

Structural development studies of nuclear receptor ligands*

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Abstract: Studies in our laboratory are focused on structural development studies of biological response modifiers (BRMs), including nuclear receptor (NR) ligands, which act directly on cells at the gene expression level, and thalidomide (and related molecules), which modulates a variety of physiological processes. Our studies on the molecular design of ligands for retinoic acid receptor (RAR), androgen receptor (AR), vitamin D receptor (VDR), farnesoid X receptor (FXR), and peroxisome proliferator-activated receptor (PPAR) are reviewed.

Keywords: nuclear receptors; ligands; structural development; retinoid; androgens; vitamin D; antagonists.

INTRODUCTION

The commercial availability of antibiotics from the mid-20th century is one of the factors that increased longevity in advanced nations. The highest mortality rates in Japan before the 1950s were due to infectious diseases such as tuberculosis, but these fell dramatically in the 1950s, and instead, the death rate from cancer has been rising. In other words, the widespread use of antibiotics, drugs based on species-selective toxicity, has shifted the nature of our lethal diseases from infectious and acute to noninfectious and chronic. Biological response modifiers (BRMs), which are not based on species-selective toxicity, might provide a means to meet this new challenge. We have been engaged in development studies of two types of BRMs, i.e., nuclear receptor (NR) ligands, which act directly on cells at the gene expression level to regulate their behavior [1–5], and thalidomide-related molecules, which modulate physiological processes of our body to restore a normal state [4–8]. Of course, studies of these two types of BRMs are mutually inter-related. For example, our studies on anti-androgens were derived from our thalidomide research [6–12].

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 [13]. Based on the elucidated human genome sequence, 48 NRs are thought to exist in humans [13]. So far, the ligands of only 20–25 of them, including steroid hormone receptors [estrogen receptors (ERs)/estradiol, progesterone receptors (PRs)/progesterone, androgen receptors (ARs)/testosterone, glucocorticoid receptors (GRs)/cortisone, and mineral corticoid receptors (MRs)/aldosterone], retinoid receptors [retinoic acid receptors (RARs)/all *trans*-retinoic acid (ATRA: **1**) and retinoid X receptors (RXRs)/9-*cis* retinoic acid], thyroxine hormone receptors (TRs)/thyroxine, vitamin D receptors (VDRs)/1,25-dihydroxyvitamin D₃, peroxisome proliferator-activated receptors (PPARs)/fatty acid,

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liver X receptors (LXRs)/oxysterol, farnesoid receptors (FXRs)/bile acid, and steroid xenobiotic receptors (SXR)/steroids, have been identified. In this paper, our researches on structural development of ligands for RAR, AR, VDR, PPAR, and FXR are reviewed.

RETINOIC ACID RECEPTOR LIGANDS (RETINOIDS)

RAR is a receptor of ATRA: **1**, which is an active form of vitamin A (except for its function in vision), and its bioisosters [called retinoids, including ATRA (**1**)]. The ability of retinoids to modulate the growth and differentiation of a wide variety of cells has been reported, with ATRA (**1**) shown to be significantly involved in the control of embryonic development and cell differentiation [1,2]. Retinoids have received much attention from a clinical standpoint because they are useful in the treatment of vitamin A deficiency, proliferative dermatological diseases, leukemia, and several types of tumors. ATRA (**1**) has been established as the first-choice medicament for the treatment for acute promyelocytic leukemia (APL) [14]. Subsequently, a large number of retinoids has been synthesized for potential clinical application [1–5,15]. As regards the target molecule of retinoids, there are three subtypes of RARs, i.e., RAR α , RAR β , and RAR γ . Each of them is a retinoid-dependent transcription factor which acts as a heterodimer with another member of the NRs, RXR. A close relationship between aberrancy of RARs and malignancy of cells has been well documented [2,16,17], i.e., truncated RAR α fused with another gene (PML or PLZF) as a result of chromosome translocation [t(15;17) and t(11;17), respectively] is found in APL, loss of RAR β is seen in some types of lung tumors, expression of a dominant negative isoform of RAR β in some breast cancers, and so on.

We have been engaged in structural development studies of retinoids aiming at superior RAR subtype-selectivity and amelioration of the clinical disadvantages of ATRA (**1**) and other conventional retinoids with a hydrocarbon skeleton. The major disadvantage of ATRA (**1**)/conventional retinoids is their high lipophilicity and very slow elimination from the body, which cause long-lasting toxicity (hypervitaminosis A). Our initial strategy for overcoming these disadvantages was introduction of a heteroatom(s) into a structural mimic of ATRA (**1**), leading to benzanilide derivatives, of which a typical example is Am80 (tamibarotene: **2**) (Fig. 1) [1–5,15,18]. Another potent synthetic retinoid prepared in our laboratory is TAC101 (**3**) (Fig. 1), which possesses two trimethylsilyl groups [1–5,15,19]. One of the unique features of Am80 (**2**) and TAC-101 (**3**) is the lack of binding affinity toward cellular retinoic acid binding protein (CRABP) [20,21]. Among the anilide-type retinoids, Am80 (**2**) possesses potent cell differentiation-inducing activity and was approved officially for the treatment of APL (launched in June 2005 in Japan), while TAC101 (**3**) possesses potent anti-angiogenic activity and anti-hepato-metastasis activity, and was successful in a phase II clinical study for the treatment of solid tumors in the United States (a phase III study is starting). Both Am80 (**2**) and TAC101 (**3**) are RAR α / β -selective retinoids [1,2,15,21]. Further structural development based on computer-assisted docking (CAD) studies resulted in non-benzoic acid types of retinoids (**10–12**) (Fig. 1).

