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Nuclear receptor antagonists designed based on the helix-folding inhibition hypothesis

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Abstract—Here we review our studies on the molecular design of nuclear receptor antagonists, including retinoic acid receptor (RAR) antagonists, retinoid X receptor (RXR) antagonists, androgen receptor (AR) antagonists, and vitamin D receptor (VDR) antagonists, based on inhibition of folding of helix 12, which contains a co-activator binding site. Recent progress in structural development studies of peroxisome proliferator-activated receptor (PPAR) ligands is also reviewed.

1. Introduction

Nuclear receptors (NRs) are ligand-dependent transcription factors, which regulate the expression of responsive genes and thereby affect diverse processes, including cell growth, development, and metabolism.[1,2] Based on the elucidated human genome sequence, 48 NRs are thought to exist in humans.[1] So far, the ligands of only 20–25 of them, including steroid hormone receptors [estrogen receptors (ERs), progesterone receptor (PR), androgen receptor (AR), glucocorticoid receptor (GR), and mineral corticoid receptor (MR)], retinoid receptors [retinoic acid receptors (RARs) and retinoid X (9-*cis*-retinoic acid) receptors (RXRs)], thyroxine hormone receptors (TRs), vitamin D receptor (VDR), peroxisome proliferator-activated receptors (PPARs), liver X (oxysterol) receptors (LXRs), farnesoid (bile acid) receptor (FXR), and steroid xenobiotic receptor (SXR), have been identified.[1,3]

Broadly speaking, the structural/functional features of NRs are similar in spite of the wide variation in their ligands' structures, that is, NRs generally consist of an amino-terminal region, which encodes a ligand-independent transcriptional activation function (AF-1), a DNA-binding domain (DBD) with a motif structure of two zinc fingers, which has a sequence-specific DNA (response element: RE)-binding function, and a large carboxyl-terminal region (ligand-binding domain: LBD), which incorporates a specific ligand-binding function, a dimerization function, and a ligand-dependent activation function (AF-2).[1–6]

With some exceptions, NRs bind with one of the so-called co-repressors in the absence of their ligands (*apo*-form NRs), and act as transcriptional suppressors of the responsive genes. As a simple example, co-repressors have affinity for histone deacetylase (HDAC) and act as adaptors combining an NR and an HDAC, resulting in transcriptional repression. Ligand binding to NRs (*holo*-form NRs) results in dissociation of the co-repressor, and the transcriptional level of the responsive gene recovers to the basal level. At the same time, one of the so-called co-activators is recruited and binds to a specific site located in the LBD, which results in transcriptional enhancement of the responsive gene (Fig. 1).[4] Generally, co-activators possess affinity for RNA polymerase II (RNA pol II), acting as a connector combining an NR with the transcriptional machinery, and resulting in transcriptional activation. Various co-repressors and co-activators exist, some of them being cell-type/tissue specific.

In the ligand-dependent activation of NRs, a general and important structural feature has been elucidated based on the X-ray crystal analysis of several LBDs with (*holo*-form) or without (*apo*-form) the ligand, that is, the ligand-induced folding of the helix 12 (H12), which is one of the LBD substructures. 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).[7]

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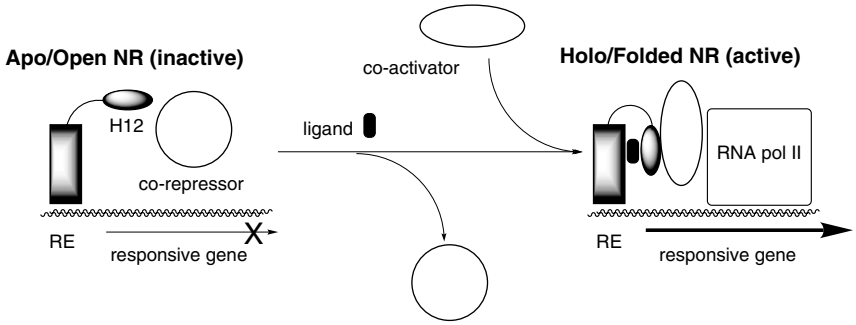

Figure 1. A schematic illustration of the transcriptional activation function of NR.

