



Contents lists available at ScienceDirect

# Bioorganic & Medicinal Chemistry Letters

journal homepage: [www.elsevier.com/locate/bmcl](http://www.elsevier.com/locate/bmcl)

## Novel selective anti-androgens with a diphenylpentane skeleton

Keisuke Maruyama, Tomomi Noguchi-Yachide, Kazuyuki Sugita, Yuichi Hashimoto, Minoru Ishikawa\*

Institute of Molecular &amp; Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

### ARTICLE INFO

#### Article history:

Received 9 August 2010

Revised 30 August 2010

Accepted 2 September 2010

Available online 9 September 2010

#### Keywords:

Anti-androgen

Steroid surrogate

Diphenylpentane

### ABSTRACT

We have proposed a multi-template approach for drug development, focusing on similar fold structures of proteins, and have effectively generated lead compounds for several drug targets. Modification of these polypharmacological lead compounds is then needed to generate target-selective compounds. In the work presented here, we aimed at separation of the anti-androgen activity and vitamin D activity of previously identified diphenylpentane lead compounds. Based on the determined X-ray crystal structures of androgen receptor and vitamin D receptor, bulky substituents were introduced at the *t*-butyl group in the lead compounds **2** and **3**. As a result of this structural development, we obtained **16c**, which exhibits more potent anti-androgen activity ( $IC_{50}$ : 0.13  $\mu$ M) than clinically used anti-androgen bicalutamide ( $IC_{50}$ : 0.67  $\mu$ M) with 30-fold selectivity over vitamin D activity. This result indicates that lead compounds obtained via the multi-template approach can indeed be structurally modified to generate target-selective compounds.

© 2010 Elsevier Ltd. All rights reserved.

Nuclear receptors (NRs) are ligand-dependent transcription factors which regulate the expression of responsive genes and thereby affect diverse processes, including cell growth, development, differentiation, and metabolism.<sup>1</sup> Based on the elucidated human genome sequence, 48 NRs are thought to exist in humans.<sup>1</sup> Examples of NRs and their ligands include androgen receptors (ARs)/testosterone, vitamin D receptors (VDRs)/1,25 $\alpha$ -dihydroxyvitamin D<sub>3</sub>, farnesoid receptors (FXRs)/bile acid, and estrogen receptors (ERs)/estradiol.

Steroid hormones act via NRs. For example, AR is a receptor of androgens, typically testosterone and/or its active form, 5 $\alpha$ -dihydrotestosterone, which are endogenous ligands essential for the development and maintenance of the male reproductive system and secondary male sex characteristics. Androgens play diverse physiological and pathophysiological roles in both males and females. Among the pathophysiological effects elicited by androgens, a role as endogenous tumor promoters, especially for prostate tumor, is well known. This action is considered to be mediated by androgen-binding to AR. Thus, AR antagonists are used for treatment of androgen-dependent tumors, especially prostate tumors. Steroidal anti-androgens, however, also have progestational properties with central actions on the pituitary gland. Steroidal anti-androgens significantly lower the levels of both serum testosterone and luteinizing hormone, which might reduce libido and sexual potency.<sup>2</sup>

To reduce side effects of steroidal NR ligands, non-steroidal NR ligands have been developed. In the case of AR, non-steroidal anti-

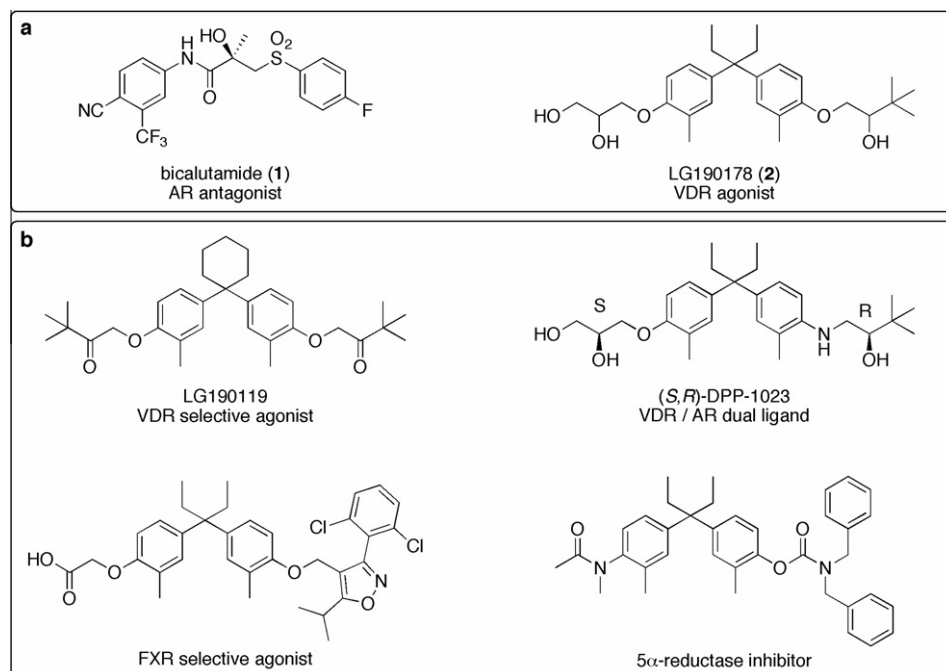
androgens are more favorable for clinical applications because of the lack of cross-reactivity with other NRs and improved oral bioavailability. Of this structural class of anti-androgens, clinically used bicalutamide (**1**) is the most potent and best tolerated (Fig. 1a).<sup>3,4</sup> Bicalutamide (**1**) has little effect on serum testosterone and luteinizing hormone levels, at least in an animal model.<sup>2</sup>