CAD studies also afforded valuable information concerning the molecular design of antagonists. In the ligand-dependent activation of NRs, a general and important structural feature has been elucidated based on the X-ray crystal structure analysis of the ligand-binding domain (LBD) of several NRs with (*holo*-form) or without (*apo*-form) the ligand, i.e., the ligand-induced folding of helix 12 (H12), which is one of the substructures located in the LBD. In the *apo*-form, H12 takes an open conformation, while in the *holo*-form, it functions as a lid covering the ligand binding pocket (closed conformation) [3,22]. This major conformational change induced by ligand-binding is thought to be the key structural feature in the activation of NRs. According to this concept, a compound that binds with the ligand-binding pocket, but interferes with the folding of H12, should be an antagonist of the corresponding NR (H12-folding inhibition hypothesis for molecular design of NR antagonists) [4]. Based on this concept, the first retinoid antagonist, TD550 (**13**), was created by introduction of a bulky diamantyl group [3,22,23]. As expected, CAD studies using the H12-closed conformation of the LBD of RAR α suggested that ATRA (**1**) and Am80 (**2**) precisely fit into the ligand-binding pocket, while TD550 (**13**)

cannot fit into the pocket, i.e., collision between the diamantyl moiety of TD550 (**13**) and H12 of the LBD was suggested to occur (Fig. 2).

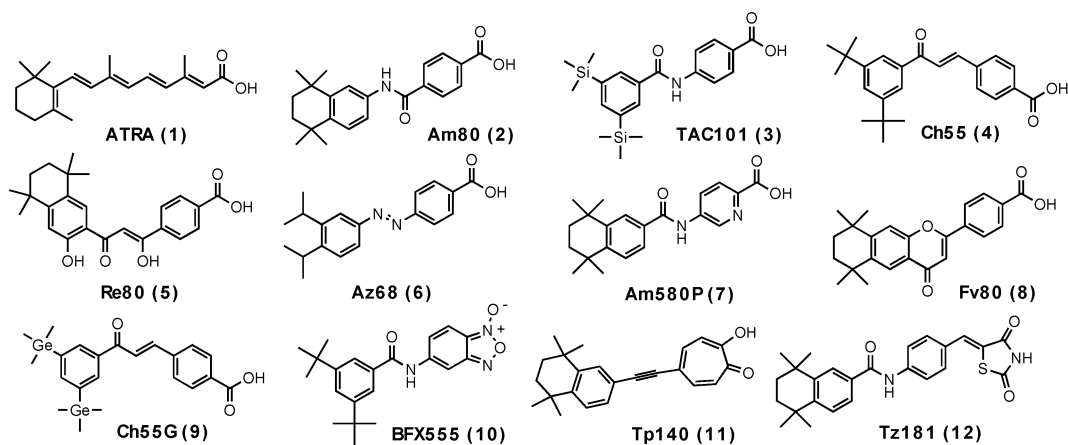


Fig. 1 Structures of ATRA (**1**) and typical retinoids synthesized in our laboratory (**2–12**).

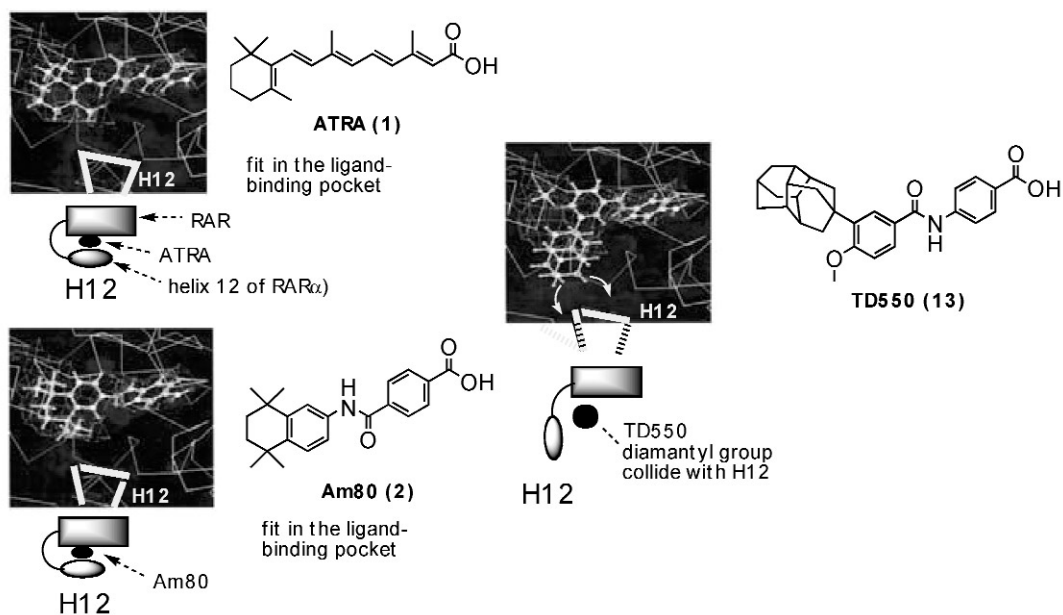


Fig. 2 CAD studies of retinoids with the LBD of RAR α .

