Diphenylpentane Skeleton as a Multi-Template for Steroid Skeleton-Recognizing Receptors/Enzymes

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Abstract: As an example of a multi-template approach, we focused on the 3,3-diphenylpentane (DPP) skeleton, which has been demonstrated to act as a steroid skeleton substitute. Various ligands for nuclear receptors (NRs), including vitamin D receptor (VDR), androgen receptor (AR) and farnesoid X receptor (FXR), and inhibitors of steroid metabolism-related enzymes, including 5α-reductase and HMG-CoA reductase (HMGR), have been efficiently created by introducing various substituents onto the DPP skeleton.

Key Words: Multi-template, steroids, diphenylpentane skeleton, steroid skeleton substitute, nuclear receptor, vitamin D receptor, androgen receptor, farnesoid X receptor, 5α-reductase, HMG-CoA reductase.

MULTI-TEMPLATE APPROACH

Chemical genetics, which emerged in the 1990's, is a field based on the idea that the functions of proteins can be analyzed/understood by the observation of phenomena caused by small molecules (ligands) that specifically bind to or regulate the proteins. For studies based on this idea, as well as for efficient discovery of lead compounds for drug development, it is crucial to obtain high-quality chemical libraries of candidate ligands. The number of human proteins with unique sequences is estimated to be 50,000-70,000. This means that for human chemical genetics studies, we require at least 50,000-70,000 ligands.

Although the number of human proteins is very large from the standpoint of amino acid sequences, the three-dimensional spatial structures (fold structures) of proteins are thought to be far better conserved in evolution than are the amino acid sequences [1,2]. The number of fold structure types that comprise all the domains occurring in human proteins is thought to be as few as approximately 1,000 [1-3]. A given fold structure might be characteristic of 50-70 human proteins on average, and therefore, ignoring physical/chemical interactions, one might expect that a template/scaffold structure which is spatially complementary to one fold structure might serve as a multi-template for structural development of ligands that would interact specifically with 50-70 different human proteins. In other words, the structures of ligands that bind to a protein having a certain fold structure may be useful for the development of novel ligands for other proteins possessing the same fold structure [3,4].

One example of such a multi-template is the mother skeleton of steroid hormones. Although the amino acid sequence homology among nuclear receptors (*vide infra*) is quite low, the spatial structures of the ligand-binding domains of the nuclear receptors are quite similar [5]. In addition, there exists a wide range of biologically active steroids, including neurosteroids (ligands for GABA receptor), cardiotonic steroids (ligands for Na^+/K^+-ATPase), and so on. Therefore, a skeleton that corresponds structurally to the steroid skeleton would be expected to be a good candidate as a multi-template for structural development of ligands for a number of proteins. In this paper, we describe the application of the diphenylpentane skeleton as a multi-template for creation of novel ligands of nuclear receptors and steroid-related enzymes.

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NUCLEAR RECEPTORS AND THEIR STEROIDAL/NONSTEROIDAL LIGANDS

Steroid hormones act *via* nuclear receptors (NRs) (Fig. (**1**)). NRs are ligand-dependent transcription factors that regulate the expression of responsive genes and thereby affect diverse processes, including cell growth, development, differentiation, and metabolism [5]. Based on the elucidated human genome sequence, 48 NRs are thought to exist in humans [5]. So far, the ligands of only 20–25 of them have been identified, including steroid hormone receptors [estrogen receptors (ERs)/estradiol, progesterone receptor (PR)/progesterone, androgen receptor (AR)/testosterone, glucocorticoid receptor (GR)/cortisone, and mineral corticoid receptor (MR)/aldosterone] (Fig (**1**)), retinoid receptors [retinoic acid receptors (RARs)/all *trans*-retinoic acid and retinoid X receptors (RXRs)/9-*cis*-retinoic acid], thyroxine hormone receptors (TRs)/thyroxine, vitamin D receptor (VDR)/1α,25-dihydroxyvitamin D_3, peroxisome proliferator-activated receptors (PPARs)/fatty acid, liver X receptors (LXRs)/oxysterol, farnesoid receptor (FXR)/bile acid, and steroid xenobiotic receptor (SXR)/steroids. In this paper, we focus on ligands for VDR, AR and FXR.

