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The discovery of a potent orally efficacious indole androgen receptor antagonist through in vivo screening

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Abstract—A series of novel 2-(1H-indol-2-yl)-propan-2-ols have been designed, synthesized, and screened for their ability to inhibit testosterone-induced prostate weight increases in immature rats. Through the use of this paradigm, we were able to identify compounds that exhibited in vivo potency equal to that of the marketed antiandrogen Casodex® when orally administered.

The androgen receptor[1] (AR) belongs to a subclass of the nuclear hormone receptor superfamily known as the nuclear steroid hormone receptors. The principal circulating endogenous AR ligand testosterone (T) and its more potent tissue metabolite 5α-dihydrotestosterone (DHT) are the compounds[2] responsible for both the development of male sex characteristics and the modulation of androgen receptor mediated myotropic events. The stimulatory effects of T and DHT have been identified as key facilitators of proliferative disease states such as benign prostatic hyperplasia (BPH) and prostate carcinoma (PC). Side effects attributed to the non-specific effects of steroidal antiandrogens[3] prompted research into non-steroidal scaffolds as a means of mitigating the potential for crosstalk within the endocrine system. The first compound to be commercialized from these efforts was the anilide flutamide (**1**, Eulexin®), the active metabolite of which, hydroxy anilide **2**, was demonstrated to have strong androgen receptor binding affinity (Fig. 1).

Subsequent work in the area leading to marketed products involved two major thrusts: bioisosteric replacement of the hydroxy anilide culminating in the launch of nilutamide (**3**, Nilandron®) and elaboration of one of the methyl groups leading to the discovery of bicalutamide (**4**, Casodex®). Of these second generation

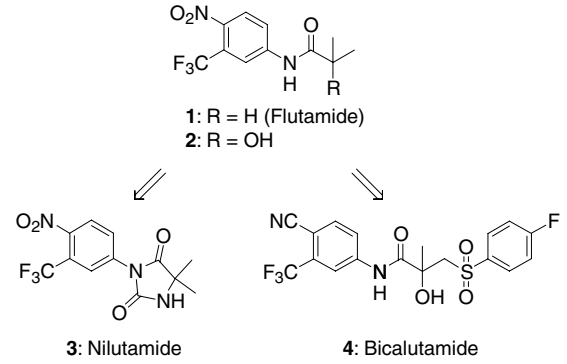

Figure 1. Currently marketed non-steroidal antiandrogens.

molecules, the latter has become the gold standard in antiandrogen therapy. Research in the field has continued with a number of interesting leads[4] but to date no third generation non-steroidal antiandrogens have made it to market.

As part of our interest[5] in the area, we prepared a series of indole carbinols (**5**) to investigate their potential as bioisosteric[6] replacements for the hydroxy amide of bicalutamide (Fig. 2). During our SAR studies on these structures we found that intermediate **6a** was active not only in our in vitro binding assay but also in vivo when dosed orally. Noting its structural similarity to **2**, we began to explore the SAR of these lower molecular weight compounds. As an alternative to the in vitro screening we conducted on the other series, we used a

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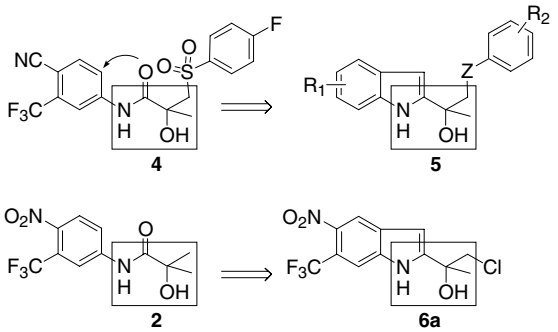
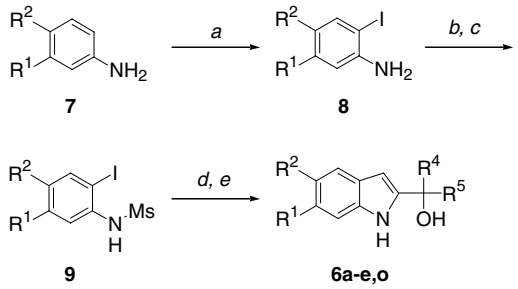


Figure 2. Bioisosteric replacement of the amide moiety in **2**.