NUCLEAR RECEPTOR ANTAGONISTS DESIGNED BASED ON THE HELIX 12-FOLDING INHIBITION HYPOTHESIS

Androgen receptor antagonists

AR is a receptor of androgens, typically testosterone and/or its active form, 5 α -dihydrotestosterone, which are endogenous ligands essential for the development and maintenance of the male reproductive system and secondary male sex characteristics [24]. Androgens play diverse physiological and pathophysiological roles in both males and females [24,25]. 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.

We have been engaged in structural development studies of thalidomide (**14**) [4–8,26], and have developed various kinds of biologically active compounds (Fig. 3), including AR antagonists [9–12]. Thalidomide (**14**) was developed in the 1950s as a nontoxic sedative/hypnotic drug, but was withdrawn from the market in the early 1960s because of its serious teratogenicity. However, it was subsequently identified as an effective agent for the treatment of multiple myeloma (MM), AIDS, Hansen's disease, and various cancers [27]. The U.S. Food and Drug Administration (FDA) approved it for the treatment of erythema nodosum in Hansen's disease in 1998, and (in combination with dexamethasone) for the treatment of MM in 2006. Official approval for the use of thalidomide (**14**) to treat MM has also been applied for in Japan.

Our initial studies yielded phthalimide-type AR antagonists, including S-FPTN (**15**) (Fig. 3) [3–9]. AR-antagonistic activity of the compounds was assessed by growth-inhibition assay using the androgen-dependent cell line SC-3 and by AR transcriptional activation assay [9]. However, the phthalimide-type AR antagonists we prepared were thought not to be H12-folding inhibitors, judging from CAD studies. They were considered to elicit AR-antagonistic activity by inducing misfolding of H12 [3,10,11].

The major obstacle in the treatment of prostate tumor with AR antagonists is the sudden appearance of AR antagonist-resistant cells. One major molecular mechanism of such resistance is point mutation of AR, as found in human prostate cancer cell line LNCaP. AR of LNCaP cells possess a point mutation T877A, and is considered to take an H12-folded conformation that is constitutively active even in the absence of the cognate ligand, androgen. Of course, the mutated AR can bind androgens and misfolding inducer-type AR antagonists, which stabilize the active conformation of the AR, leading to super-activation of the mutated AR. In fact, our phthalimide-type AR antagonists were ineffective as antagonists toward LNCaP cells, and acted as AR agonists. Therefore, to overcome the problem of such resistance based on AR mutation, H12 folding-inhibitor types of AR antagonists, which bind to the mutated ARs and induce unfolding (or inhibit folding) of H12, would be useful.

CAD studies present only misfolding inducer-type antagonists because the *holo*-form LBD (H12 folded conformation) has been used as a template for docking calculations. Therefore, candidates into which a bulky substituent can be introduced at the region where H12 interacts should be chosen from the group of compounds identified by CAD. One such compound is the isoxazolone derivative **17** (Fig. 4) [10]. Using this skeleton as a scaffold, we introduced various substituents at the position at which H12 interacts, aiming to obtain H12 folding inhibitor-type AR antagonists, including ISOP-4 (**16**) (Figs. 3 and 4) and its derivatives (**17–23**) (Fig. 4) [10]. ISOP-4 (**16**) and its derivatives (**17–23**) possess high binding affinity for normal and T877A-mutated ARs, and have been shown to be active as AR antagonists in LNCaP cells [11].

