaphthyl; C = 4-cyanonaphthyl.

[b] Binding determined through direct displacement of ligand with [$^3$H]-DHT in the MDA-453 cell line ($K_i$).[5]

[c] Functional antagonist activity determined through a transiently transfected reporter system utilizing a secreted alkaline phosphatase (SEAP) reporter construct and a PSA AR promoter domain.[5]

performed very well in binding and functional antagonism to WT AR. When the aniline portion was changed to the 4-nitronaphthyl or 4-cyanonaphthyl, **27**, and **28**, respectively, the binding improved but the functional activity decreased.

The benzamide analogs (**29**–**33**) also showed tight binding and moderate antagonist activity against the AR. Both examples contain electronically neutral aromatic portions, found to be essential for potent antagonist activity in the amide series. The benzamides **29** and **30** were very different in terms of binding and function, suggesting a different conformation of the LBD for each ligand. Compound **30** bound very tightly but had no antagonist function under our assay conditions. Whereas compound **29** demonstrated reduced binding but reasonable antagonist activity against MDA-453. The butyl-substituted benzamides **31**–**33** also showed promising activity. Of the library compounds, **32** showed the most potent antagonist activity against the WT cell line. However, against the MT cell line, it did not compare well to bicalutamide. Interestingly, alkyl amides and carbamates were not as potent as their aryl analogs, suggesting a common interaction in the LBD, which will be investigated further. Overall, compounds **26** and **32** were the most promising, and related compounds are under investigation.

In summary, we have developed a versatile, enantiospecific synthesis of a novel series of androgen receptor antagonists. These modified [2.2.1]-bicyclic hydantoins were proposed based on the biological activity of the parental ring-system and molecular modeling utilizing the crystal structure of DHT bound to the WT AR. Several of the new compounds demonstrated potent binding to the WT AR but fell short of the desired functional antagonist activity in the cell lines expressing the WT and especially the MT AR. However, several of these compounds compared favorably to bicalutamide. We are currently investigating modifications of this series based on the most active compounds and will report these results in due course.

## References and notes


1. Jemal, A.; Thomas, H.; Murray, T.; Thun, M. *CA Cancer J. Clin.* **2002**, *52*, 23.
2. (a) Blackledge, G. *Cancer* **1993**, *1*, 3830; (b) Maucher, A.; von Angerer, E. *J. Cancer Res. Clin. Onc.* **1993**, *119*, 669; (c) Sufrin, G.; Coffey, D. S. *Invest. Urol.* **1976**, *13*, 429; (d) Narayana, A. S.; Loening, S. A.; Culp, D. A. *Brit. J. Urol.* **1981**, *53*, 152.
3. (a) Huggins, C.; Stevens, R.; Hodges, C. V. *Arch. Surg.* **1947**, *43*, 209; (b) Westin, P.; Stattin, P.; Damber, J.-E.; Bergh, A. *Am. J. Pathol.* **1995**, *146*, 1368.
4. Salvati, M. E.; Balog, A.; Pickering, D. A.; Giese, S.; Fura, A.; Li, W.; Patel, R.; Hanson, R. L.; Mitt, T.; Roberge, J.; Corte, J. R.; Spergel, S. H.; Rampulla, R. A.; Misra, R.; Xiao, H.-Y. World Patent Application WO 2003062241, 2003.
5. New analogs were assayed for effect on AR function in MDA-453, a cell line expressing WT AR and LNCaP, a cell line expressing MT AR.
   Transactivation assays were done in whole cells and $IC_{50}$'s were calculated. Both cell lines were obtained from American Type Culture Collection (Rockville, MD). Binding constants ($K_i$) were determined in whole cells measuring displacement by tritiated DHT in the MDA-453 cell line. See Ref. 6 below for experimental details on assays.
6. Salvati, M.; Balog, A.; Shan, W.; Geise, S. U.S. Patent 885,798, 2003.
7. Sack, J. S.; Kish, K. F.; Wang, C.; Attar, R. M.; Kiefer, S. E.; An, Y.; Wu, G. Y.; Scheffler, J. E.; Salvati, M. E.; Krystek, S. R., Jr.; Weinmann, R.; Einspahr, H. M. *Proc. Natl. Acad. Sci.* **2001**, *98*, 4904.
8. Molecular modeling was performed using ICM software (Molsoft LLC, San Diego, CA).[9] The docking of the ligands into WT AR LBD was carried out using the ICM docking procedure which is a two step process. Initial docking of ligands were carried out using grid potential representation of the receptor and flexible ligand. Five grid potentials describe the shape, hydrophobicity, electrostatics, and hydrogen-bonding potential of the receptor. The conformations from the grid were then optimized with a full atom representation of the receptor and flexible ligand, by an ICM stochastic global optimization algorithm as implemented in version 2.7 the MOLSOFT ICM program[9].
9. (a) Molsoft ICM 2.7. *Program Manual*, **1998** (Molsoft, San Diego, CA), (b) Totrov, M.; Abagyan, R. *Proteins Suppl.* **1997**, *1*, 215–220.
10. (a) Poujol, N.; Wurtz, J.-M.; Tahiri, B.; Lumbroso, S.; Nicolas, J.-C.; Moras, D.; Sultan, C. *J. Biol. Chem.* **2000**, *275*, 24022; (b) Marhefka, C. A.; Moore, B. M.; Bishop, T. C.; Kirkovsky, L.; Mukherjee, A.; Dalton, J. T.; Miller, D. D. *J. Med. Chem.* **2001**, *44*, 1729.
11. Wurtz, J.; Bourguet, W.; Renaud, J.; Vivat, V.; Chambon, P.; Moras, D.; Gronemeyer, H. *Nat. Struct. Funct.* **1991**, *3*, 87.
12. The Aldrich Chemical Company, Inc.
13. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
14. Kim, Y. J.; Kido, M.; Bando, M.; Kitahara, T. *Tetrahedron* **1997**, *53*, 7501.
15. (a) Jordis, U.; Sauter, F.; Siddiqi, S. M.; Kuenburg, B.; Bhattacharaya, K. *Synthesis* **1990**, 925; (b) Rosen, T.; Chu, D. T. W.; Lico, I. M.; Fernandes, P. B.; Marxh, K.; Shen, L.; Cepa, V. G.; Pernet, A. G. *J. Med. Chem.* **1988**, *31*, 1598.
16. Isocyanate was generated by treatment of the corresponding aniline with phosgene and $NaHCO_3$ in $CH_2Cl_2$. The isocyanate was isolated and used in crude form directly.
17. The solution phase library was run in 96-well format with amine **24** (1.0 equiv) and an acylating reagent (3.0 equiv) in $CH_2Cl_2$ utilizing polymer-bound Hunig's base (5.0 equiv) for each series. Workup included tris-(2-aminoethyl)amine polystyrene (5.0 equiv) to scavenge excess acylating reagents. Eighty percent of the products generated did not require purification and isolated yields ranged from 35% to 99%.