Non-secosteroidal VDR agonists such as LG190178 (**2**)<sup>5,6</sup> with greater stability, easier synthesis and reduced calcium-raising effects have also been developed (Fig. 1a). Some of them do not bind to serum vitamin D binding protein,<sup>5</sup> a property that has been correlated with lower calcemic potential in vivo.<sup>7</sup> However, these non-steroidal NR ligands were discovered individually, and no common template for steroid surrogates is known.

The multi-template approach is based on the fact that the number of three-dimensional spatial structures (fold structures) of human proteins is much smaller (more than 50 times smaller) than the number of human proteins (50,000–70,000).<sup>8–12</sup> Therefore, ignoring physical/chemical interactions, a template/scaffold structure which is spatially complementary to onefold structure might serve as a multi-template for structural development of ligands that would interact specifically with more than 50 different human proteins. In other words, the structures of ligands that bind to one member of the fold structures may be used for the development of novel lead compounds for other members of the same fold structure group. Obtained lead compounds probably possess polypharmacologic character when the target proteins share fold structures with other proteins. Therefore, in order to extend the multi-template approach, structural modification of polypharmacological lead compounds, aimed at optimizing target-selectivity, is required.

\* Corresponding author.

E-mail address: [m-ishikawa@iam.u-tokyo.ac.jp](mailto:m-ishikawa@iam.u-tokyo.ac.jp) (M. Ishikawa).



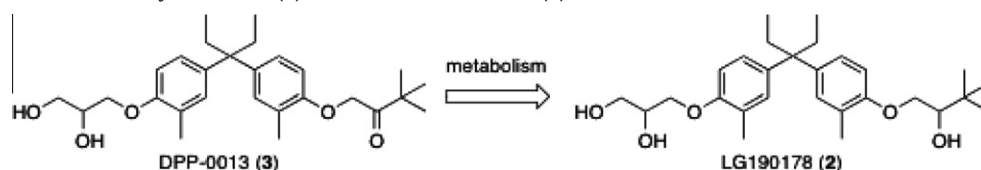
**Figure 1.** (a) Chemical structures of representative non-steroidal NR ligands. (b) Diphenylpentane-based ligands of NR and steroid-related enzyme generated by means of the multi-template approach.

One of the multi-templates that we have adopted is a surrogate for the steroid skeleton.<sup>12–15,11,16</sup> A skeleton that corresponds structurally to the steroid skeleton would be a potential multi-template for structural development of ligands for a number of proteins. For example, we have found that the non-steroidal VDR agonist **2** also possesses AR-antagonistic activity,<sup>12,13</sup> while LG190119 possesses VDR-selective agonistic activity.<sup>13</sup> We have also employed the diphenylpentane skeleton as a multi-template for creation of novel ligands of nuclear receptors and steroid-related enzymes, such as VDR/AR dual ligands (VDR agonists/AR antagonists),<sup>12,13</sup> FXR agonists,<sup>14</sup> and inhibitors of steroid metabolism-related enzymes, including 5 $\alpha$ -reductase<sup>15</sup> (Fig. 1b). These results indicate that the diphenylpentane skeleton can act as steroid surrogate. However, AR-selective ligands with the diphenylpentane skeleton have not been generated. It has been suggested that polypharmacological lead compounds generated by means of the multi-template approach might be difficult to optimize toward target-selective drug candidates, simply because the multiple target proteins share similar fold structures. Therefore, we focused here on separation of vitamin D and anti-androgen activities, and on the creation of selective anti-androgens. Here, we describe the design, synthesis and structure–activity relationship study of diphenylpentane derivatives, focusing on generation of selective anti-androgens.