Scheme 1. Synthesis of the indole carbinols. Reagents and conditions: (a) NIS, pTsOH (cat.), MeOH–THF, rt; (b) 5 equiv MsCl/pyridine, heat; (c) NaOH/MeOH–H_2O; (d) HCCC(OH)R_4R_5, 10% CuI, 5% $Pd(PPh_3)_2Cl_2$, Et_3N/THF rt; (e) NaOMe/MeOH.

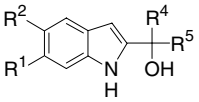
modified Hersherberger assay as an in vivo screening tool to evaluate our analogs. We found that in this particular program the in vivo screening paradigm provided a substantial time savings because it did not require in vitro binding assays, functional assays, and in vivo ADME screening before moving to an oral efficacy model. This model required only about 40 mg of material and delivered our screening results in less than a week. In our experience, this ability to screen compounds for oral efficacy offset the substantially more complex data analysis.

In our hands a five-day Hershberger assay[7] provided the best balance between turnaround time, compound requirements, and assay resolution. We found that the measurement of prostate rather than the seminal vesicle weights provided more accurate data due to the highly variable water retention of the latter tissue. When using such a screening protocol, it is important to bear in mind that the results are a combination of a test compound's ADME properties and intrinsic efficacy. As a result, this method of screening compounds is most useful when working in a series that is analogous to a compound of known molecular mechanism or the molecular mechanism of a series has been established. To mitigate differences in the test compounds' rate of dissolution, we selected 30% hydroxy propyl β-cyclodextrin (HPBCD) as our oral dosing vehicle as it dissolved all compounds in the series.

We initiated our efforts by examining the SAR of the indole 5- and 6-positions as well as substitution of the pendant carbinol. To prepare these analogs, we iodinated[8,9] the aniline ring using acid catalyzed reaction with *N*-iodosuccinimide to afford an iodoaniline **8** (Scheme 1). Conversion to the iodo mesylamide **9** was achieved in a one-pot two-step fashion by reacting **8** with an excess of mesyl chloride in hot pyridine followed by mono demesylation with aqueous hydroxide. Compound **9** was converted to the target structures by Sonogashira coupling-cyclization[10] with the appropriate propargyl alcohol followed by N-deprotection.

Simplifying the initial lead by replacement of the chlorine atom with hydrogen (**6b**) did not improve the efficacy and removal of the chloromethyl group entirely (**6c**) abolished activity (Table 1). Replacement of the chlorine atom with the isosteric methyl group (**6d**), a thiomethyl

Table 1. In vivo screening results for compounds **6a**–**k**



Compound	R^1	R^2	R^4	R^5	% Pros Wt. Redn[a]
6a	CF_3	NO_2	CH_2Cl	CH_3	38
6b	CF_3	NO_2	CH_2H	CH_3	32
6c	CF_3	NO_2	H	CH_3	na
6d	CF_3	NO_2	CH_2CH_3	CH_3	63
6e	CF_3	NO_2	CH_2SMe	CH_3	66
6f	CF_3	NO_2	CH_2OH	CH_3	58
6g	CF_3	NO_2	O	O	na
6h	CF_3	NO_2	CF_3	CH_3	78
6i	CF_3	CN	CF_3	CH_3	82
(+)-**6i**	CF_3	CN	CF_3	CH_3	80
(−)-**6i**	CF_3	CN	CF_3	CH_3	76
6j	CF_3	Cl	CF_3	CH_3	30
6k	H	CN	CF_3	CH_3	55
4	—	—	—	—	75

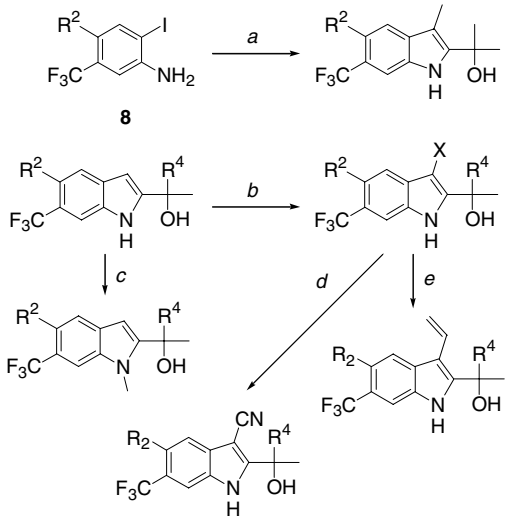
[a] Values are means of three animals, adjusted for the body weight of each animal; standard deviation is given in parentheses (na, not active).