We considered that this major conformational change induced by ligand binding is the key structural feature in the activation of NRs. Based on this consideration, a compound which 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). In this article, we first describe our structural development studies of antagonists for RAR, RXR, AR, and VDR, and then we review recent progress in structural development studies of PPAR ligands, including work by other researchers.

2. Classification of nuclear receptor antagonists

The validity of the H12-folding inhibition hypothesis for molecular design of NR antagonists mentioned above is supported by computer-assisted docking studies (CADS) using reported X-ray crystal structures of the LBD of *holo*-form NRs and known antagonists. For example, CADS of a synthetic estrogen, diesthylstilbestrol (**2**), using the atomic coordinates of the LBD, prepared by deletion of estradiol (**1**) from the reported X-ray structure of ER's LBD complexed with estradiol (**1**) as a template, results in good fitting of diethylstilbestrol (**2**) in the binding pocket. However, in the case of tamoxifen (**3**), a potent estrogen antagonist, CADS indicates a collision of the dimethylaminoethyloxyphenyl moiety of tamoxifen (**3**) with the H12 moiety of ER's LBD, suggesting that the bulky dimethylaminoethyloxyphenyl group inhibits H12 folding.

From the planar-structural viewpoint, the position of the dimethylaminoethyloxyphenyl group of tamoxifen (**3**) corresponds to the 7- and/or 11-position of the steroid skeleton (Fig. 2). Therefore, introduction of a bulky group at the 7- or 11-position of steroid hormones is expected to yield antagonists of the corresponding steroid hormone (ligand superfamily concept).[8] Indeed, a number of known steroid antagonists possess a bulky group at the 7- or 11-position, including ICI154,384 (anti-

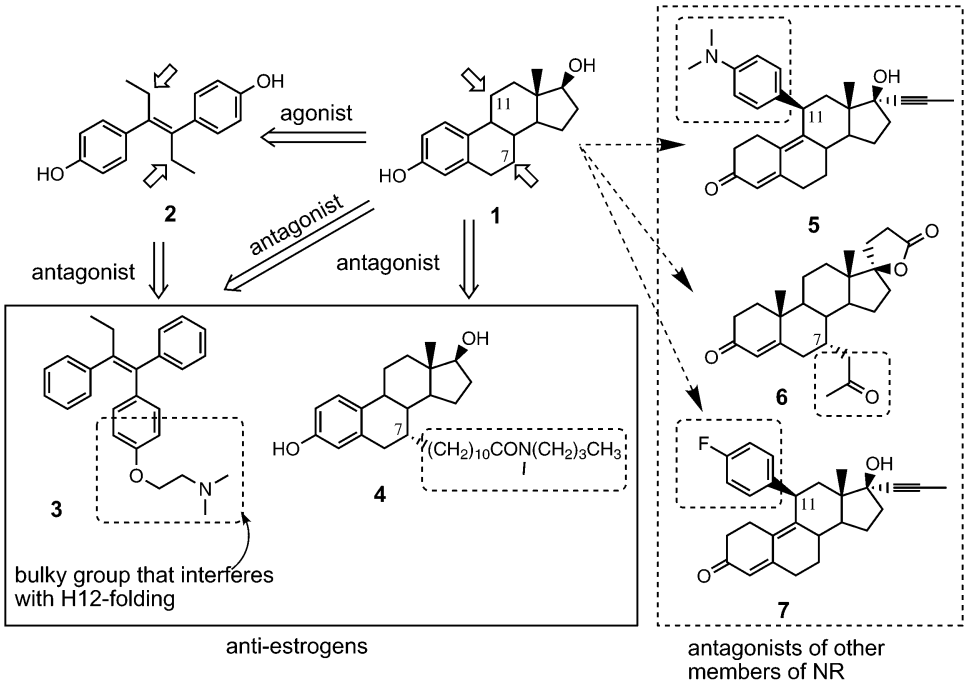

Figure 2. Ligand superfamily concept for molecular design of NR antagonists.