NUCLEAR RECEPTOR (VDR AND AR) LIGANDS WITH A 3,3-DIPHENYLPENTANE SKELETON

VDR is a receptor of 1α,25-dihydroxyvitamin D_3, which is an active form of vitamin D and plays critical roles in a

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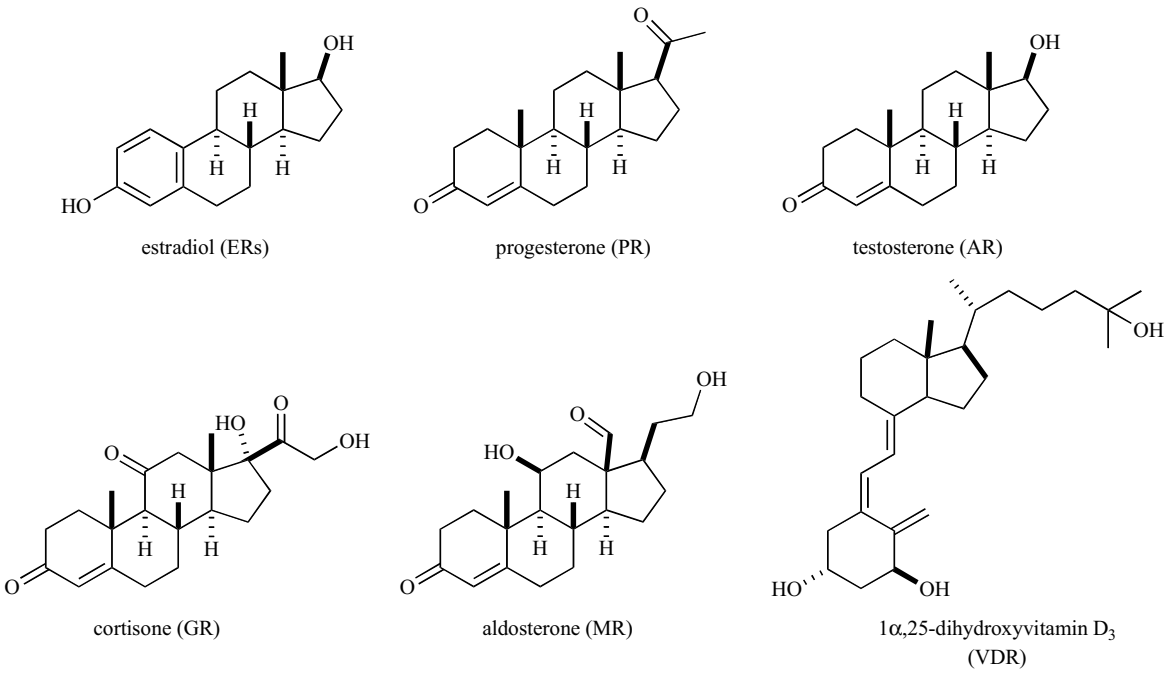


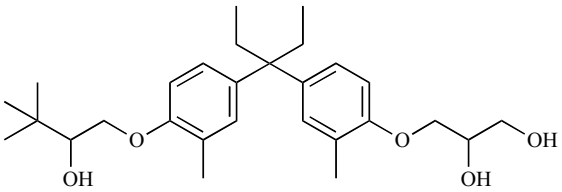
Fig. (1). Natural ligands of steroid hormone receptors.

variety of biological activities, including regulation of calcium homeostasis, bone mineralization, and control of cellular growth, differentiation, and apoptosis. So far, more than 3000 derivatives of 1α,25-dihydroxyvitamin D_3 with a secosteroidal skeleton have been synthesized. Almost all of the known VDR ligands prepared so far possess a secosteroidal skeleton. However, in 1999, Boehm *et al.* reported non-secosteroidal VDR modulators with a 3,3-diphenylpentane (DPP) skeleton, including LG190178 (**1**), which was a mixture of four stereoisomers [6]. Other groups also reported DPP (or 3,3-*bis*-arylpentane) derivatives as VDR agonists [7-9]. We focused on aza-analogs of LG190178 (**1**), each of which possesses two asymmetric carbons, and synthesized/obtained optically pure forms of aza-analogs of LG190178 (**1**) and of LG190178 (**1**) itself. Among the synthesized stereo-isoforms of each compounds, the (*R,S*)-isomers, including (*R,S*)-LG190178 (**5**), show the most potent activities in terms of both VDR affinity and HL-60 cell differentiation-inducing activity [10,11]. This result indicated that the (*R,S*)-isoforms of DPP-type VDR ligands are the eutomers for vitamin D activity; this conclusion was also supported by the results of computational chemistry and transcriptional reporter gene assays performed by another group [12]. A well-established specific biological activity of vitamin D_3 is human leukemia HL-60 cell monocytic differentiation-inducing activity. In this cellular assay system, (*R,S*)-DPP1023 (**6**) showed more potent activity (EC_{50} = 4.1 nM) than 1α,25-dihydroxyvitamin D_3 (EC_{50} = 9 nM).