group (**6e**) and, surprisingly, a hydroxyl moiety (**6f**) all increased the efficacy relative to the parent structure. Further oxidation of **6f** to the carboxylic acid (**6g**) abolished activity while perfluorination of one methyl group in **6b** led to a compound (**6h**) with as good efficacy as **4** at the screening dose. The nitro group could be replaced with a nitrile (**6i**) without loss of potency but replacement with a chlorine (**6j**) was not as well tolerated. Likewise removal of the indole 6-trifluoromethyl group from **6i** resulted in erosion of the potency (**6k**). After separation of the enantiomers of **6i** by chiral HPLC (ChiralPak AD column), we noted that both compounds reached the same level of efficacy as **4** at the testing dose. This is in contrast to the results for optically resolved **4** in which one enantiomer was reported to be active while the other is essentially inactive. Finally, it had been reported in the literature[16] that replacement of one of the methyl groups in compound **2** with a trifluoromethyl moiety led to partial agonism in vivo. Given the structural similarity between the indole carbinols and **2**, we screened compounds **6h**–**k** using an agonist format

Hershberger assay by dosing the castrated rats in the absence of testosterone propionate. In contrast to the reported results for fluorinated analogs of **2**, we found no agonist activity in our group of screened compounds.

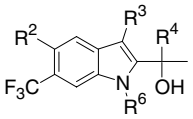
We next turned our attention to the indole 1- and 3-positions. Installation of a 3-methyl group was achieved starting from iodoanilines **8** by use of Larock's coupling-annulation protocol[11] (Scheme 2). N-Methylation of the indoles was achieved by treatment with a slight excess of sodium hydride in DMF[12] followed by the addition of methyl iodide. Halogenation of the 3-position could be accomplished by treating the unsubstituted indoles **6** with the corresponding *N*-halosuccinimide in the presence of sodium methoxide in methanol.[13] Treatment of 3-bromoindoles with CuCN[14] in heated DMF delivered the analogous 3-cyano compound. Palladium catalyzed coupling[15] of 3-iodo structures with triethoxy vinyl silane in the presence of TBAF produced 3-vinyl indoles.

Installation of a chlorine atom at the 3-position of **6b** increased efficacy dramatically (**6l**) but increasing the atom size to bromine (**6m**) and iodine (**6n**) caused moderate erosion in potency (Table 2). For the corresponding nitrile 3-iodo (**6s**) and chloro (**6u**) compounds then trend seemed to be reversed. Replacement of the 3-chloro moiety in **6l** with the isosteric methyl group (**6o**) led to a substantial loss in potency, while the corresponding change in **6u** produced an equipotent compound (**6r**). Installation of a nitrile (**6p**) at the indole C-3 position of **6b** improved potency somewhat while introduction of a vinyl moiety (**6q**) abolished activity. Finally, introduction of an iodine to the three position of **6i** produced an equipotent compound (**6v**) while methylation of the indole nitrogen (**6w**) afforded a compound that was toxic, though potent. As a result, indole nitrogen alkylation was not further pursued.



Scheme 2. Elaboration of the indole 1- and 3-positions. Reactions and conditions: (a) 5% $Pd(OAc)_2$, 10% PPh_3, $MeCCC(OH)Me_2$, KOAc, LiCl/DMF, heat; (b) NXS, NaOMe/MeOH, rt; (c) NaH/DMF, then MeI; (d) X = Br: CuCN/DMF, heat; (e) X = I: $(CH_2{=}CH)Si(OEt)_3$, $Pd_2(dba)_3$ (cat.), TBAF, DMF, heat.