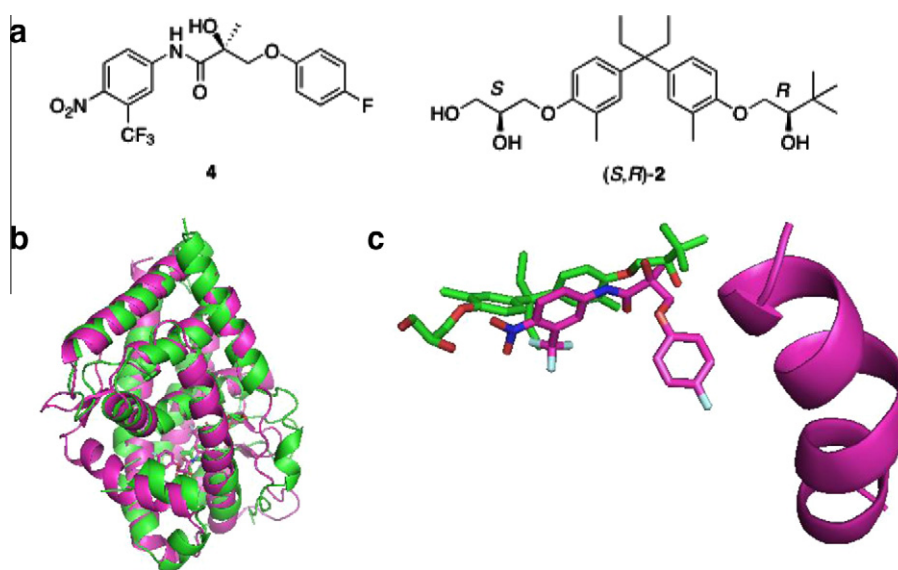
As a clue for the molecular design, we first reconsidered our previous findings regarding metabolism.<sup>13,17</sup> DPP-0013 (**3**) has been designated as a selective AR ligand, based on AR and VDR binding assay data (Table 1). However, **3** showed both anti-androgen activity and vitamin D activity in cell-based assay, that is, it inhibited testosterone-induced cell growth of the androgen-dependent cell line SC-3 (anti-androgen activity) and HL-60 cell differentiation-inducing activity (vitamin D activity). Further, a reductive metabolite of **3**, LG190178 (**2**), showed both anti-androgen activity and vitamin D activity. Thus, **3** is not a selective anti-androgen in a cellular system, apparently as a result of metabolism. Therefore, we designed compounds possessing a bulky group introduced at the *t*-butyl group of **3** in order to block metabolism of the carbonyl group.

Secondly, we utilized X-ray crystal structure data. X-Ray crystal structures of the complex of VDR with (S,R)-**2**,<sup>18</sup> and the complex of AR with a bicalutamide analog **4**<sup>19</sup> (Fig. 2a) have been reported. We compared their X-ray crystal structures and confirmed that AR and VDR possess very similar fold structures (Fig. 2b). These similar fold structures are considered to be the basis of not only effective lead generation based on the multi-template approach, but also the dual function of **2**. Next, we carefully analyzed the active conformation of both ligands. In general, a compound which binds with the ligand-binding pocket (LBD) but interferes with the folding of helix 12 (one of the LBD substructures) acts as an antagonist of the corresponding NR.<sup>20</sup> As shown in Figure 2c, the fluorophenyl group in **4** is located near helix 12 of AR, and is important for AR antagonism. On the other hand, VDR antagonism is thought not to be straightforward, because only a few series of secosteroidal VDR antagonists and no non-steroidal VDR antagonists<sup>21–27</sup> have been created, even though thousands of VDR ligands are known. Thus, we hypothesized that introduction of substituents into the *t*-butyl group in **2** would decrease VDR-agonistic activity and would not lead to VDR-antagonistic activity. We also speculated that the designed ketone series would retain selective AR-antagonistic activity even if the carbonyl group was metabolized to a hydroxyl group. From these two ideas, we designed novel compounds containing a bulky substituent at the *t*-butyl group of **2** and **3** with the aim of both increasing metabolic stability and reducing VDR affinity. We decided to synthesize (S)-isomers of the diol, because (S)-isomers of **2** exhibited stronger anti-androgen activity than the corresponding (R)-isomers (Table 1). On the other hand, there are no great distinction between the activity of (S,R)-**2** and the activity of (S,S)-**2** (Table 1). Therefore, we designed alcohol series as diastereomixtures of the hydroxyl group.

Right-hand fragments bearing a bulky moiety were synthesized as shown in Scheme 1. Nitrile anion was reacted with bromides **5a–d,f** to generate **6a–d,f**. Methyl lithium was reacted with nitriles **6a–d,f** to afford ketones **7a–d,f**. Alpha-bromination of ketones **7a–d,f** gave **8a–d,f**. Next, benzylpyrrolone **9g** was reacted with CbzCl in the presence of KHCO<sub>3</sub> to give **9h**. Epoxidation<sup>28</sup> of **9g–h** afforded racemic epoxides **10g–h**.

**Table 1**Anti-androgen activity and vitamin D activity of DPP-0013 (**3**) and its metabolite LG190178 (**2**)

Compound	AR		VDR		VDR/AR <sup>c</sup>
	Binding ( $K_i$ nM)	Antiandrogen activity <sup>a</sup> $IC_{50}$ (nM)	Binding ( $K_i$ nM)	Vitamin D activity <sup>b</sup> $EC_{50}$ (nM)	
( <i>R</i> )- <b>3</b>	1100	15	nt <sup>d</sup>	nt <sup>d</sup>	
( <i>S</i> )- <b>3</b>	1800	32	nt <sup>d</sup>	nt <sup>d</sup>	
Racemic- <b>3</b>	nt <sup>d</sup>	nt <sup>d</sup>	nd <sup>e</sup>	90	
( <i>R,R</i> )- <b>2</b> <sup>f</sup>	1000	14	320	26	1.9
( <i>R,S</i> )- <b>2</b> <sup>f</sup>	1100	68	740	120	1.8
( <i>S,R</i> )- <b>2</b> <sup>f</sup>	1000	3.0	12	8.6	2.9
( <i>S,S</i> )- <b>2</b> <sup>f</sup>	1100	13	11	23	1.8