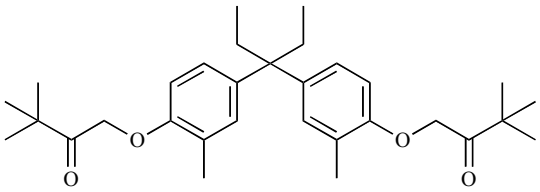
On the other hand, our group noticed that some DPP derivatives also possessed AR-antagonistic activity [10,11,13]. AR is a receptor of androgens, typically testosterone and/or its active form, 5α-dihydrotestosterone, which are endogenous ligands essential for the development and maintenance of the male 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 expected to be effective for treatment of androgen-dependent tumors, especially prostate tumor.

Among the aza-analogs of LG190178 (**1**), the (*S,S*)-isomers seemed to be the eutomers for AR-binding activity, in contrast to VDR-binding affinity, for which the (*R,S*)-isoforms are the eutomers. (*S,S*)-DPP0123 (**7**), containing an amino group at the side of the 1,2-diol moiety, possessed the most potent AR-antagonistic activity among the compounds examined, and also showed moderate vitamin D_3 activity, *i.e.,* this compound appears to be a dual ligand for both AR and VDR. DPP derivatives containing a pivaloyl group [LG190155 (**2**) and (*S*)-DPP0113 (**8**), Figs. (**2**) and (**3**)] were found to possess no VDR-binding activity, though they showed rather high affinity for AR. In spite of the lack of the VDR-binding affinity of DPP derivatives bearing a pivaloyl group, these compounds show HL-60 cell differentiation-inducing activity, because the pivaloyl group is reduced metabolically in HL-60 cells to give a metabolite with high VDR-binding affinity. Therefore, (*S*)-DPP0113 (**8**) binds only to AR, but it acts as a prodrug for a VDR agonist. (*S*)-DPP0113 (**8**) inhibits growth of androgen-dependent Shionogi carcinoma SC-3 cells with higher potency (IC_{50} = 32 nM) than hydroxyflutamide (IC_{50} = 180 nM), an active form of the therapeutically applied anti-androgen flutamide.

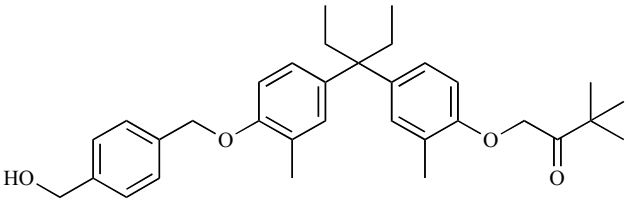
Structure-activity relationships (SAR) of the central quaternary carbon structure for both VDR-agonistic and AR-antagonistic activities were analyzed (Table **1**). To simplify the analysis, side chains were chosen to be the same as those of LG190155 (**2**) here. As shown in Table **1**, LG190155 (**2**) possessing a DPP skeleton (*i.e.*, containing a bis-ethyl moiety) showed the most potent VDR-agonistic and AR-



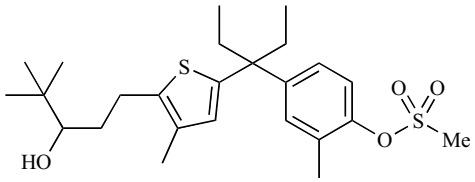
LG190178 (**1**)



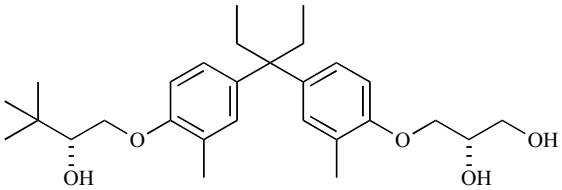
LG190155 (**2**)



