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3,3-Diphenylpentane skeleton as a steroid skeleton substitute: Novel inhibitors of human 5α-reductase 1

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Abstract—We designed and synthesized novel type 1 5α-reductase inhibitors by using 3,3-diphenylpentane skeleton as a substitute for the usual steroid skeleton. 4-(3-(4-(*N*-Methylacetamido)phenyl)pentan-3-yl)phenyl dibenzylcarbamate (**11k**) is a competitive 5α-reductase inhibitor with the IC_{50} value of 0.84 μM.

Steroid hormones regulate a wide range of physiological processes, including reproduction, development, and homeostasis, by binding to and activating the nuclear receptors (NRs).[1] Testosterone, which is biosynthesized from cholesterol, is converted by 5α-reductase to the more active metabolite, 5α-dihydrotestosterone (DHT), which is considered to play a major role in androgenic signal transduction.[2] Since DHT production aggravates several diseases, including prostate cancer,[3,4] benign prostatic hyperplasia (BPH)[5] and androgenic alopecia,[6,7] several 5α-reductase inhibitors have been developed for the treatment of these disorders.[8–10]

Recently, two types of human 5α-reductases have been identified.[11,12] Type 1 5α-reductase (5αR-1) is expressed predominantly in liver and sebaceous glands of skin, whereas type 2 5α-reductase (5αR-2) is found in prostate, seminal vesicles, epididymis, liver, and hair follicles. Interestingly, some human prostate cancer cell lines, DU-145 and LNCaP, express only 5αR-1.[13,14] 5α-Reductase inhibitors down-regulate DHT and suppress prostate cancer cell proliferation.[15] Therefore, 5αR-1 inhibitors may selectively inhibit the growth of prostate tumors.

Steroidal 5α-reductase inhibitors, including finasteride (**1**) and dutasteride (**2**) (Fig. 1), are used clinically to treat BPH.[16,17] However, undesired adverse effects occur owing to cross-reactivity with other steroid hormone receptors, including progesterone receptor (PR).[18] Moreover, steroidal compounds are rapidly modified by several steroid-metabolizing enzymes. Therefore, development of 5αR-1 inhibitors with a non-steroidal skeleton is expected to be an effective strategy.

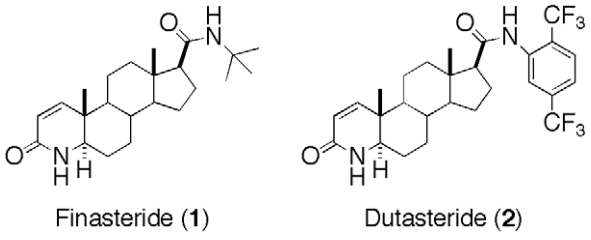

Figure 1. Structures of finasteride (**1**) and dutasteride (**2**).

In our previous study, we showed that 3,3-diphenylpentane (DPP) derivatives act as ligands of several NRs. Typical examples are shown in Figure 2, that is, a vitamin D receptor (VDR) agonist **3**,[19,20] an androgen receptor (AR) antagonist **4**,[19,20] a farnesoid X receptor (FXR) agonist **5**,[21] and a PR antagonist **6**.[22]

Because natural ligands of these NRs have steroidal (or secosteroidal) structure, it is suggested that the DPP skeleton may act not only as a multi-template for NR ligands, but also as a steroid skeleton substitute that would be recognized by steroid-metabolizing enzymes. In this paper, we report the synthesis of novel 5αR-1 inhibitors developed based on a DPP skeleton.

Keywords: 3,3-Diphenylpentane; Steroid; 5α-Reductase; Inhibitor.
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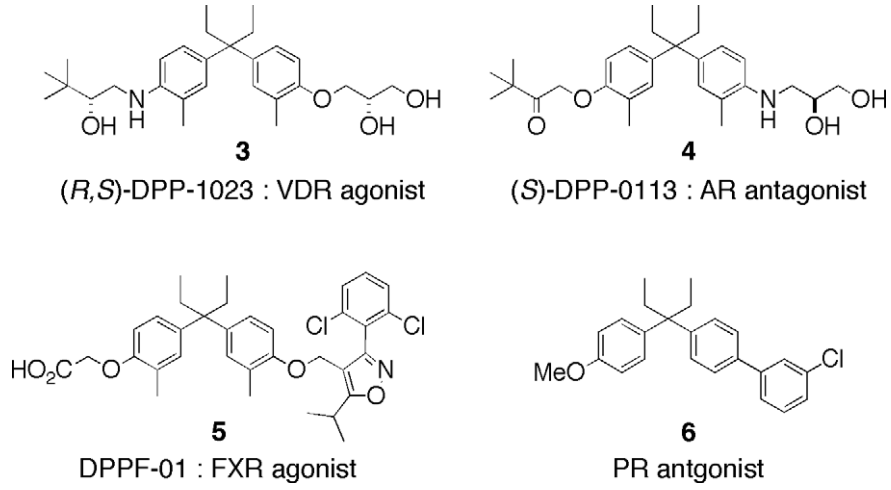