estrogen) (**4**), RU486 (anti-progestin) (**5**), spironolactone (anti-mineral corticoid) (**6**), and RU25,593 (anti-glucocorticoid) (**7**). These steroid hormone antagonists can be regarded as inhibitors of H12 folding owing to the bulky group introduced at the 7- or 11-position of the steroid skeleton.[8]

Based on this concept, the first retinoid antagonist, TD550 (**10**) was created.[9] As expected, CADS of TD550 (**10**) with RAR's LBD suggested collision of the diamantyl moiety of TD550 (**10**) with H12 of the LBD, while all-*trans*-retinoic acid (ATRA: **8**), a natural ligand of RAR, and Am80 (**9**), a synthetic retinoid, precisely fitted into the ligand pocket (Fig. 3).

There are also other NR antagonists, which appear to fit into the ligand-binding pocket as far as investigated by CADS, and which are structurally similar in size to the corresponding agonist. A possible interpretation is that these antagonists induce misfolding of H12, making the receptor unable to bind with co-activator(s) (Fig. 4).[10] Such antagonists are expected to act as partial antagonists/agonists, because NRs with misfolded H12 might still bind some kinds of co-activator(s).

Of course, this classification of NR antagonists is just a formal/expedient one. The former type of antagonists, that is, H12-folding inhibitors, are defined as ligands, which do not fit into the ligand-binding pocket owing to collision with the H12 moiety as far as calculated by CADS. These antagonists are expected to act as full antagonists. On the other hand, the latter type of antagonists, that is, H12-misfolding inhibitors, are defined as antagonists, which can fit into the ligand-binding pocket as far as calculated using CADS.

3. Retinoic acid receptor (RAR) antagonists

RAR is a receptor of ATRA (**8**), which is an active form of vitamin A (except for function in vision), and its bioisosters [called retinoids, including ATRA (**8**)]. The ability of retinoids to modulate the growth and differentiation of a wide variety of normal and transformed

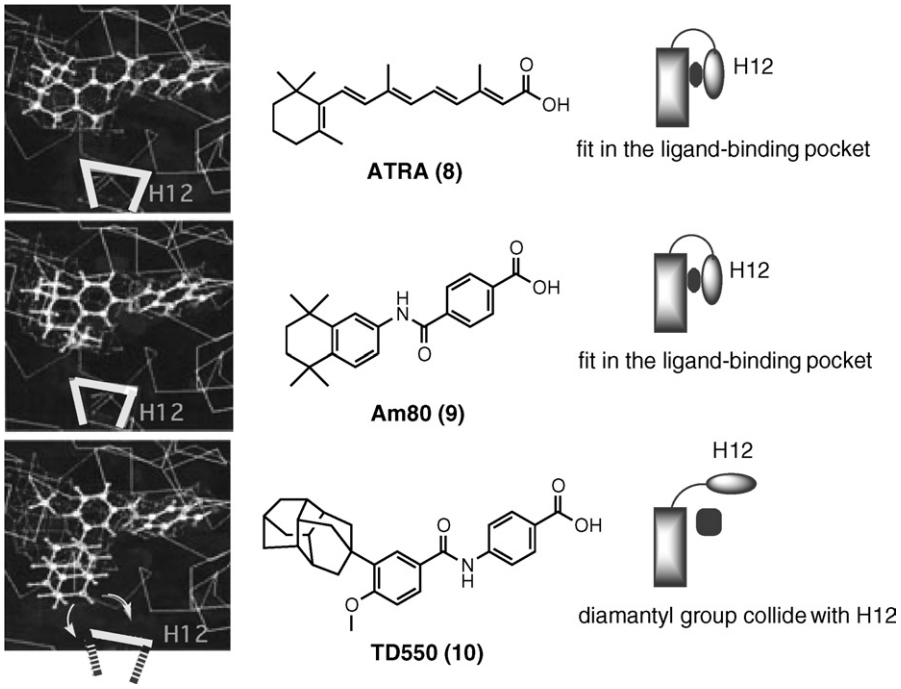

Figure 3. CADS of retinoids with the LBD of RARα.