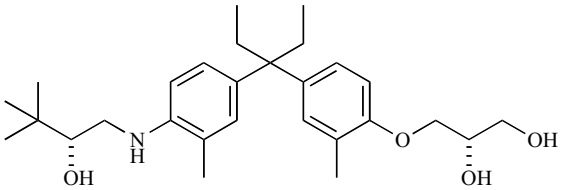
A11 (**3**)



LY2108491 (**4**)



(*R,S*)-LG190178 (**5**)



(*R,S*)-DPP1023 (**6**)

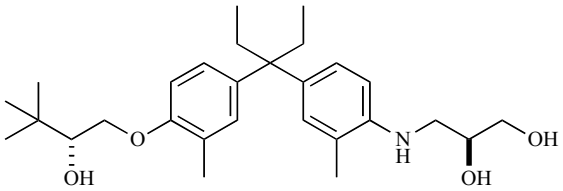
Fig. (2). DPP derivatives with VDR-agonistic activity.

antagonistic activities, suggesting that the DPP skeleton represents a favorable ligand structure for both VDR and AR.

FARNESOID X RECEPTOR LIGANDS WITH A DPP SKELETON

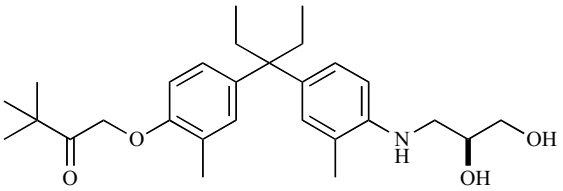
All members of NRs are thought to have evolved from a single molecular ancestor, and they form the so-called "nuclear receptor superfamily". It seems likely that a ligand superfamily also exists [14,15]. These results suggest that the DPP skeleton may act as a general template structure of NR ligands, like the steroidal-type skeleton, *i.e.,* the DPP skeleton might be utilized as a superior multi-template for creation of novel NR ligands. To test this idea, we focused on another type of NR, FXR. FXR is a well-characterized member of the so-called metabolic subfamily of NRs, and is a transcriptional sensor for bile acids. Its ligands, including chenodeoxycholic acid (CDCA: **15**), act as signaling molecules and participate in an intricate network of interactions that ultimately govern lipid, steroid, and cholesterol homeostasis and are involved in processes such as glucose utilization, inflammation, and carcinogenesis. Typical natural and synthetic ligands of FXR are shown in Fig. (**4**).

Maloney *et al.* reported GW4064 (**16**) as a potent nonsteroidal agonist for FXR [16]. Considering the side chain structures of GW4064 (**16**), and the DPP skeleton as a steroid skeleton substitute, we designed and synthesized DPPF-01 (**17**) as an FXR ligand candidate [17]. In transcriptional activation reporter gene assay, DPPF-01 (**17**) showed more potent agonistic activity for transcriptional activation of FXR



(*S,S*)-DPP0123 (**7**)

Ki (VDR) = 420 nM
Ki (AR) = 400 nM
IC_{50} (SC-3) = 55 nM

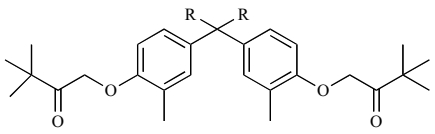


(*S*)-DPP0113 (**8**)

Ki (VDR) > 5000 nM
Ki (AR) = 720 nM
IC_{50} (SC-3) = 32 nM

Fig. (3). DPP derivatives with AR-antagonistic activity.

Table 1. VDR and AR Activity of LG190155 and its Derivatives



	R	R	VDR-agonistic activity[a] (EC_{50}, μM)	AR-antagonistic activity[b] (IC_{50}, μM)
9	methyl	methyl	4.0	3.2
2 (LG190155)	ethyl	ethyl	0.41	1.9
10	*n*-propyl	*n*-propyl	inactive	15
11	cyclobutyl		>10.0	11
12	cyclopentyl		inactive	inactive
13 (LG190119)	cyclohexyl		2.0	inactive
14	cycloheptyl		inactive	inactive

[a]Evaluated from HL-60 cell differentiation-inducing activity.

[b]Evaluated from SC-3 cell growth-inhibitory activity.

than that of the physiological ligand, chenodeoxycholic acid (CDCA, **15**). The EC_{50} values were 3.4 μM for DPPF-01 (**17**) and 11.7 μM for CDCA (**15**) under the experimental conditions employed.