Figure 2. Typical NR ligands with a DPP skeleton.

We designed a candidate lead compound **8a** with a DPP skeleton based on the structure of 4-MA (**7**) (Fig. 3), a potent 5αR-1 inhibitor (at least in vitro), which is structurally related to finasteride (**1**) and dutasteride (**2**).[23] The *N*-methyl-*N*-phenylacetamide moiety of **8a** was chosen as a mimic of the steroid A-ring structure of 4-MA (**7**). The DPP skeleton possessing an amino group was synthesized as previously described (Scheme 1).[19,20]

The inhibitory potency of the prepared compounds toward human 5αR-1 was evaluated by the method described by Picard et al.,[24] except that human prostatic carcinoma LNCaP cells[14] were used instead of human prostatic carcinoma DU 145 cells. Briefly, [4-^{14}C]androstenedione ([^{14}C]A-en) (50 nM) was incubated with LNCaP cells for 18 h, and metabolites were extracted with Et_2O. The metabolite mixture thus obtained was separated by means of thin layer chromatography (Merck 25 TLC plates silica gel 60 F_{254}) with ethyl acetate/hexane (1:1 v/v). The radioactivity of each spot on the plate was measured with a bioimaging analyzer (BAS-2000, Fuji Film, Tokyo, Japan). 5αR-1-inhibitory activity was calculated from the ratio of the radioactivities of starting material ([^{14}C]A-en) and its reduced metabolite 5α-androstandione ([^{14}C]A-an). The assay was performed in duplicate or triplicate, and the mean values were taken. The experiments were repeated at least two times, and a typical set of data was shown in this paper.

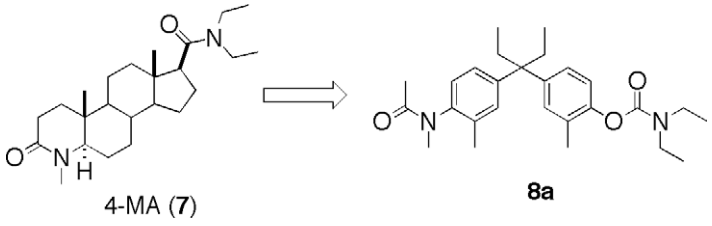
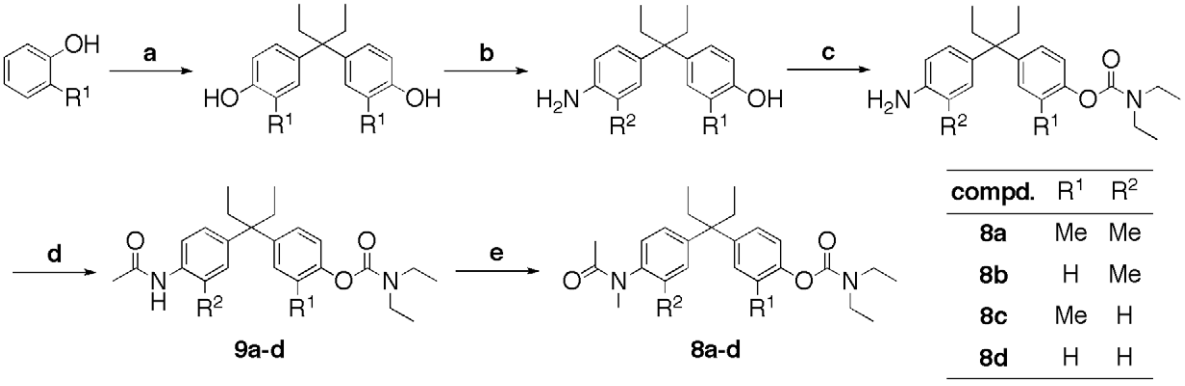
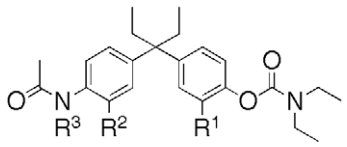


Figure 3. Molecular design of a DPP type 5αR-1 inhibitor.