Table 2. In vivo screening results for compounds **6l–w**



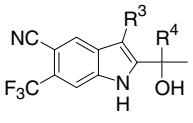
Compound	R^2	R^3	R^4	R^6	% Pros Wt. Redn[a]
6b	NO_2	H	CH_3	H	32
6l	NO_2	Cl	CH_3	H	81
6m	NO_2	Br	CH_3	H	71
6n	NO_2	I	CH_3	H	59
6o	NO_2	CH_3	CH_3	H	37
6p	NO_2	CN	CH_3	H	56
6q	NO_2	$CHCH_2$	CH_3	H	na
6r	CN	CH_3	CH_3	H	55
6s	CN	I	CH_3	H	81
6t	Cl	I	CH_3	H	na
6u	CN	Cl	CF_3	H	66
6v	CN	I	CF_3	H	79
6w	CN	H	CF_3	CH_3	90
4	—	—	—	—	75

[a] Values are means of four animals, body weight adjusted (na, not active).

With the screening results in hand we brought the SAR of the 5-cyano-6-trifluoromethyl indole substitution pattern into clearer focus by generating ID_{50} data for a select group of compounds (Table 3). From this exercise we were able to determine that the screening provided reasonable guidance for selecting potent compounds. We also determined the ID_{50} of each enantiomer of our lead compound **6** (Fig. 3). From examining the curves, it is apparent that both compounds had a potency similar in to **4** (Casodex®) with (+)-**6i** the more potent of the two.

In summary, through an in vivo screening protocol we identified the indole carbinol as a viable bioisosteric replacement of the amide moiety in non-steroidal anilide antiandrogens. Through systematic modification of this scaffold, we have identified a compound with higher potency in vivo when dosed orally in an immature rat model. These data provide an impetus for further inves-

Table 3. Single dose and ID_{50} comparison



Compound	R^3	R^4	ID_{50} (mpd)	% Pros Wt. Redn[a]
6x	Cl	CH_3	>3.00	nt
6y	Br	CH_3	0.80	nt
6t	I	CH_3	1.10	81
6s	CH_3	CH_3	>3.00	55
6j	Cl	CF_3	6.80	66
6i	H	CF_3	nt	82
(+)-**6i**	H	CF_3	0.13	80
(−)-**6i**	H	CF_3	0.43	76
4	—	—	0.29	75

[a] Values are means of four animals, body weight adjusted (nt, not tested).

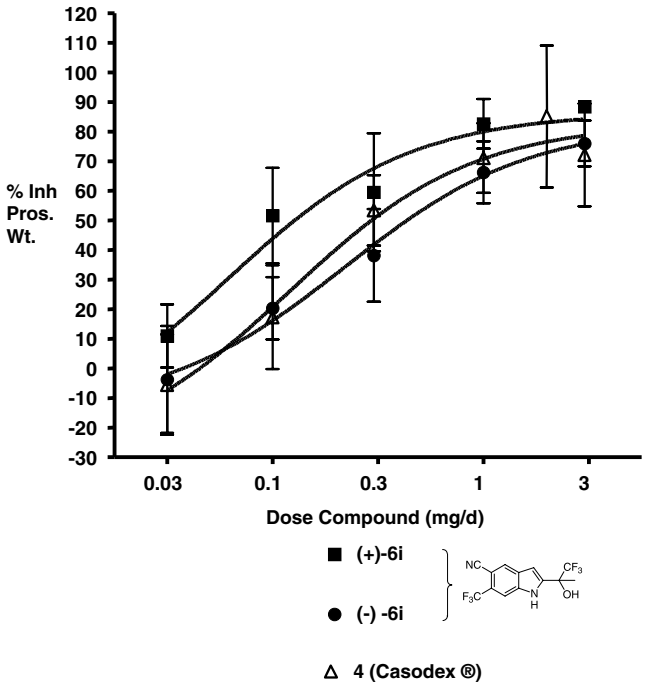


Figure 3. Dose–response curves of prostate weight reduction for bicalutamide, (+)-**6i**, and (−)-**6i** in immature rats.

tigation of the indole carbinol nucleus for the purpose of uncovering novel antiandrogens. This approach also highlights the utility of the Hershberger assay as a means of rapidly developing in vivo androgen receptor SAR.