In many cases, steroidal derivatives show crosstalk among NRs [18,19], but DPPF-01 (**17**) seems to be specific for FXR; at least, it did not activate VDR, PPARs, LXR, RARα or RXRα at 10 μM (Fig. (**5**)). This specificity of DPPF-01 for FXR may be attributed to the structure of the side chain, which had been optimized by Maloney *et al.* for GW4064 (**16**) [16].

VDR, AR and FXR all recognize steroid (or secosteroid) derivatives as endogenous ligands. Based on the above results, the DPP skeleton may be able to replace the steroid skeleton as a general structure of NR ligands. This led us examine extending the range of target molecules of DPP derivatives, from NRs to steroid-related enzymes (*vide infra*).

5α-REDUCTASE INHIBITORS WITH A STEROIDAL OR DPP SKELETON

Because metabolites of the steroid hormones regulate the production of hormones as a feedback mechanism, some steroidal derivatives possess inhibitory activity toward enzymes related to steroid synthesis or metabolism. Some kinds of steroid hormone synthetase, such as 5α-reductase,

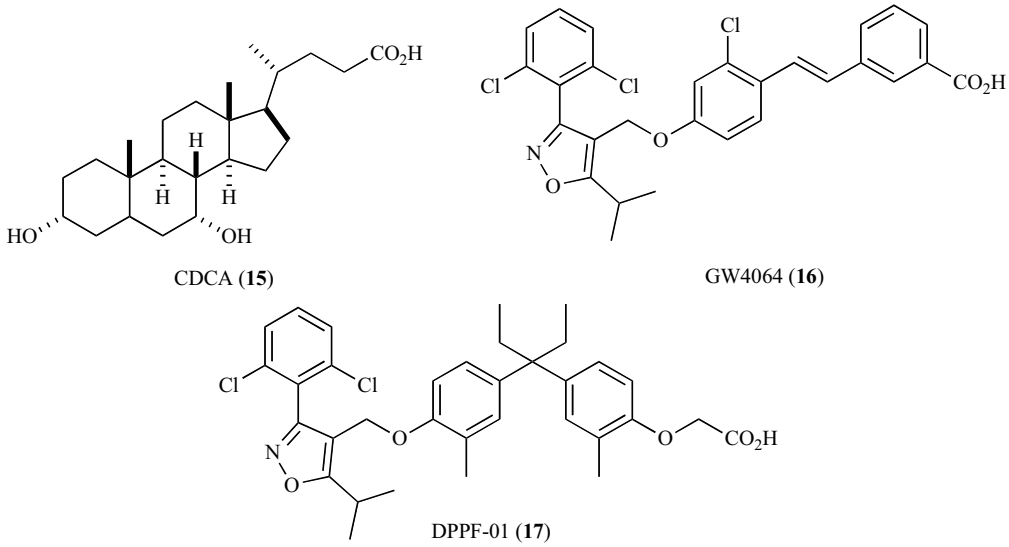


Fig. (4). Structure of FXR agonists: The natural ligand chenodeoxycholic acid (CDCA, **15**), synthetic ligand GW4064 (**16**) and DPP ligand DPPF-01 (**17**).