Scheme 1. Reagents and conditions: (a) 3-pentanone, methanesulfonic acid, 3 d, 36–53%; (b) 2-R^2-aniline, neat, 180 °C, 3 h, 41–85%; (c) NaH, diethylcarbamoyl chloride, DMF, 2 h; (d) Ac_2O, pyridine, CH_2Cl_2, 1 h, 44–90% in two steps; (e) NaH, MeI, DMF, 18 h, 75–100%.

Table 1. 5αR-1 inhibitory activity of DPP derivatives



8a-8d, 9a

Compound	R^1	R^2	R^3	Inhibition (%) at 10 μM
9a	Me	Me	H	17
8a	Me	Me	Me	22
8b	Me	H	Me	56
8c	H	Me	Me	25
8d	H	H	Me	57

In our assay system, the IC_{50} value for inhibition of 5αR-1 activity of finasteride (**1**) was calculated to be 24 nM. This value is consistent with the reported IC_{50} value (39 nM) of finasteride (**1**).[24] The designed DPP-based compound **8a** and its synthetic precursor **9a** showed only slight inhibition, 22% and 17% at 10 μM, respectively (Table 1).

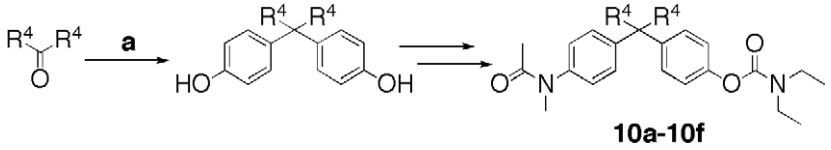
Next, structural modification of **8a** was performed. First, the effect of aromatic methyl substituents, that is, **8a–d**, was analyzed (Scheme 1). As shown in Table 1, **8b** and **8d** showed more potent inhibitory activity (56% and 57%, respectively) than **8a**, indicating that methyl groups on the DPP skeleton are not required. Therefore, further optimization of the structure was performed based on **8d**, and compounds **10a–g** were prepared (Scheme 2).

Table 2 summarizes the structure–activity relationships (SAR) of the **8d** derivatives, **10a–f**. The prepared derivatives possessing dialkyl (**10a**, **10b**) or cycloalkyl (**10c–f**) groups were less potent than **8d**. This feature of the SAR is similar to that found for derivatives of VDR ligand (**3**), AR ligand (**4**), and FXR ligand (**5**). The results suggest that the diethyl–diphenyl moiety (i.e., a DPP skeleton) is an appropriate substitute for a steroid skeleton.

Thus, we focused our attention on the synthesis and SAR of the side chain analogs (Scheme 3). The diethylcarbamoyl group of **8d** was changed to several other substituents as shown in Table 3. Introduction of bulky groups (**11b–c**, **11e–f**) resulted in more potent inhibitory activity, as compared with **8d**. On the other hand, groups possessing an additional heteroatom (**11g**, **11h**) resulted in weaker inhibitory activity.

Based on these results, more bulky and hydrophobic groups were introduced (Table 3, **11i–l**). The inhibitory activity of these compounds was screened at the lower concentration of 1 μM. The chain-elongated derivative, **11i**, showed slightly more potent activity than did **11b**, while the ring-enlarged derivative, **11l**, was less potent. The dibenzylamino derivative, **11k**,[25] showed the most potent inhibitory activity among our compounds, and its IC_{50} was 0.84 μM, so that it is 11 times more potent than **8d**.