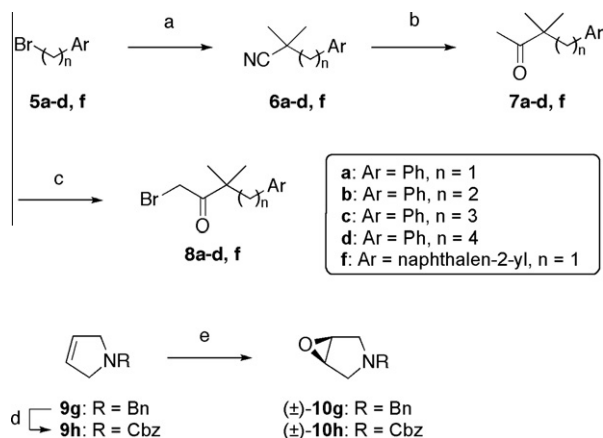
<sup>a</sup> Anti-androgen activity was measured in terms of testosterone-induced SC-3 proliferation-inhibitory activity.<sup>b</sup> Vitamin D activity was measured in terms of HL-60 differentiation-inducing activity.<sup>c</sup> Vitamin D activity/anti-androgen activity.<sup>d</sup> nt: not tested.<sup>e</sup> nd: not detected.<sup>f</sup> The first *S* or *R* indicates the stereochemistry of the diol, while the second indicates that of the other hydroxyl group.**Figure 2.** (a) Chemical structures of bicalutamide analog **4** and (*S,R*)-**2**. (b) Superposition of X-ray crystal structures of the complex of AR with **4** (magenta, PDB ID: 2AXA) and the complex of VDR with (*S,R*)-**2** (green, PDB ID: 2ZFX). (c) Overlay of active conformation of (*S,R*)-**2** (green stick) and **4** (magenta stick), and helix 12 of AR (magenta ribbon). The images were drawn with PYMOL.

The left-hand common intermediate **13** was synthesized as shown in Scheme 2. Bisphenol **11**<sup>12</sup> was reacted with (*S*)-glycidol in the presence of CsF<sup>29</sup> to give optically pure diol **12**. This diol **12** was protected with acetonide to obtain the left-hand fragment **13**. The right-hand fragments **8a–f** were coupled with optically pure phenol **13** to give **14a–f** (Scheme 2). 1-Adamantyl bromomethyl ketone (**8e**) is commercially available. Deprotection of the acetonide group afforded a series of desired ketones (*S*)-**15a–f**. Reduction of the carbonyl group of **15a–f** gave another series of desired alcohols **16a–f**. These alcohols were thought to be diastereomixtures from the reaction mechanism. However, they did not be observed as diastereomixtures by means of <sup>1</sup>H NMR.

Cyclic compounds **16g–h** were synthesized as shown in Scheme 3. Epoxides **10g–h** were coupled with phenol **13**,<sup>30</sup> followed by deprotection of the acetonide group to provide the desired **16g–h**.

Vitamin D activity was measured as human promyelocytic leukemia cell line (HL-60) differentiation-inducing activity, as has commonly been done.<sup>12,13,23,31–33</sup> Briefly, HL-60 cells can be induced to differentiate into monocytic cells by 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>. Differentiation of HL-60 cells was quantified in terms of morphology using nitroblue tetrazolium (NBT) reduction assay. HL-60 cell differentiation-inducing activity is believed to correlate well with the binding/activation of VDR by its ligands. Anti-androgen activity was evaluated in terms of inhibitory activity on testosterone-induced cell growth of the androgen-dependent mouse mammary carcinoma cell line SC-3.<sup>12,34–37</sup> SC-3 proliferation was measured by WST-1 assay. Under our assay conditions, the clinically used AR antagonist **1** exhibited an  $IC_{50}$  value of 0.67  $\mu$ M for inhibition of proliferation.

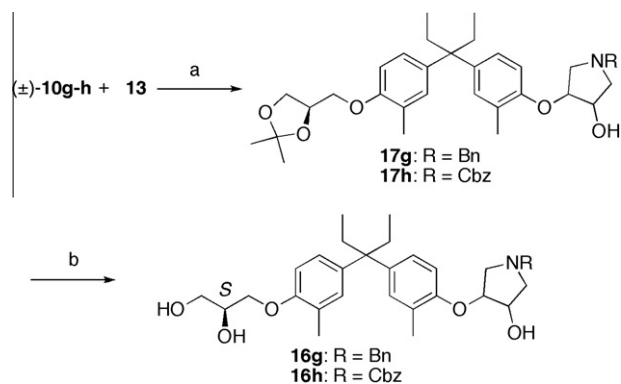
First, ketones **15a–d**, containing alkyl phenyl groups introduced at the *t*-butyl group of **2**, were evaluated (Table 2). Although all compounds tested showed decreased anti-androgen activity, the