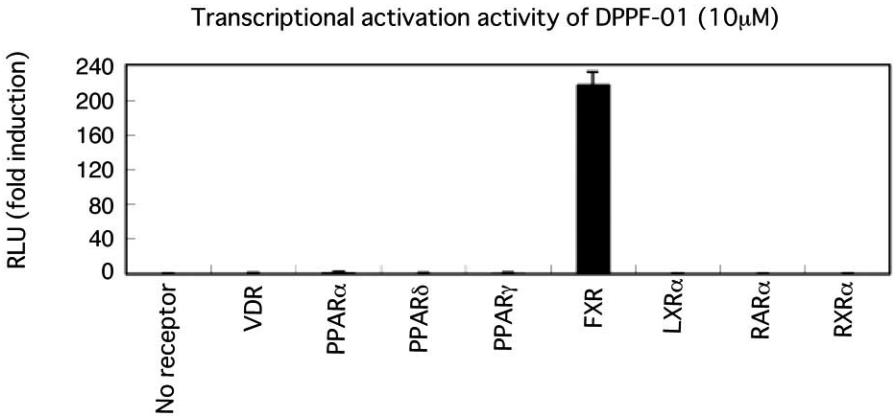
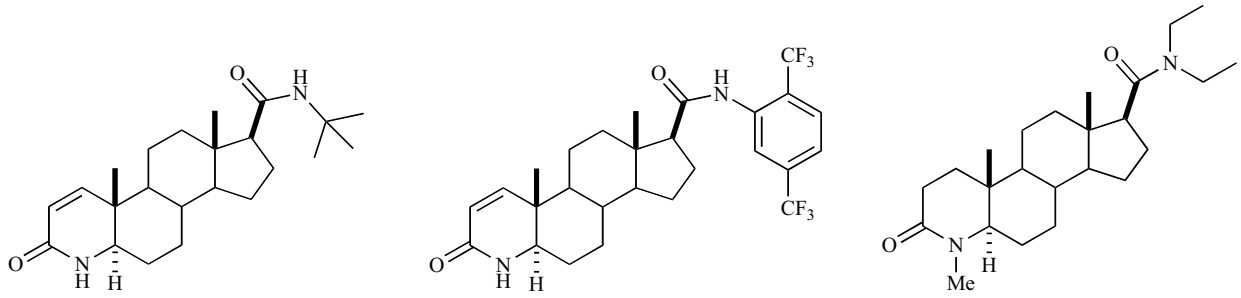


Fig. (5). Transcriptional activation of various NRs by DPPF-01 (**11**).



Finasteride (**18**) Dutasteride (**19**) 4–MA (**20**)

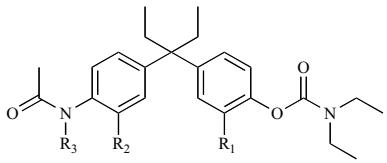
Fig. (6). 5α-Reductase inhibitors with a steroidal skeleton.

have been considered to be pharmaceutical targets, and so steroidal inhibitors have been used clinically.

Testosterone 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 [20]. Since DHT production aggravates various diseases, including prostate cancer [21,22], several 5α-reductase inhibitors possessing a steroidal skeleton have been developed, as shown in Fig. (**6**) [23-25]. Recently, two types of human 5α-reductase have been identified [26,27]. Some human prostate cancer cell lines, DU-145 and LNCaP, express only type 1 5α-reductase (5αR-1) [28,29]. Therefore, selective 5αR-1 inhibitors may preferentially inhibit the growth of prostate tumors.

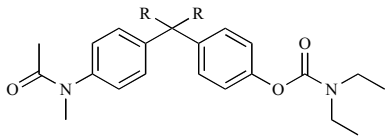
As in the case of NRs, we expected that the DPP skeleton could substitute for the steroidal core structure found in steroidal 5αR-1 inhibitors. Based on the structure of the steroi-

Table 2. 5αR-1 Inhibitory Activities of DPP Derivatives



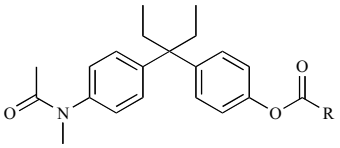
Compound	R_1	R_2	R_3	Inhibition (%) at 10 μM
21	Me	Me	H	17
22	Me	Me	Me	22
23	Me	H	Me	56
24	H	Me	Me	25
25	H	H	Me	57

Table 3. SAR of Diphenyl-X Skeleton analogs



Cmpd.	R	R	5αR-1 inhibition (10 μM, %)
26	methyl	methyl	16
25	ethyl	ethyl	56
27	*n*-propyl	*n*-propyl	27
28	cyclobutyl		16
29	cyclopentyl		5
30	cyclohexyl		46
31	cycloheptyl		25

Table 4. SAR of Carbamoyl Moiety



Cmpd.	R	5αR-1 Inhibition (IC_{50}, μM)
25	NEt_2	9.2
32	$N(n\text{-}Pr)_2$	2.9
33	*N*-piperidyl	4.7
34	*N*-morpholyl	>10.0
35	NBn_2	0.84