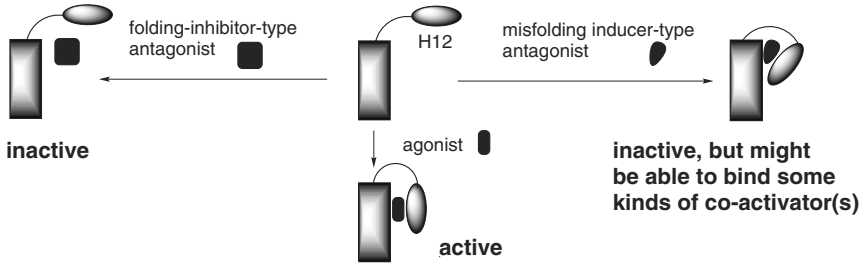

Figure 4. Classification of NR antagonists.

cells has been reported, with ATRA (**8**) shown to be significantly involved in the control of embryonic development and cell differentiation.[5,6] Retinoids have received much attention from a clinical standpoint because they are useful in the treatment of vitamin A deficiency, proliferative dermatological diseases including skin cancer and psoriasis, leukemia, and several types of tumors, as well as for chemoprevention of cancer. ATRA (**8**) has been established as the first-choice medicament for the treatment of acute promyelocytic leukemia (APL).[10] Consequently, a large number of retinoids has been synthesized for potential clinical application.[5,6]

As regards the target molecule of retinoids, there are three subtypes of RAR, that is, RARα, RARβ, and RARγ. Each of them is a retinoid-dependent transcription factor, which acts as a heterodimer with another member of the NRs, RXR (vide infra). A close relationship between aberrancy of RARs and malignancy of cells has been well documented,[6,11,12] that is, truncated RARα 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 RARβ in some types of lung tumors, expression of a dominant negative isoform of RARβ 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 (**8**) and other conventional retinoids with a hydrocarbon skeleton. The major disadvantage of ATRA (**8**)/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 (**8**), leading to benzanilide derivatives, of which a typical example is Am80 (**9**) (Fig. 5).[5,6,13–18] One of unique features of Am80 (**9**) is the lack of binding affinity toward cellular retinoic acid binding protein (CRABP).[19–21] Among the anilide-type retinoids, Am80 (**9**) possesses potent cell differentiation-inducing activity and was successful in a phase II clinical study for the treatment of APL (it is currently passing through the official approval process in Japan), while TAC101 (**11**),[5,6,15–18,22] with two trimethylsilyl groups in its structure, possesses potent anti-angiogenic activity and anti-hepato-metastasis activity, and is now under phase I/II clinical study for the treatment of solid tumors in the United States. Both Am80 (**9**) and TAC101 (**11**) are RARα/β-selective retinoids.[20,23–26] Further structural development based on CADS resulted in non-benzoic acid types of retinoids (Fig. 5).[15,16]