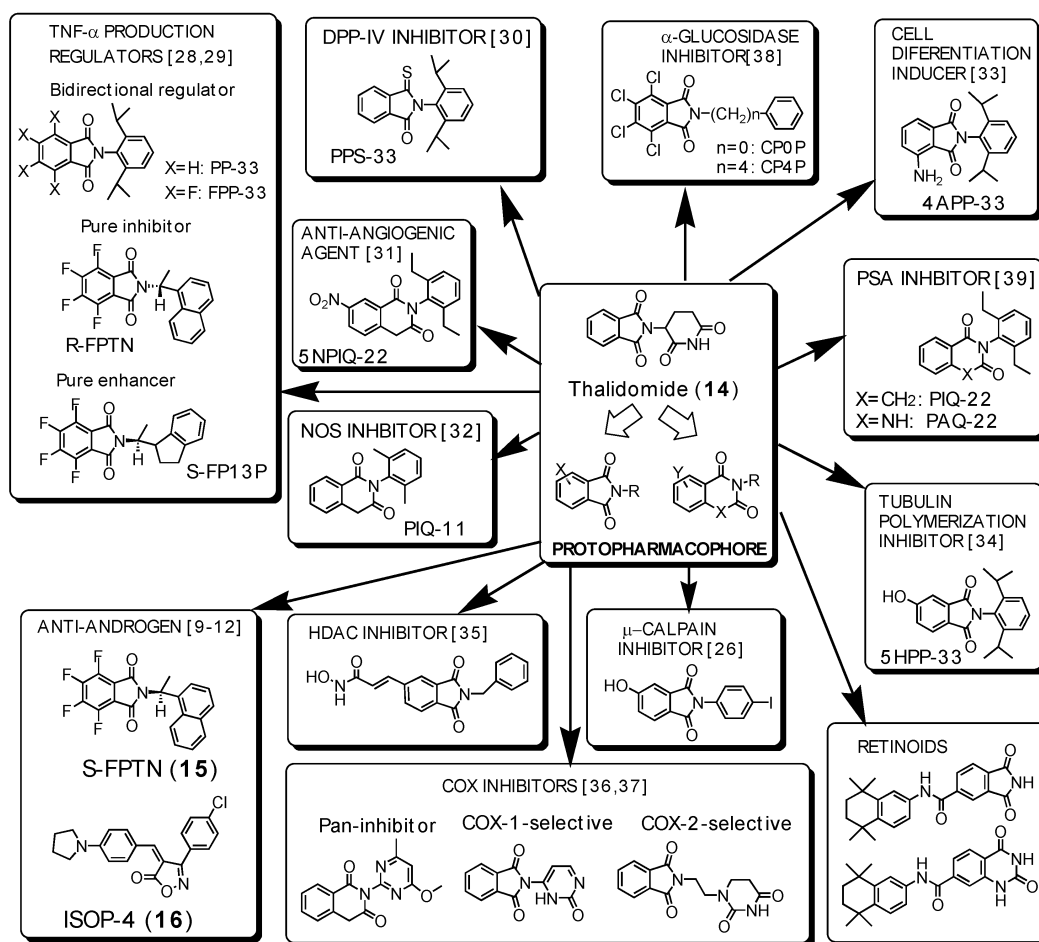


Fig. 3 Biologically active compounds derived from thalidomide (14).

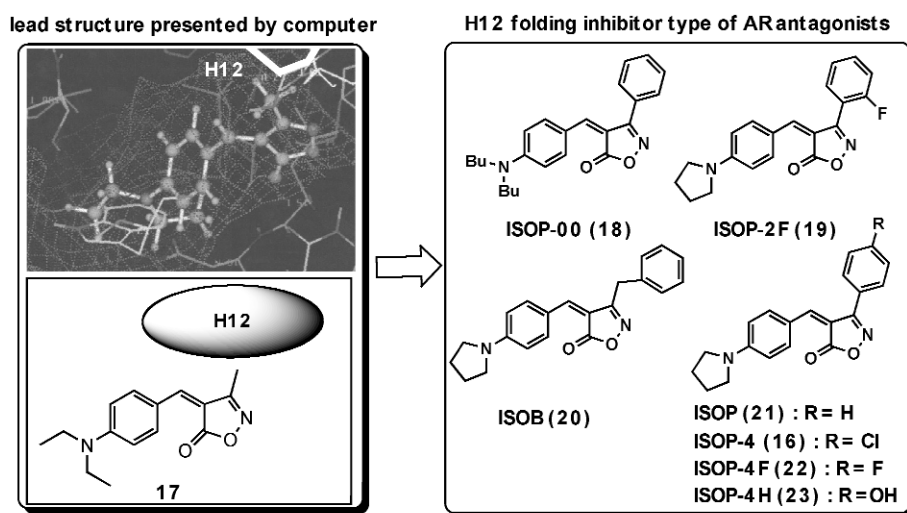


Fig. 4 Molecular design of H12 folding inhibitor type of AR antagonists.

Vitamin D receptor antagonists

VDR is a receptor of 1,25-dihydroxyvitamin D₃ (**24**), which is an active form of vitamin D and plays critical roles in a variety of biological activities, including regulation of calcium homeostasis, bone mineralization, and control of cellular growth, differentiation, and apoptosis. VDR antagonists can be expected to be useful for the treatment of Paget's disease [40,41]. So far, more than 3000 derivatives of 1,25-dihydroxyvitamin D₃ (**24**) have been synthesized, but only a few types of compounds, including TEI-9647 (**25**) and ZK168281 (**26**) and its analogs (Fig. 5), have been reported as VDR antagonists [40,41].

On the basis of CAD studies using the *holo*-form LBD of VDR, we designed a novel VDR antagonist possessing a nitrogen atom in its structure, i.e., DLAM-1P (**27a–d**) (Fig. 5) [42]. The nitrogen atom was chosen as a key component to install in the side chain, which is expected to inhibit the folding of H12. Among the four configurational isomers of DLAM-1P (**27a–d**), the (23*S*,25*S*)-isomer (**27a**) showed the highest affinity for VDR and only this isomer showed VDR antagonistic activity with similar potency to that of TEI-9647 (**25**) for inhibition of 1,25-dihydroxyvitamin D₃ (**24**)-induced cell differentiation of HL-60 cells and in a VDR transcriptional activation reporter gene assay system [30,31]. In the VDR transcriptional activation reporter gene assay, TEI-9647 (**25**) showed slight activation at a very high concentration (10 μM), while (23*S*,25*S*)-DLAM-1P (**27a**) did not show any VDR activation. This result suggests that (23*S*,25*S*)-DLAM-1P (**27a**) is a full VDR antagonist, i.e., an H12 folding inhibitor. In agreement with this, CAD studies of (23*S*,25*S*)-DLAM-1P (**27a**) using the *holo*-form LBD of VDR suggested collision of the *N*-benzyl group of (23*S*,25*S*)-DLAM-1P (**27a**) with phenylalanine 422 in the H12 region of VDR's LBD (Fig. 5) [43].