dal 5αR-1 inhibitor 4-MA (**20**) [30], DPP derivatives containing the corresponding carbamate side chain were designed, synthesized and evaluated [31]. The initially designed compounds, **21** and **22**, showed slight inhibitory activity toward 5αR-1 (Table **2**). In contrast to NR ligands, the aromatic methyl substituent is not required, because **23**–**25** showed more potent inhibitory activity than that of **22**. Table **3** summarizes the SAR for the central quaternary carbon moiety. Similar to the cases of VDR and AR ligands, compound **25** possessing diethyl groups showed the most potent inhibitory activity among compounds **25**–**31**. These results suggest again that the DPP skeleton is an appropriate substitute for a steroid skeleton. Furthermore, SAR of the carbamoyl group was analyzed (Table **4**). Carbamoyl derivatives containing a bulky hydrophobic moiety tended to show potent inhibition. The *N,N*-dibenzylcarbamoyl derivative **35** showed the most potent inhibitory activity toward 5αR-1 (IC_{50} = 0.84 μM). This result is consistent with the SAR of steroidal 5α-reductase inhibitors, *i.e.,* dutasteroid (**19**), possessing a very bulky side chain, is one of the most potent 5α-reductase inhibitors.

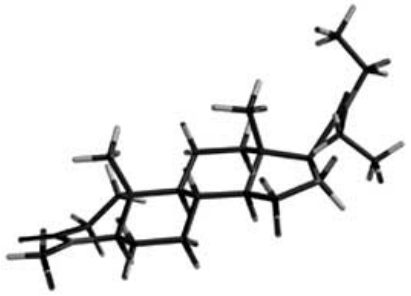


Fig. (7). 3D-structures of 5αR-1 inhibitors. For simplicity, the benzyl groups of **35** are omitted.

SIMILARITIES AND DIFFERENCES BETWEEN A STEROID SKELETON AND A DPP SKELETON

To compare the spatial structure of the DPP skeleton with that of the steroidal skeleton, ground-state structures of both 4-MA (**20**) and **35** were calculated, and results are shown in Fig. (**7**). Although the 2D-structural formula of the DPP skeleton is very different from that of the steroidal skeleton, the relative positions of the side chains are similar in 4-MA (**20**) and **35**. One of the phenyl rings of **35** lies in the steroid plane, and the other phenyl group with a *para*-substituent corresponds to the side chain of the steroidal D-ring.

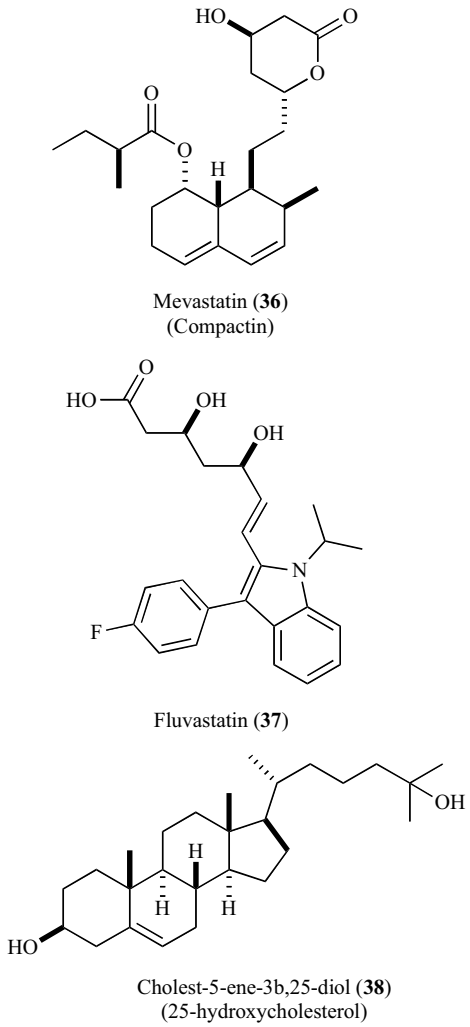


Fig. (8). Statin- and oxysterol-type HMGR inhibitors.

On the other hand, the two skeletons have very different degrees of rigidity. The MOE calculation indicated that the number of stable conformers of 4-MA (**20**) is 7, while that of **35** was calculated to be 1389. This indicates that the DPP

skeleton is much more flexible than the steroid skeleton. This flexibility may contribute the selectivity of DPP ligands, because the conformation of the DPP derivatives depends considerably on the bulkiness of the side chains.