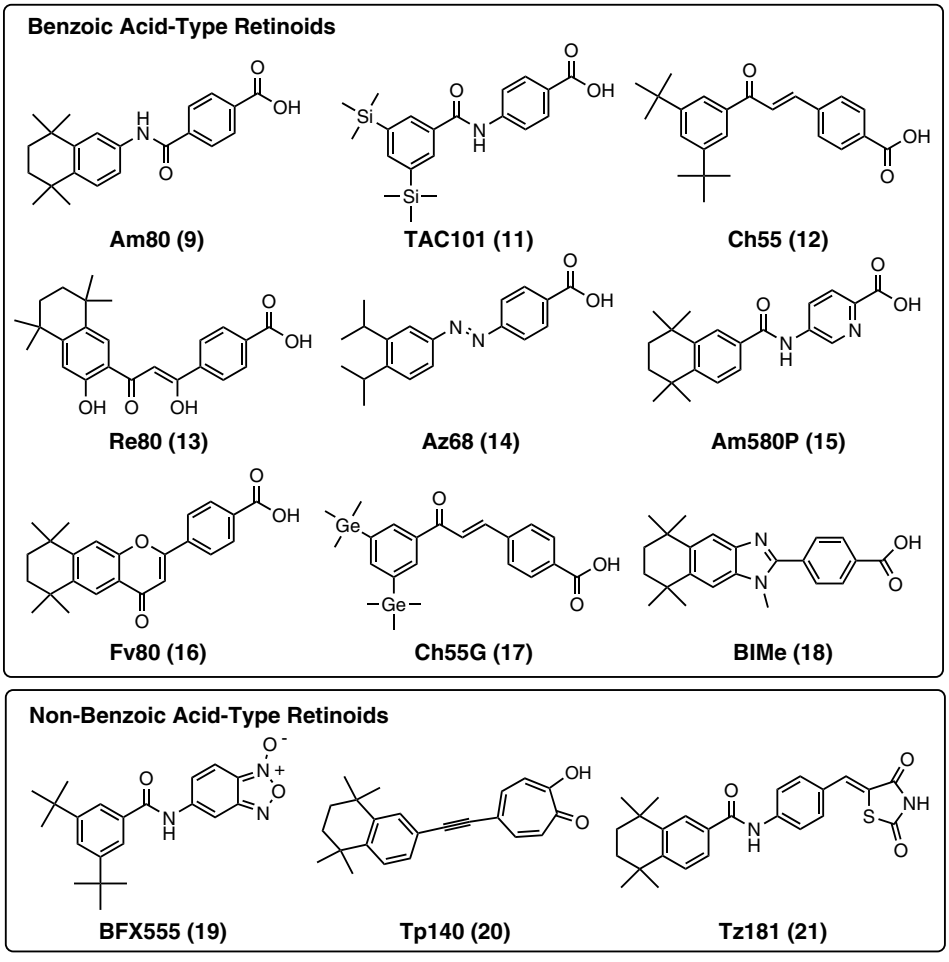

Figure 5. Structures of typical retinoids synthesized in our laboratory.