**Scheme 1.** Reagents and conditions: (a) LDA, isobutyronitrile, THF, 0 °C to rt, 67–97%; (b) MeLi, THF, 0 °C to rt, 35–94%; (c) Br<sub>2</sub>, 1,4-dioxane, 0 °C to rt, quant.; (d) CbzCl, KHCO<sub>3</sub>, CHCl<sub>3</sub>, rt, 48%; (e) mCPBA, H<sub>2</sub>SO<sub>4</sub>, acetone, H<sub>2</sub>O, rt, 12–40%.

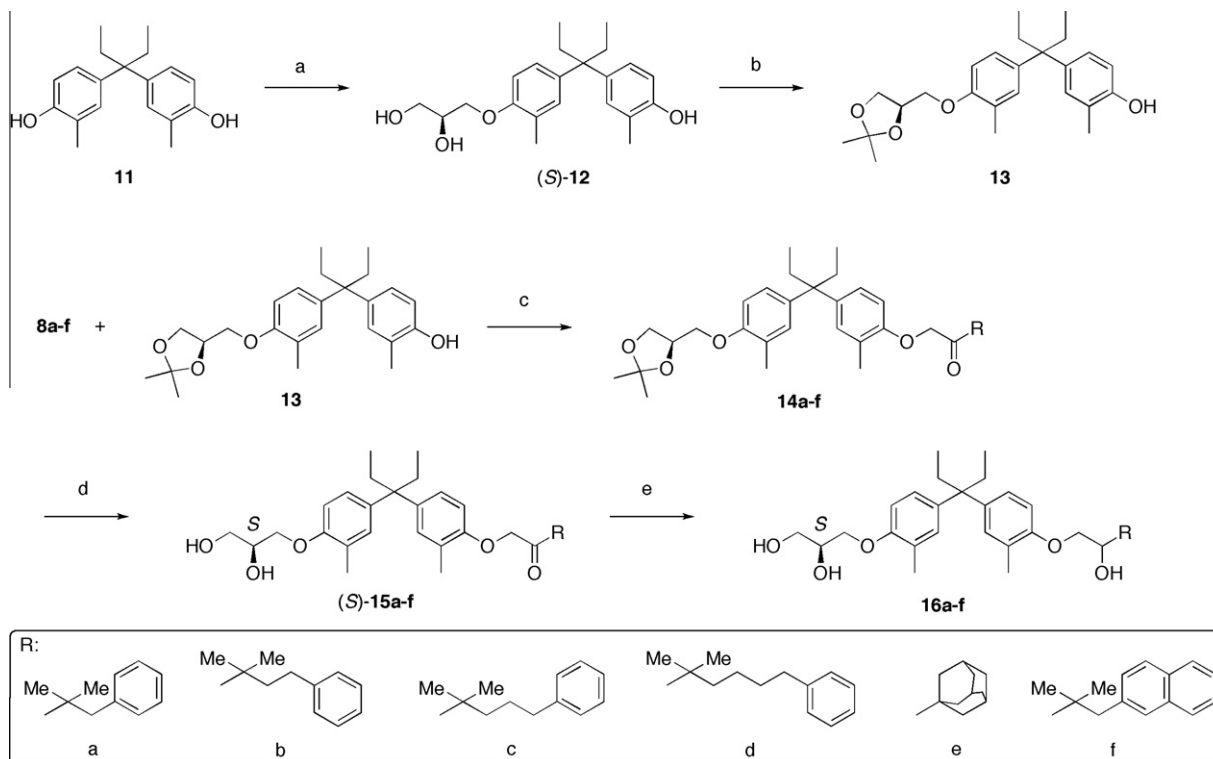
level of anti-androgen activity varied depending on the linker length: the order of anti-androgen potency was **15b** > **15c** > **15a** > **15d**. Among them, **15b** showed the strongest anti-androgen activity with an IC<sub>50</sub> value of 1.1 μM. In the case of vitamin D activity, **15a–d** all showed very weak activity (EC<sub>50</sub>: >30 μM), indicating that introduction of alkyl phenyl groups leads to reduced affinity for VDR, as expected. Concerning the selectivity of ketones **15**, **15b–c** showed improved selectivity for anti-androgen activity over vitamin D activity. Among them, **15b** is the most selective anti-androgen (21-fold selectivity). On the other hand, another bulky compound possessing the adamantyl group, **15e**, completely lacked both anti-androgen activity and vitamin D activity at the concentration tested.

In the case of alcohols **16a–e**, all compounds showed decreased anti-androgen activity. However, the anti-androgen activity varied depending on the linker length: the order of anti-androgen potency



**Scheme 3.** Reagents and conditions: (a) Cs<sub>2</sub>CO<sub>3</sub>, 18-crown-6, EtOH, reflux, 22–29%; (b) TsOH, MeOH, THF, rt, 78–80%.