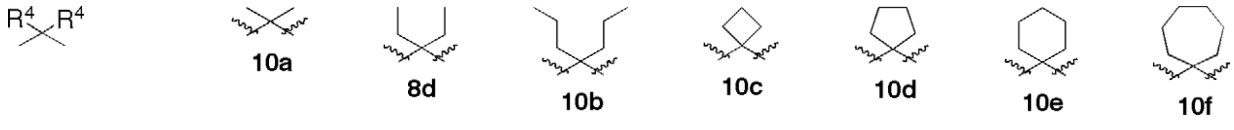
Next, the mode of inhibition of **11k** was analyzed by application of the Hanes–Woolf plot (Table 4). The plot indicated that **11k** inhibits 5αR-1 in a competitive manner (the two dose–response lines were nearly parallel), as in the case of finasteride (**1**).[26] This result



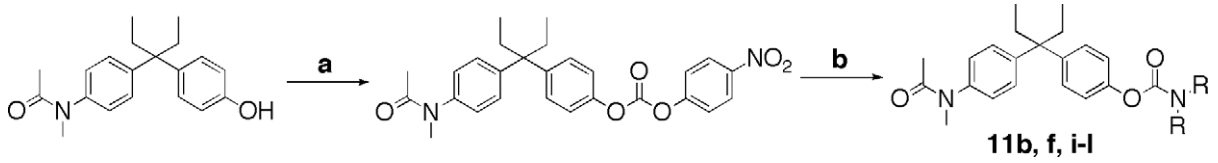
10a-10f

Scheme 2. Reagent and conditions: (a) phenol, methanesulfonic acid, 2–72 h, 31–98%.

Table 2. SAR of DPX skeleton analogs



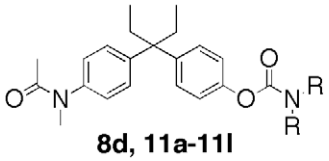
	10a	**8d**	**10b**	**10c**	**10d**	**10e**	**10f**
Inhibition (%) at 10 μM	16	56	27	16	5	46	25



11b, f, i-l

Scheme 3. Reagent and conditions: (a) 4-nitrophenylchloroformate, DMAP, DIPEA, THF, 30 min, 94%; (b) NHR_2, DMF, 1 h, 77–97%.

Table 3. SAR of carbamoyl derivatives



8d, 11a-11l

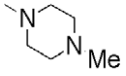
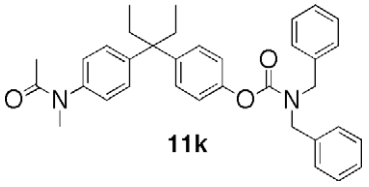
Compound	–NR_2	Inhibition (%) at 10 μM	Inhibition (%) at 1 μM	IC_{50} (μM)
11a	–NMe_2	29		
8d	–NEt_2	65		9.2
11b	–N(*n*-Pr_2)	77	26	2.9
11c	–N(*i*-Pr_2)	72		4.0
11d		57		
11e		68		4.7
11f		70		4.7
11g		15		
11h		17		
11i	–N(*n*-$Bu)_2$		32	
11j	–N(*sec*-$Bu)_2$		22	
11k	–NBn_2		51	0.84
11l			20	

Table 4. Inhibitory parameters of **11k**



11k

	Without inhibitor	With 1 μM **11k**
V_{max} (nM/h)	8.2	13.4
K_m (nM)	2300	9100

suggests that **11k** is recognized by 5αR-1 as a substrate mimic as expected; that is, the DPP skeleton can act as a steroid skeleton substitute not only for NR ligands, but also for enzymes which recognize steroidal structures.

In summary, we developed novel 5αR-1 inhibitors with a DPP skeleton. Compound **11k** with a dibenzylamino group possessed the most potent inhibitory activity among our derivatives. This is the first example of a compound in which the DPP skeleton can replace a steroid skeleton for target molecules other than NRs, to our knowledge. Further structural development of **11k** and a search for new candidates mimicking other biologically active steroids are in progress.