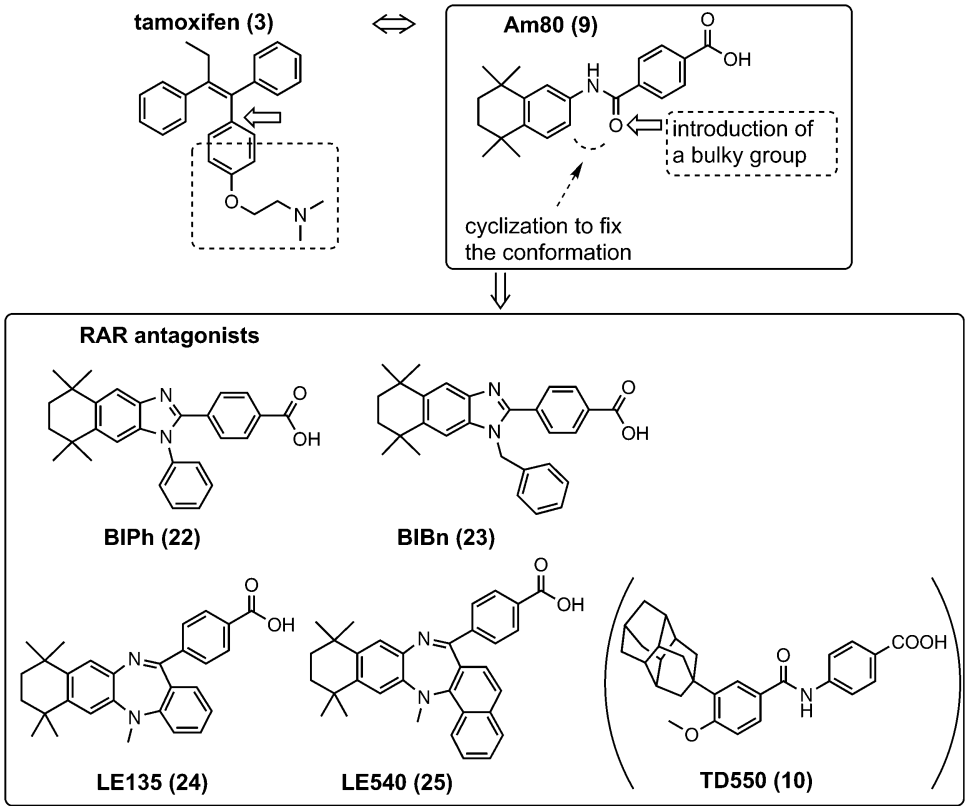

Figure 6. Design of RAR antagonists based on the ligand superfamily concept.

Our initial structural development studies of RAR agonists indicated that a heteroatom(s), especially a nitrogen atom(s), can be introduced into a retinoid skeleton without loss of the retinoidal activity. By using this fact together with the above-mentioned ligand superfamily concept for molecular design of NR antagonists (Fig. 2), the structure of Am80 (**9**) can be modified to yield RAR antagonists (Fig. 6). [8,17,18] Namely, we designed structural mimics of Am80 (**9**) in which a bulky group is introduced at the position corresponding to the dimethylaminoethyloxyphenyl-substituted position of tamoxifen (**3**), followed by cyclization to fix the conformation (Fig. 6).[8,27,28] The compounds thus designed proved to be RAR antagonists, as expected, based on inhibition of retinoid-induced cell differentiation of human leukemia cell line HL-60 and inhibition of retinoid-induced transcriptional activation of RARs.[9,27,28] Among them, LE135 (**24**) is an RARβ -selective antagonist.[27,29] By the use of these RAR antagonists, it was shown that RARβ plays a critical role in the growth-inhibitory effects of retinoids by promoting apoptosis in breast cancer cells.[12] RAR antagonists should be useful for the treatment of hypervitaminosis A and malignant diseases caused by expression of a dominant negative isoform of RARβ.

4. Retinoid X receptor (RXR) antagonists

RXR is a receptor whose ligand has been identified as 9-*cis*-retinoic acid (9cRA: **26**) (Fig. 7). 9cRA (**26**) can also bind and activate RARs, while the RAR ligand ATRA (**8**) does not bind to RXR. Although there is a ligand for RXR, the major role of RXR seems to be as a partner in heterodimer formation with other NRs, including RARs, TRs, VDR, PPARs, and some other orphan receptors.[1,5,6] The ligand 9cRA (**26**) is not necessary for the heterodimerization of RXR. Steroid hormone receptors (ERs, PR, AR, GR, and MR) do not form heterodimers, but act as homodimers. There are three subtypes of RXR, that is, RXRα, RXRβ, and RXRγ.