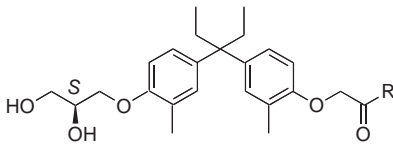
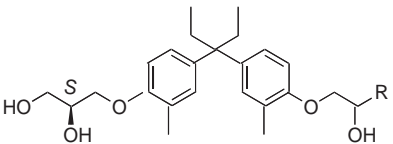
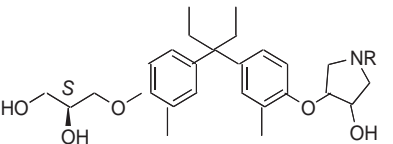
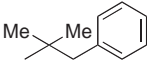
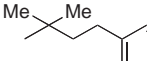


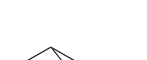

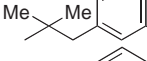
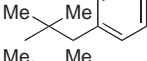
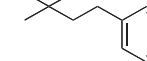
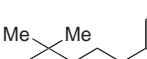
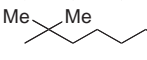

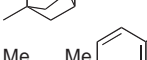

was **16c** > **16b** > **16a** > **16d**. Among these compounds, **16c**, possessing a propyl linker, showed the strongest anti-androgen activity with the IC<sub>50</sub> value of 0.13 μM, being more potent than bicalutamide (IC<sub>50</sub>: 0.67 μM). The butyl linker compound **16d** lacked anti-androgen activity. This result indicates that the butyl phenyl group in **16d** might be too bulky to be accommodated in the pocket around helix 12 in the AR. Concerning vitamin D activity, **16a–e** showed decreased vitamin D activity compared to **2**, as expected. The order of potency is the same as that of the anti-androgen activity, that is, **16c** > **16b** > **16a** > **16d**. However, this structural development changed the selectivity, also as expected. Specifically, **16a–c** showed better selectivity than **2**, and the propyl linker compound **16c** showed the best selectivity, that is, 28-fold selectivity for anti-androgen activity over vitamin D activity. However, adamantyl analog **16e** completely lacked anti-androgen activity, suggesting that bulky cyclic alkyl ring is unfavorable for the interaction with AR.



**Scheme 2.** Reagents and conditions: (a) (S)-glycidol, CsF, DMF, 85 °C, 47%; (b) acetone dimethyl acetal, CSA, MeCN, rt, 87%; (c) K<sub>2</sub>CO<sub>3</sub>, DMF, rt or 90 °C, 21–71%; (d) TsOH, MeOH, THF, rt, 30–69%; (e) NaBH<sub>4</sub>, MeOH, rt, 37–83%.

**Table 2**

Anti-androgen activity and vitamin D activity of synthesized compounds

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>(S)-15a-f</p> </div> <div style="text-align: center;">  <p>16a-f</p> </div> <div style="text-align: center;">  <p>16g,h</p> </div> </div>				
Compound	R	Anti-androgen activity <sup>a</sup> IC <sub>50</sub> (μM)	Vitamin D activity <sup>b</sup> EC <sub>50</sub> (μM)	VDR/AR <sup>c</sup>
15a		>10 <sup>d</sup>	21	<2.1
15b		1.1	23	21
15c		9.8	>30	>3.1
15d		>30	>30	—
15e		>30	>30	—
15f		>30	>30	—
16a		1.7	11	6.5
16b		0.88	7.1	8.1
16c		0.13	3.6	28
16d		>10 <sup>d</sup>	>30	—
16e		>10 <sup>d</sup>	22	<2.2
16f		>10 <sup>d</sup>	>30	—
16g		>10 <sup>d</sup>	>30	—
16h		>30	>30	—

<sup>a</sup> Anti-androgen activity was measured in terms of testosterone-induced SC-3 proliferation-inhibitory activity.<sup>b</sup> Vitamin D activity was measured in terms of HL-60 differentiation-inducing activity.<sup>c</sup> Vitamin D activity/anti-androgen activity.<sup>d</sup> Cytotoxicity at 30 μM.

Alcohols **16a–e** showed stronger anti-androgen activity and vitamin D activity than the corresponding ketones **15a–e**. This result indicates that the hydroxyl group in the right part is important for both anti-androgen activity and vitamin D activity. On the other hand, the vitamin D activity of ketone **15c** was more than eight times weaker than that of the corresponding alcohol **16c**, whereas **3** and its metabolite **2** possess similar vitamin D activity. Thus, introduction of the alkyl phenyl group into **3** might lead to increased metabolic stability.

The structure–activity relationships mentioned above, that is, the finding that the propyl linker **16c** is the best for anti-androgen activity, prompted us to restrict the space occupied by the terminal

phenyl group of **16c**, because the terminal phenyl group is expected to be important for the interaction with helix 12 in AR. Thus, ketone **15f** and alcohols **16f–h** were designed and synthesized. All these cyclic analogs completely lacked vitamin D activity. However, they also lacked anti-androgen activity. This result suggests that the flexibility of the propyl linker might be important for the interaction with AR, or the space occupied by the terminal phenyl group might be different from that occupied by the fluoro-phenyl group in bicalutamide.