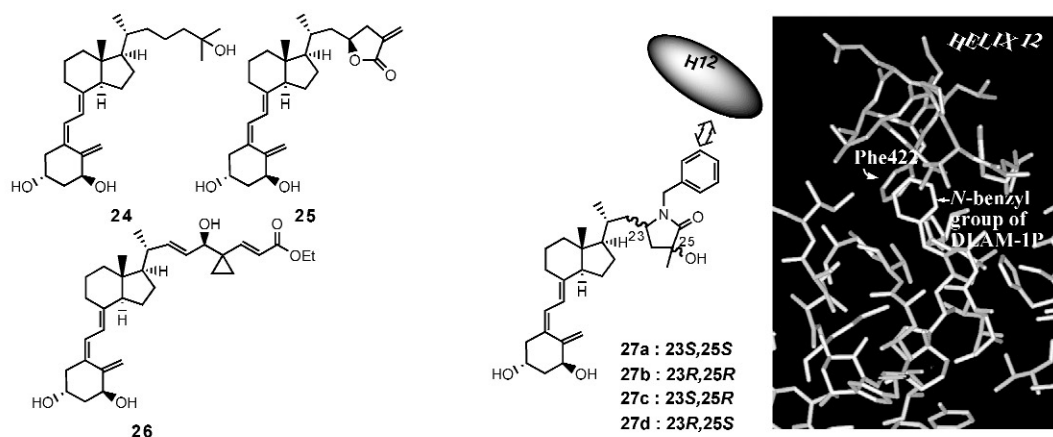
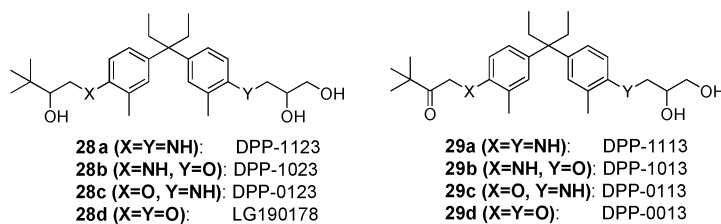


Fig. 5 Structures of 1,25-dihydroxyvitamin D₃ (**24**), TEI-9647 (**25**), ZK168281 (**26**), molecular design of DLAM-1P (**27**) based on H12 folding inhibition hypothesis and CAD results of 23*S*,25*S*-DLAM-1P (**27a**) with VDR's LBD.

NUCLEAR RECEPTOR LIGANDS WITH A 3,3-DIPHENYLPENTANE SKELETON

Vitamin D receptor/androgen receptor ligands

Almost all of the known VDR ligands prepared so far possess a secosteroidal skeleton, including our VDR antagonist DLAM-1P. However, Boehm et al. reported a novel structural type of VDR modulators, i.e., compounds with a bis-phenol skeleton/3,3-diphenylpentane skeleton, including LG190178 (**28d**) (Fig. 6) [44,45]. We focused on aza-analogs of LG190178 (**28d**), i.e., compounds **28a–c** and their pivaloyl derivatives, **29a–d** (Fig. 6) [46,47]. The biological activities were evaluated by receptor-binding assay and human leukemia cell line HL-60 monocytic cell differentiation-inducing assay (Fig. 6).



cmpd	VDR affinity (Ki, nM)	HL-60 differentiation induction (EC ₅₀ , nM)	cmpd	VDR affinity (Ki, nM)	HL-60 differentiation induction (EC ₅₀ , nM)
DPP-1123 (28a) ^b	260	37	LG190178 (28d) ^b	360	44
(<i>R,R</i>)-DPP-1123 [(<i>R,R</i>)- 28a]	190	48	(<i>R,R</i>)-LG190178 [(<i>R,R</i>)- 28d]	320	26
(<i>S,R</i>)-DPP-1123 [(<i>S,R</i>)- 28a]	580	170	(<i>S,R</i>)-LG190178 [(<i>S,R</i>)- 28d]	740	120
(<i>R,S</i>)-DPP-1123 [(<i>R,S</i>)- 28a]	120	6.6	(<i>R,S</i>)-LG190178 [(<i>R,S</i>)- 28d]	12	8.6
(<i>S,S</i>)-DPP-1123 [(<i>S,S</i>)- 28a]	350	43	(<i>S,S</i>)-LG190178 [(<i>S,S</i>)- 28d]	11	23
DPP-1023 (28b) ^b	52	48	DPP-1113 (29a) ^a	>5000	850
(<i>R,R</i>)-DPP-1023 [(<i>R,R</i>)- 28b]	150	30	DPP-1013 (29b) ^a	3600	360
(<i>S,R</i>)-DPP-1023 [(<i>S,R</i>)- 28b]	180	55	DPP-0113 (29c) ^a	>5000	110
(<i>R,S</i>)-DPP-1023 [(<i>R,S</i>)- 28b]	9.5	4.1	DPP-0013 (29d) ^a	>5000	90
(<i>S,S</i>)-DPP-1023 [(<i>S,S</i>)- 28b]	20	16	1,25-VD ₃	0.17	9
DPP-0123 (28c) ^b	930	52			
(<i>R,R</i>)-DPP-0123 [(<i>R,R</i>)- 28c]	340	320			
(<i>S,R</i>)-DPP-0123 [(<i>S,R</i>)- 28c]	1100	770			
(<i>R,S</i>)-DPP-0123 [(<i>R,S</i>)- 28c]	220	47			
(<i>S,S</i>)-DPP-0123 [(<i>S,S</i>)- 28c]	420	110			

Fig. 6 VDR agonistic activity of 3,3-diphenylpentane derivatives. (a: enantiomeric mixture, b: diastereomeric mixture).