Our RAR agonists listed in Figure 5 are all RAR-specific and possess no affinity toward RXRs. Our RAR antagonists listed in Figure 6 are also RAR-specific, except for LE540 (**25**), that is, LE135 (**24**) is RAR-specific, but fusion of an additional benzene ring to it afforded LE540 (**25**), which exhibits a weak affinity for RXR (Fig. 7). This suggests that LE540 (**25**) can be regarded as a structural mimic of both ATRA (**8**) and 9cRA (**26**). On this basis, we designed HX600 (**27**) and its analogs (**28**, **29**), in which a tetramethylcyclohexyl moiety corresponding to that in LE540 (**25**) is transferred to the equivalent position in 9cRA (**26**) (Fig. 7), aiming at an RXR-specific ligand.[17,18,28,30,31] These HX compounds (**27**–**29**) indeed proved to be RXR-specific agonists with no affinity for RARs. They act as a potent retinoid synergists in cell differentiation-inducing assay using HL-60 cells and in RAR transcriptional activation assay.[15–18,28,30,31] Other types of RXR-specific agonists have been created similarly and by the use of CADS (Fig. 7).[15–18,32,33]

CADS of HX600 (**27**) with the LBD of RXRα indicated that the region around the D-phenyl ring moiety (Fig. 8)

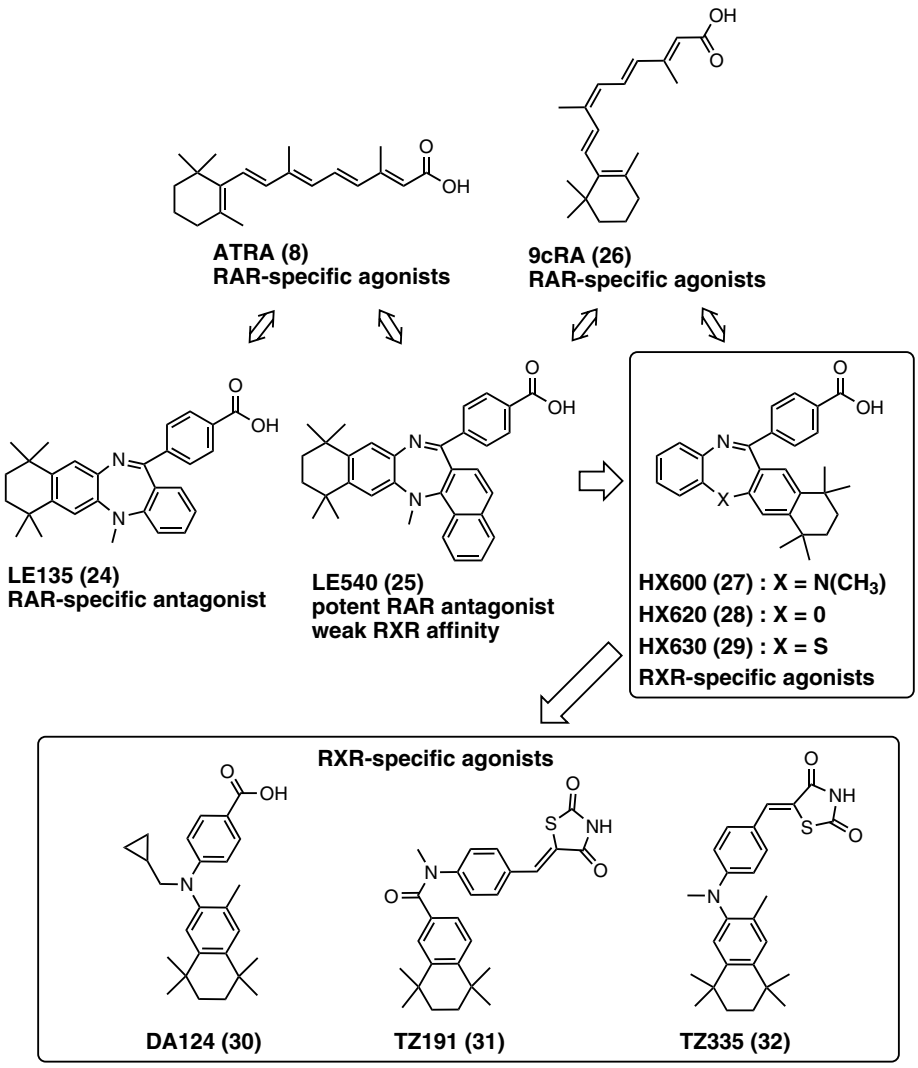

Figure 7. Conversion of RAR ligands to RXR ligands.