Overall, the propyl linker compound **16c** exhibited the best profile, showing six times stronger anti-androgen activity than bicalutamide, and 28-fold selectivity for anti-androgen activity over



vitamin D activity. The androgen activity and antivitamin D activity of **16c** were further evaluated to confirm these results. Compound **16c** did not show growth-promoting activity on the androgen-dependent cell line SC-3 at 10  $\mu\text{M}$ , suggesting that it does not possess androgen activity. In addition, it did not inhibit differentiation of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$ -induced HL-60 cells at 30  $\mu\text{M}$ , suggesting that it is also not a VDR antagonist. Thus, **16c**<sup>38</sup> is considered to be a selective anti-androgen.

In summary, we aimed to separate the anti-androgen activity and vitamin D activity of the lead compounds **2** and **3** identified by means of the multi-template approach. Bulky substituents were introduced at the *t*-butyl group in **2** and **3** with the aim of (1) increasing the metabolic stability of **3** and (2) decreasing the affinity for VDR. This structural development afforded ketone **15c** with increased metabolic stability. In addition, alcohols **16a–c** showed improved selectivity for anti-androgen activity over vitamin D activity. Among them, **16c** possessed stronger anti-androgen activity ( $\text{IC}_{50}$ : 0.13  $\mu\text{M}$ ) than bicalutamide ( $\text{IC}_{50}$ : 0.67  $\mu\text{M}$ ), with about 30-fold selectivity for anti-androgen activity over vitamin D activity.

Our results demonstrate that target-selective compounds can be derived from multi-target lead compounds generated via the multi-template approach. We have also obtained LXR $\alpha$ -selective antagonists by structural development of a lead compound possessing LXRs dual antagonistic activity and  $\alpha$ -glucosidase-inhibitory activity.<sup>39</sup> Thus, we believe that the multi-template approach is a useful method for not only generating lead compounds, but also creating target-selective drug candidates.

## Acknowledgments

The work described in this Letter was partially supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Japan Society for the Promotion of Science.