HL-60 cell differentiation-inducing activity has been believed to correlate well with the binding/activation of VDR by its ligands. In fact, though only a few 1,25-dihydroxyvitamin D₃ antagonists have been reported [40–43], they were prepared based on VDR binding assay and activity to repress 1,25-dihydroxyvitamin D₃-induced transcriptional activation in reporter gene assay, and they have been shown to possess inhibitory activity toward 1,25-dihydroxyvitamin D₃-induced HL-60 differentiation [40–43], indicating that induction of HL-60 cell differentiation by 1,25-dihydroxyvitamin D₃ and at least some VDR modulators is mediated by binding to and activation of VDR.

As shown in Fig. 6, there seemed to be a reasonably good correlation between affinity for VDR and HL-60 cell differentiation-inducing activity for all optical isomers of **28a–d**. For DPP-1123 (**28a**), DPP-1023 (**28b**), and DPP-0123 (**28c**), the (*R,S*)-isomers showed the most potent activities among the series, as regards both affinity for VDR and HL-60 cell differentiation induction. (*R,S*)-LG190178 [(*R,S*)-**28d**] showed the most potent HL-60 cell differentiation-inducing activity among the optical isomers, though its VDR-binding activity was comparable with that of (*S,S*)-LG190178 [(*S,S*)-**28d**]. In HL-60 cell differentiation-inducing assay, the (*R,S*)-isomers were the most potent VDR agonists among the optical isomers in each series, and the enantiomers, i.e., the (*S,R*)-isomers, showed the lowest activities in both VDR-binding and HL-60 cell differentiation-inducing assays. (*R,S*)-DPP-1023 [(*R,S*)-**28b**] showed the most potent activity among the (*R,S*)-isomers for both VDR-binding and HL-60 cell differentiation, with the latter activity being more potent (EC₅₀ = 4.1 nM) than that of 1,25-dihydroxyvitamin D₃ the (EC₅₀ = 9 nM) [47].

On the other hand, although compounds **29a–d** showed moderate to potent HL-60 cell differentiation-inducing activity, they lacked in VDR-binding activity (except **29b**, which showed very weak binding activity). This discrepancy has been interpreted in terms of fast metabolism of **29a–d** in HL-60 cells, i.e., the pivaloyl compounds **29a–d** were found to be metabolically reduced in HL-60 cells to the corresponding VDR-binding triol derivatives **28a–d** [47]. The importance of the hydroxyl groups of **28a–d** in VDR-binding was also suggested by CAD studies [47].

During the studies, we found that the 3,3-diphenylpentane derivatives mentioned above also possess AR-binding affinity (Table 1) [46,47]. The AR-binding activities of the triol derivatives (**28a–d**) in their optically pure forms were evaluated. For DPP-1123 (**28a**), DPP-1023 (**28b**), and DPP-0123 (**28c**), the (*S,S*)-isomers showed the most potent AR-binding activity in each series of compounds, in contrast to the case of VDR-binding activity, where the (*R,S*)-isomers are the eutomers. (*S,S*)-DPP-0123 [(*S,S*)-**28c**] showed the most potent activity, with a K_i value of 400 nM. Nevertheless, all optical isomers of LG190178 (**28d**) showed similar AR-binding affinity, with K_i values of 1000–1100 nM, suggesting that the exchanged nitrogen atom(s) interacts with some residue(s) in the ligand-binding pocket of AR.

Table 1 AR-binding activity of 3,3-diphenylpentane derivatives.