Acknowledgment

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References and notes

1. (a) Wilson, E. M. *Pure Appl. Chem.* **2003**, *75*, 1685; (b) Gelmann, E. P. *J. Clin. Oncol.* **2002**, *20*, 3001.
2. Gao, W.; Bohl, C. E.; Dalton, J. T. *Chem. Rev.* **2005**, *105*, 3352.
3. Bruggemeier, R. W.. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; John Wiley & Sons: NY, 1996; Vol. 3, pp 445–510.
4. (a) Mohler, M. L.; Nair, V. A.; Hwang, D. J.; Rakov, I. M.; Patil, R.; Miller, D. D. *Exp. Opin. Ther. Patents* **2005**, *15*, 1565; (b) Chen, J.; Kim, J.; Dalton, J. T. *Mol. Interventions* **2005**, *5*, 173.
5. (a) Lanter, J. C.; Sui, Z.; Fiordeliso, J. J.; Jiang, W.; Zhang, X. U.S. Pat. Appl. Publ. 200525074, 2005; (b) Lanter, J. C.; Sui, Z.; Fiordeliso, J. J.; Jiang, W.; Zhang, X. U.S. Pat. Appl. Publ. 2005250740, 2005; (c) Lanter, J. C.; Sui, Z.; Fiordeliso, J. J.; Jiang, W.; Zhang, X. U.S. Pat. Appl. Publ. 2005245485, 2005; (d) Lanter, J. C.; Sui, Z.; Fiordeliso, J. J. U.S. Patent 6,858,621, 2005.
6. (a) Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23; (b) Kier, L. B.; Hall, L. H. *Chem. Biodivers.* **2004**, *1*, 138; (c) Wermuth, C. G. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed., 2nd ed.; Academic Press: San Diego, CA, 2003; p 189; (d) Patani, G. A.; LaVoie, E. *J. Chem. Rev.* **1996**, *96*, 3147.
7. A typical procedure: A modified Hershberger assay (Hershberger, L. G.; Shipley, E. G.; Meyer, R. K.; Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. *Proc. Soc. Exp. Biol. Med.* **1953** *83*, 175) was performed to assess androgen antagonist activity in orchidectomized rats. Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were castrated at three weeks old. They were then dosed once daily for five days with test compound (oral dosing by gavage needle; vehicle: 30% (w/v) hydroxypropyl-β-D-cyclodextrin [Cargil Inc., Cedar Rapids, IA]) in the presence of 0.1 mg/day (approximately 1.3 mg/kg) testosterone propionate (subcutaneous dosing; vehicle: sesame oil). At necropsy, ventral prostates and seminal vesicles were removed and their wet weights were measured. Tissue weights were divided by whole body weight. Data were expressed as percentage of inhibition relative to testosterone alone (0% inhibition) and cyclodextrin vehicle alone (100% inhibition).
8. Gladiali, S.; Pinna, L. *Tetrahedron: Asymmetry* **1991**, *2*, 623.
9. Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271.
10. (a) Campbell, I. B. In *Organocopper Reagents*; Taylor, R. J. K., Ed.; IRL Press: Oxford, UK, 1994; pp 217–235; (b) Sakamoto, Takao; Kondo, Yoshinori; Iwashita, Shigeki; Nagano, Tatsuo; Yamanaka, Hiroshi. Pharm. Inst., Tohoku Univ., Sendai, Japan. *Chem. Pharm. Bull.* **1988**, *36*, 1305.
11. Larock, R. C.; Yum, E. K.; Refvik, M. D. *J. Org. Chem.* **1998**, *63*, 7652.
12. Ho, B.-T.; Li, K.-C.; Walker, K. E.; Tansey, L. W.; Kralik, P. M.; McIsaac, W. M. *J. Pharm. Sci.* **1970**, *59*, 1445.
13. Walsh, D. A.; Moran, H. W.; Shamblee, D. A.; Uwaydah, I. M.; Welstead, W. J., Jr.; Sancilio, L. F.; Dannenburg, W. N. *J. Med. Chem.* **1984**, *27*, 1379.
14. Das, B. P.; Boykin, D. W. *J. Med. Chem.* **1977**, *20*, 531.
15. Hosomi, A.; Kohra, S.; Tominaga, Y. *Chem. Pharm. Bull.* **1988**, *36*, 4622.
16. Wakeling, A. E.; Furr, B. J. A.; Glen, A. T.; Hughes, L. R. *J. Steroid Biochem.* **1981**, *15*, 355.