## References and notes

- Chawla, A.; Pepa, J. J.; Evans, R. M.; Mangelsdorf, D. J. *Science* **2001**, 294, 1866.
- Guidelines on prostate cancer (European Association of Urology) (<<http://www.urotoday.com/prod/pdf/eau/prostatecancer.pdf>>).
- Schellhammer, P. *Expert Opin. Investig. Drugs* **1999**, 8, 849.
- Fradet, Y. *Exp. Rev. Anticancer Ther.* **2004**, 4, 37.
- Boehm, M. F.; Fitzgerald, P.; Zou, A.; Elgort, M. G.; Bischoff, E. D.; Mere, L.; Mais, D. E.; Bissonnette, R. P.; Heyman, R. A.; Nadzan, A. M.; Reichman, M.; Allegretto, E. A. *Chem. Biol.* **1999**, 6, 265.
- Swann, S. L.; Bergh, J.; Farach-Carson, M. C.; Ocasio, C. A.; Koh, J. T. *J. Am. Chem. Soc.* **2002**, 124, 13795.
- Dusso, A. S.; Negrea, L.; Gunawardhana, S.; Lopez-Hilker, S.; Finch, J.; Mori, T.; Nishii, Y.; Slatopolsky, E.; Brown, A. J. *Endocrinology* **1991**, 128, 1687.
- Koonin, E. V.; Wolf, Y. I.; Karev, G. P. *Nature* **2002**, 420, 218.
- Grishin, N. V. *J. Struct. Biol.* **2001**, 134, 167.
- Koch, M. A.; Wittenberg, L.-O.; Basu, S.; Jeyaraj, D. A.; Gourzoulidou, E.; Reinecke, K.; Odermatt, A.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, 101, 16721.
- Hosoda, S.; Matsuda, D.; Tomoda, H.; Hashimoto, Y. *Mini-Rev. Med. Chem.* **2009**, 9, 572.
- Hosoda, S.; Tanatani, A.; Wakabayashi, K.; Nakano, Y.; Miyachi, H.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2005**, 15, 4327.
- Hosoda, S.; Tanatani, A.; Wakabayashi, K.; Makishima, M.; Imai, K.; Miyachi, H.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem.* **2006**, 14, 5489.
- Kainuma, M.; Kasuga, J.; Hosoda, S.; Wakabayashi, K.; Tanatani, A.; Nagasawa, K.; Miyachi, H.; Makishima, M.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2006**, 16, 3213.
- Hosoda, S.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2007**, 17, 5414.
- Hashimoto, Y. *Arch. Pharm. Chem. Life. Sci.* **2008**, 341, 536.
- Hosoda, S. et al., unpublished data.
- Kakuda, S.; Okada, K.; Eguchi, H.; Takenouchi, K.; Hakamata, W.; Kurihara, M.; Takimoto-Kamimura, M. *Acta Crystallogr., Sect. F* **2008**, 64, 970.
- Bohl, C. E.; Miller, D. D.; Chen, J.; Bell, C. E.; Dalton, J. T. *J. Biol. Chem.* **2005**, 280, 37747.
- Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2005**, 13, 5080.
- Herdick, M.; Steinmeyer, A.; Carlberg, C. *Chem. Biol.* **2000**, 7, 885.
- Miura, D.; Manabe, K.; Ozono, K.; Saito, M.; Gao, Q.; Norman, A. W.; Ishizuka, S. *J. Biol. Chem.* **1999**, 274, 16392.
- Nakano, Y.; Kato, Y.; Imai, K.; Ochiai, E.; Namekawa, J.; Ishizuka, S.; Takenouchi, K.; Tanatani, A.; Hashimoto, Y.; Nagasawa, K. *J. Med. Chem.* **2006**, 49, 2398.
- Kato, Y.; Nakano, Y.; Sano, H.; Tanatani, A.; Kobayashi, H.; Shimazawa, R.; Koshino, H.; Hashimoto, Y.; Nagasawa, K. *Bioorg. Med. Chem. Lett.* **2004**, 14, 2579.
- Saito, N.; Matsunaga, T.; Saito, H.; Anzai, M.; Takenouchi, K.; Miura, D.; Namekawa, J.; Ishizuka, S.; Kittaka, A. *J. Med. Chem.* **2006**, 49, 7063.
- Nakabayashi, M.; Yamada, S.; Yoshimoto, N.; Tanaka, T.; Igarashi, M.; Ikura, T.; Ito, N.; Makishima, M.; Tokiwa, H.; Deluca, H. F.; Shimizu, M. *J. Med. Chem.* **2008**, 51, 5320.
- Inaba, Y.; Yoshimoto, N.; Sakamaki, Y.; Nakabayashi, M.; Ikura, T.; Tamamura, H.; Ito, N.; Shimizu, M.; Yamamoto, K. *J. Med. Chem.* **2009**, 52, 1438.
- Ji, H.; Stanton, B. Z.; Igarashi, J.; Li, H.; Martásek, P.; Roman, L. J.; Poulos, T. L.; Silverman, R. B. *J. Am. Chem. Soc.* **2008**, 130, 3900.
- Kitaori, K.; Furukawa, Y.; Yoshimoto, H.; Otera, J. *Tetrahedron* **1999**, 55, 14381.
- Holsworth, D. D.; Stier, M.; Wang, W.; Edmunds, J. J.; Li, T.; Maiti, S. N. *Synth. Commun.* **2004**, 34, 4421.
- Fujishima, T.; Konno, K.; Nakagawa, K.; Kurobe, M.; Okano, T.; Takayama, H. *Bioorg. Med. Chem.* **2000**, 8, 123.
- Imae, Y.; Manaka, A.; Yoshida, N.; Ishimi, Y.; Shinki, T.; Abe, E.; Suda, T.; Konno, K.; Takayama, H.; Yamada, S. *Biochim. Biophys. Acta* **1994**, 1213, 302.
- Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem.* **1988**, 31, 2839.
- Takahashi, H.; Ishioka, T.; Koiso, Y.; Sodeoka, M.; Hashimoto, Y. *Biol. Pharm. Bull.* **2000**, 23, 1387.
- Roehborn, C. G.; Zoppi, S.; Gruber, J. A.; Wilson, C. M.; McPhaul, M. J. *Mol. Cell. Endocrinol.* **1992**, 84, 1.
- Sawada, T.; Hashimoto, Y.; Li, Y.; Kobayashi, H.; Iwasaki, S. *Biochem. Biophys. Res. Commun.* **1991**, 178, 558.
- Sawada, T.; Kobayashi, H.; Hashimoto, Y.; Iwasaki, S. *Biochem. Pharmacol.* **1993**, 45, 1387.
- <sup>1</sup>H NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.29–7.27 (m, 2H), 7.19–7.16 (m, 3H), 6.96–6.93 (m, 2H), 6.90–6.89 (m, 2H), 6.69 (d,  $J$  = 8.5 Hz, 1H), 6.66 (d,  $J$  = 8.5 Hz, 1H), 4.10 (br s, 1H), 4.04–4.03 (m, 3H), 3.86–3.82 (m, 2H), 3.78–3.76 (m, 2H), 2.62–2.57 (m, 2H), 2.167 (s, 3H), 2.162 (s, 3H), 2.01 (q,  $J$  = 7.3 Hz, 4H), 1.71–1.60 (m, 2H), 1.34–1.29 (m, 2H), 0.97 (s, 3H), 0.94 (s, 3H), 0.59 (t,  $J$  = 7.3 Hz, 6H); HRMS (FAB,  $m/z$ ,  $M^+$ ) calcd for  $\text{C}_{36}\text{H}_{50}\text{O}_5$ , 562.3568; found 562.3587.
- Motoshima, K.; Noguchi-Yachide, T.; Sugita, K.; Hashimoto, Y.; Ishikawa, M. *Bioorg. Med. Chem.* **2009**, 17, 5001.