cmpd	AR affinity (K_i , nM) [and SC-3 growth inhibition (IC ₅₀ , nM)]	cmpd	AR affinity (K_i , nM) [and SC-3 growth inhibition (IC ₅₀ , nM)]
testosterone	9	(<i>R,R</i>)-LG190178 [(<i>R,R</i>)- 28d]	1000
hydroxyflutamide	940 [180]	(<i>S,R</i>)-LG190178 [(<i>S,R</i>)- 28d]	1100
(<i>R,R</i>)-DPP-1123 [(<i>R,R</i>)- 28a]	1100	(<i>R,S</i>)-LG190178 [(<i>R,S</i>)- 28d]	1000
(<i>S,R</i>)-DPP-1123 [(<i>S,R</i>)- 28a]	1100	(<i>S,S</i>)-LG190178 [(<i>S,S</i>)- 28d]	1100 [13]
(<i>R,S</i>)-DPP-1123 [(<i>R,S</i>)- 28a]	2300	(<i>R</i>)-DPP-1113 [(<i>R</i>)- 29a]	2200
(<i>S,S</i>)-DPP-1123 [(<i>S,S</i>)- 28a]	540 [22]	(<i>S</i>)-DPP-1113 [(<i>S</i>)- 29a]	7400
(<i>R,R</i>)-DPP-1023 [(<i>R,R</i>)- 28b]	1200	(<i>R</i>)-DPP-1013 [(<i>R</i>)- 29b]	1500
(<i>S,R</i>)-DPP-1023 [(<i>S,R</i>)- 28b]	1200	(<i>S</i>)-DPP-1013 [(<i>S</i>)- 29b]	2800
(<i>R,S</i>)-DPP-1023 [(<i>R,S</i>)- 28b]	910	DPP-0113 (29c) ^a	1170
(<i>S,S</i>)-DPP-1023 [(<i>S,S</i>)- 28b]	730 [4.7]	(<i>R</i>)-DPP-0113 [(<i>R</i>)- 29c]	3400
(<i>R,R</i>)-DPP-0123 [(<i>R,R</i>)- 28c]	2500	(<i>S</i>)-DPP-0113 [(<i>S</i>)- 29c]	720 [32]
(<i>S,R</i>)-DPP-0123 [(<i>S,R</i>)- 28c]	1100	(<i>R</i>)-DPP-0013 [(<i>R</i>)- 29d]	1100
(<i>R,S</i>)-DPP-0123 [(<i>R,S</i>)- 28c]	1900	(<i>S</i>)-DPP-0013 [(<i>S</i>)- 29d]	1800
(<i>S,S</i>)-DPP-0123 [(<i>S,S</i>)- 28c]	400 [55]		

a: enantiomeric mixture.

In contrast to the case of VDR-binding activity, all the pivaloyl analogs (**29a–d**) showed moderate to potent AR-binding activity with K_i values of 720–7400 nM (Table 1), suggesting that metabolic activation (reduction of the pivaloyl group) is not necessary to elicit AR-binding activity. Among the compounds, (*S*)-DPP-0113 [(*S*)-**29c**] showed the most potent AR-binding activity ($K_i = 720$ nM); this compound is more potent than hydroxyflutamide ($K_i = 940$ nM), an active form of the clinically useful anti-androgenic drug, flutamide.

None of the compounds examined above (**28a–d** and **29a–d**) showed growth-promoting activity on the androgen-dependent cell line SC-3, suggesting they are not androgen agonists, but androgen antagonists [46,47]. In fact, the (*S,S*)-isomers of **28a–d**, i.e., eutomers for AR-binding, were more potent growth inhibitors of testosterone-induced SC-3 cells than was hydroxyflutamide, which is in accordance with their higher affinity for AR than that of hydroxyflutamide (Table 1). (*S,S*)-DPP-0123

[(*S,S*)-**28c**] showed very potent activity, with an IC_{50} value of 4.7 nM, being almost 40 times more potent than hydroxyflutamide [47].

The pivaloyl derivatives (**29a–d**) can be regarded as intrinsically AR-selective ligands, because their reduction was shown to be necessary to bind VDR (*vide supra*). Therefore, non-metabolizable analog(s) (the compounds without a precursor of a hydroxyl group at the corresponding position) might be AR-specific ligand(s). Based on this consideration, we prepared the decarbonylated derivative (**30**) and the ethylene analog (**31**) (Fig. 7). Decarbonylation, i.e., **30**, resulted in complete loss of both VDR-agonistic and AR-antagonistic activities. However, the ethylene analog (**31**) retained AR-antagonistic activity with IC_{50} value of 500 nM for SC-3 cell growth inhibition, and the VDR-agonistic activity estimated in terms of HL-60 cell differentiation induction was reduced to 6360 nM. As for AR/VDR-selectivity based on SC-3 cell growth-inhibiting and HL-60 cell differentiation-inducing activity, compounds **28a–d** and **29a–d** are 1.5- to 100-fold selective for VDR over AR, except (*S,S*)-DPP-0123 [(*S,S*)-**28c**], which is 1.1-fold almost non selective for AR over VDR [47]. In contrast, the non-metabolizable ethylene analog **31** showed 12.7-fold selectivity for AR over VDR.

The reduced analogs **28a–d** are direct ligands for both VDR and AR. Concerning **28a–c**, the (*R,S*)- and (*S,S*)-isomers are the eutomers for VDR and AR, respectively. For **28d**, the (*R,S*)- and (*S,S*)-isomers are comparably potent VDR agonists. The (*S,R*)-isomers are generally the weakest 1,25- VD_3 agonists among all of the compounds **28a–d**. In other words, the stereochemistry at the side chain is critical for the selectivity for NRs.

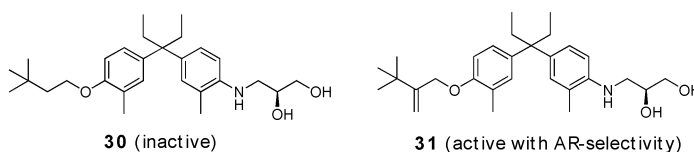


Fig. 7 Structures of nonmetabolizable analogs of 3,3-diphenylpentane-type VDR/AR ligands.