Table 5. HMGR-Inhibitory Activity of Oxysterols

Oxysterol	Inhibitory Activity (IC_{50}, μM)
Cholest-5-ene-3β,25-diol (**38**)	3.0
Cholest-5-ene-3β,20α-diol	3.2
Cholest-5-ene-3β,22(*S*)-diol	5.8
Cholest-5-ene-3β,22(*R*)-diol	7.5
20-Pentylpregn-5-ene-3β,20α-diol	10.0
27-Norcholest-5-ene-3β,25-diol	26.0
3β-Hydroxycholest-5-en-24-one	62.0
3β-Hydroxy-27-norcholest-5-en-24-one	>75.0

HMG-COA REDUCTASE INHIBITORS WITH A DPP SKELETON

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase: HMGR) is the rate-controlling enzyme in cholesterol biosynthesis. Statins, such as compactin and fluvastatin (Fig. (**8**)), inhibit HMGR and reduce low-density lipoprotein cholesterol (LDL-c) levels in the blood-stream [32-34]. It is clinically proven that statins lower the risk of cardiovascular disease [35].

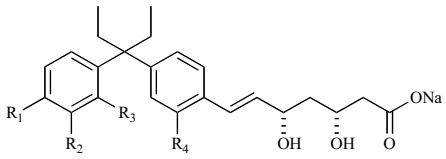
On the other hand, some kinds of oxysterols are also known to be potent inhibitors of HMGR [36,37]. The HMGR-inhibitory activity of oxysterols in primary cultures of liver cells is presented in Table **5** [36]. Oxysterols possessing a hydroxyl group in the side chain tends to show potent inhibition. Cholest-5-ene-3β,25-diol (25-hydroxycholesterol, **38**), a direct metabolite of cholesterol in the liver, is the most potent inhibitor among these oxysterols (Fig. (**8**)).

We expected that utilization of a DPP skeleton as a template would provide access to novel HMGR inhibitors. Because the HMGR-inhibitory activity of oxysterols is less potent than that of statins, we designed a hybrid structure possessing the DPP skeleton with the side-chains of statins (Table **6**). Fluorine and isopropyl substituents are widely used for synthetic statins. Although all of the DPP derivatives **39**–**44** were less potent than compactin, SAR of the substituted phenyl groups was established. Comparing **39** and the others, an isopropyl group at R_4 seems to be crucial for the inhibitory activity. On the other hand, a fluorine substituent at R_1–R_3 is not required, because **43** showed the most potent inhibitory activity among compounds **40**–**43**. The non-DPP derivative **44** is less potent than compounds **40**–**43**, which indicates that the DPP skeleton may contribute to increasing the inhibitory activity.

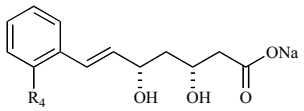
CONCLUSIONS

Our studies described above indicate that a DPP skeleton can replace a steroid skeleton, and indeed, various NR ligands and inhibitors of steroid-metabolizing enzymes were created based on the DPP skeleton. Therefore, the DPP skeleton seems to be a superior multi-template for development of various biologically active compounds. The results also suggest the utility of the multi-template approach for creation of biologically active compounds. Expansion of

Table 6. HMGR-Inhibitory Activity of DPP Derivatives



39-43



44

	R_1	R_2	R_3	R_4	Relative Inhibitory Activity
39	F	H	H	H	N.A.
40	F	H	H	*i*-Pr	0.09
41	H	F	H	*i*-Pr	0.03
42	H	H	F	*i*-Pr	0.14
43	H	H	H	*i*-Pr	0.23
44				*i*-Pr	0.03
Compactin (**36**)					1

N.A.: No inhibition at 100 μM.

target molecules beyond NRs/steroid-related enzymes will be the next step. If our hypothesis mentioned in the first section of this paper is correct, it should be possible to create specific ligands for 50-70 different proteins based on the DPP skeleton. Another example of structural development studies utilizing the multi-template approach is the creation of a range of biologically active compounds derived from thalidomide/arylphthalimides [38-42]. By using the DPP skeleton and/or thalidomide/arylphthalimide skeleton as multi-templates, it should be possible to construct high-quality chemical libraries for chemical genetics/drug discovery. Discovery of other superior multi-template structures is of great interest.

ACKNOWLEDGEMENT

The authors are grateful to all of the coauthors whose names appear in the reference list. The work described in this paper was partially supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Science, Sports and Culture, Japan.

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Received: January 30, 2009 Revised: February 04, 2009 Accepted: February 05, 2009

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