Acknowledgments

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References and notes

1. Roy, A. K.; Lavrovsky, Y.; Song, C. S.; Chen, S.; Jung, M. H.; Velu, N. K.; Bi, B. Y.; Chetterjee, B. *Vitam. Horm.* **1999**, *55*, 309.
2. Rasmusson, G. H. *Annu. Rep. Med. Chem.* **1986**, *22*, 179.
3. Lamb, J. C.; Levy, M. A.; Johnson, R. K.; Isaacs, J. T. *Prostate* **1992**, *21*, 15.
4. Brawley, O. W.; Ford, G. L.; Thompson, I.; Perlman, J. A.; Kramer, B. S. *Cancer Epidemiol. Biomarkers Prev.* **1994**, *3*, 177.
5. Geller, J. *J. Am. Geriatr. Soc.* **1991**, *39*, 1208.
6. Price, V. H. *N. Engl. J. Med.* **1999**, *341*, 964.
7. Gadwood, R. C.; Fiedler, V. C. *Annu. Rep. Med. Chem.* **1989**, *24*, 187.
8. Gormley, G. J. *Urol. Clin. North Am.* **1991**, *18*, 93.
9. Marberger, M. *Nat. Clin. Pract. Urol.* **2006**, *3*, 495–503.
10. Libecco, J. F.; Bergfeld, W. F. *Expert Opin. Pharmacother.* **2004**, *5*, 933.
11. Andersson, S.; Berman, D. M.; Jenkins, E. P.; Russel, D. W. *Nature* **1991**, *354*, 159.
12. Jenkins, E. P.; Andersson, S.; McGinley, J. I.; Wilson, J. D.; Russell, D. W. *J. Clin. Invest.* **1992**, *89*, 293.
13. Kaefer, M.; Audia, J. E.; Bruchovsky, N.; Goode, R. L.; Hsiao, K. C.; Leibovitch, I. Y.; Krushinski, J. H.; Lee, C.; Steidle, C. P.; Sutkowski, D. M.; Neubauer, B. L. *J. Steroid Biochem. Mol. Biol.* **1996**, *58*, 195.
14. Negri-Cesi, P.; Poletti, A.; Colciago, A.; Magni, P.; Martini, P.; Motta, M. *Prostate* **1998**, *34*, 283.
15. Lazier, C. B.; Thomas, L. N.; Douglas, R. C.; Vessey, J. P.; Rittmaster, R. S. *Prostate* **2004**, *58*, 130.
16. Gormley, G. H.; Stoner, E.; Bruskewitz, R. C.; ImperatoMcGinley, J.; Walsh, P. C.; McConnel, J. D.; Andriole, G. L.; Geller, J.; Bracken, B. R.; Tenover, J. S.; Darracott-Vaughan, E.; Pappas, F.; Taylor, A.; Binkowitz, B.; Ng, J. *N. Engl. J. Med.* **1992**, *327*, 1185.
17. Roehrborn, C. G.; Boyle, P.; Nickel, J. C.; Hoefner, K.; Andriole, G. *Urology* **2002**, *60*, 434.
18. Rittmaster, R. S. *N. Engl. J. Med.* **1994**, *330*, 120.
19. Hosoda, S.; Tanatani, A.; Wakabayashi, K.; Nakano, Y.; Miyachi, H.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4327.
20. Hosoda, S.; Tanatani, A.; Wakabayashi, K.; Makishima, M.; Imai, K.; Miyachi, H.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem.* **2006**, *14*, 5489.
21. 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.
22. Unpublished data; 3-(4-methoxyphenyl)-3-(4-(3-chlorophenyl)phenyÅl)pentane (**6**): ^{1}H NMR (500 MHz, $CDCl_3$/δ): 7.56 (t, *J* = 2.1 Hz, 1H), 7.46–7.43 (m, 3H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.28–7.23 (m, 3H), 7.11 (d, *J* = 9.0 Hz, 2H), 6.81 (d, *J* = 9.0 Hz, 2H), 3.79 (s, 3H), 2.11 (q, *J* = 7.3 Hz, 4H), 0.65 (t, *J* = 7.3 Hz, 6H). HRMS (FAB, M^+) calcd for $C_{24}H_{25}ClO$: 364.1594; found: 364.1587.
23. Jarman, M.; Smith, H. J.; Nicholls, P. J.; Simons, C. *Nat. Prod. Rep.* **1998**, 495.

24. Picard, F.; Schulz, T.; Hartmann, R. W. *Bioorg. Med. Chem.* **2002**, *10*, 437.
25. 4-(3-(4-(*N*-Methylacetamido)phenyl)pentan-3-yl)phenyl dibenzylcarbamate (**11k**): ^{1}H NMR (500 MHz, $CDCl_3/\delta$): 7.37–7.29 (m, 10H), 7.20 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 9.0 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 9.0 Hz, 2H), 4.56 (s, 2H), 4.53 (s, 2H), 3.25 (s, 3H), 2.10 (q, J = 7.3 Hz, 4H), 1.86 (s, 3H), 0.63 (t, J = 7.3 Hz, 6H); HRMS (FAB, $M+H^+$) calcd for $C_{35}H_{39}N_2O_3$: 535.2961; found: 535.2964.
26. Vermeulen, A.; Giagulli, V. A.; De Schepper, P.; Buntinx, A.; Stoner, E. *Prostate* **1989**, *14*, 45.