Farnesoid X receptor and peroxisome proliferator-activated receptor agonists

The above results indicate that the 3,3-diphenylpentane skeleton is an effective steroid skeleton substitute, and NR selectivity of the compounds can be attributed to the structure(s) of the side chain(s). In fact, on the basis of this consideration, we recently reported the successful creation of specific ligands for other NRs, including farnesoid X receptor (FXR) and PPAR, based on the 3,3-diphenylpentane skeleton [48].

FXR is a well-characterized member of the so-called “metabolic” subfamily of NRs, and is a transcriptional sensor for bile acids [49]. Its ligands, including chenodeoxycholic acid (CDCA: **32**) (Fig. 8), 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 [49]. Maloney et al. reported GW4064 (**33**) (Fig. 8) as a potent synthetic ligand for FXR [50]. Considering the structures of CDCA (**32**) and GW4064 (**33**), and the potential of 3,3-diphenylpentane as a steroid skeleton substitute, we designed 3,3-diphenylpentane derivatives as FXR ligand candidates, i.e., DPPF-01 (**34**) and DPPF-13 (**35**) (Fig. 8). The R group of DPPF-01 (**34**), containing a carboxylic acid group, was introduced to mimic the carboxylic acid group found in CDCA (**32**) and GW4064 (**33**). The R group of DPPF-13 (**35**), containing a diol moiety, was introduced to mimic side chains found in several oxysterols. In the transcriptional activation reporter gene assay system, the agonistic activity of DPPF-01 (**34**) for transcriptional activation of FXR was far more potent than that of the physiological ligand, CDCA (**32**). The EC_{50} values are 3.4 μ M for DPPF-01 (**34**) and 11.7 μ M for CDCA (**32**) under the experimental conditions employed. DPPF-13 (**35**) also showed agonistic activity toward FXR, though it was less potent than CDCA (**32**).

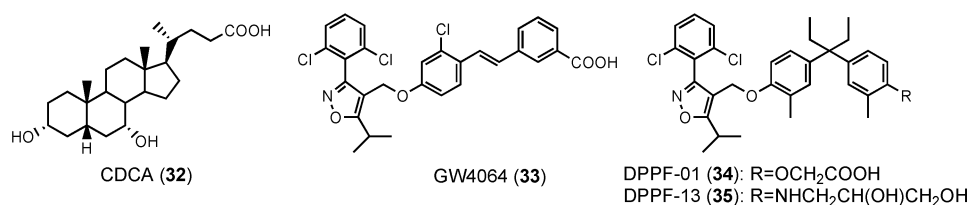


Fig. 8 Structures of CDCA (32), GW4064 (33), and designed compounds (34, 35).

PPAR α is an NR whose physiological ligands are considered to be endogenous fatty acids, and it is well known as the target molecule of fenofibrate (36) (Fig. 9), a drug used to treat dyslipidemia and type 2 diabetes, and whose active form is considered to be its hydrolyzed analog, fenofibric acid (FA: 37) (Fig. 9). Various synthetic PPAR α ligands have been reported, including our phenylpropionic acid derivatives derived from KCL (38) (Fig. 9) [51–53]. PPAR α has a large Y-shaped ligand-binding pocket of approximately 1300–1400 Å³ [54]. Based on the structures of FA (37) and KCL (38), as well as the shape of the ligand-binding pocket of PPAR α and the potential usefulness of the 3,3-diphenylmethane skeleton as a scaffold for PPAR α ligands, we designed a 3,3-diphenylpentane derivative DPPK-01 (39) and a diphenylcyclohexane derivative DPHK-01 (40) (Fig. 9), both of which possess a butyric acid moiety. The butyric acid moiety was introduced to mimic the carboxylic acid side chain of KCL (38), and a cyclohexyl moiety was adopted to provide a rigid Y-shape of the molecule, as the carbonyl group of FA (37) does. According to the transcriptional activation reporter gene assay, DPHK-01 (40) showed more potent agonistic activity for transcriptional activation of PPAR α than did FA (37). The EC₅₀ values were 3.5 μM for DPHK-01 (40) and 9.2 μM for FA (37) under the experimental conditions used. The less rigid derivative, DPPK-01 (39), showed only very weak agonistic activity toward PPAR α .

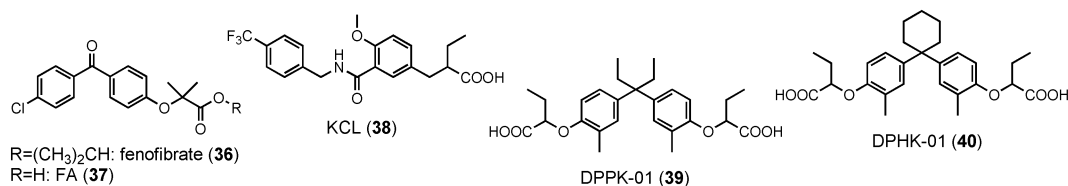


Fig. 9 Structures of fenofibrate (36), FA (37), KCL (38), and designed compounds (39, 40).

Our results suggest the usefulness of the 3,3-diphenylmethane skeleton as a multi-template for NR ligands, and furthermore, the skeleton might be also useful as a pharmacophore for preparing steroidal medicaments other than NR ligands and medicaments whose target molecule(s) are involved in steroid biosynthesis, metabolism, and homeostasis.

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