and the b-nitrogen atom (Fig. 8) is the site of interaction with H12, suggesting that introduction of a substituent into the D-phenyl ring or at the b-nitrogen atom might result in an RXR antagonist, in which the introduced substituent would inhibit the folding of H12. Among these designed compounds, HX531 (**33**) and HX603 (**34**) proved to be RXR antagonists (Fig. 8).[15–18] Other types of RXR antagonists have also been created.[34,35] These RXR antagonists act as inhibitors of not only RXR/RAR heterodimer-mediated pathways, but also PPAR/RXR heterodimer-mediated pathways. Because PPAR is an NR, which regulates lipid metabolism (vide infra), the inhibitory activity of RXR toward PPAR function suggests potential applicability of the above compounds as medicaments for the treatment of diabetes and obesity.[36,37]

5. Androgen receptor (AR) antagonists

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.[38] Androgens play diverse physiological and pathophysiological roles in both males and females.[38,39] 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 for prostate tumor.[39] There are two structural types of classical AR antagonists, which have been used in therapeutics, that is, steroidal and anilide types (Fig. 9). A typical steroidal AR antagonist is cyproterone acetate (CPA: **36**), which was the first drug to have been used orally, but CPA also interacts with other NRs, PR, and GR.[40,41] Anilide analogs were the first group of non-steroidal AR antagonists to be discovered, and they have been used therapeutically for the treatment of prostate tumor.[38,42] Flutamide (**37**) was the first non-steroidal AR antagonist to enter clinical use, but its active metabolite, 2-hydroxyflutamide (**38**) is cleared so rapidly

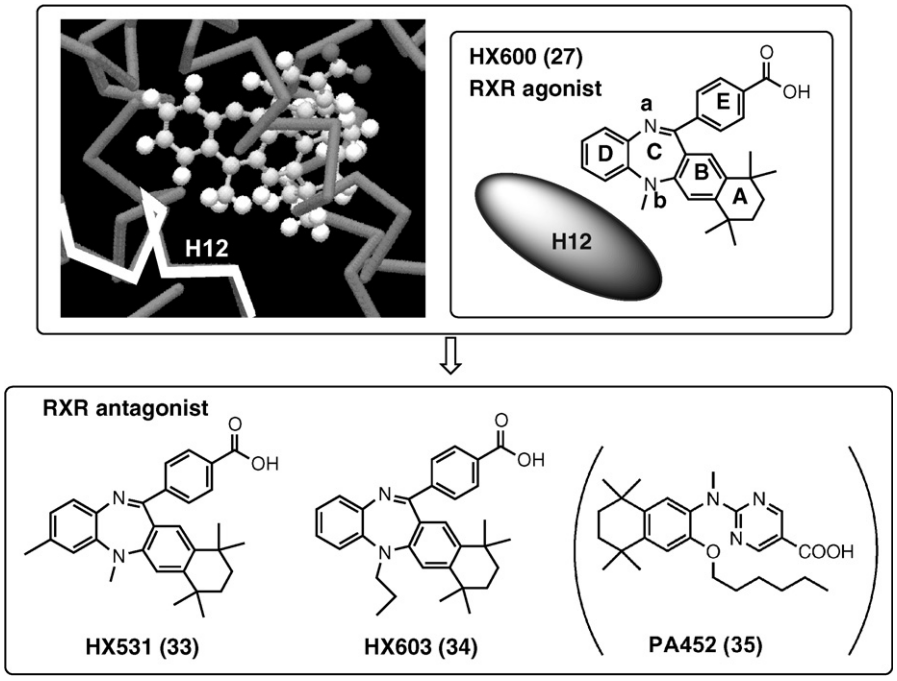

Figure 8. Design of RXR antagonists based on the ligand superfamily concept.

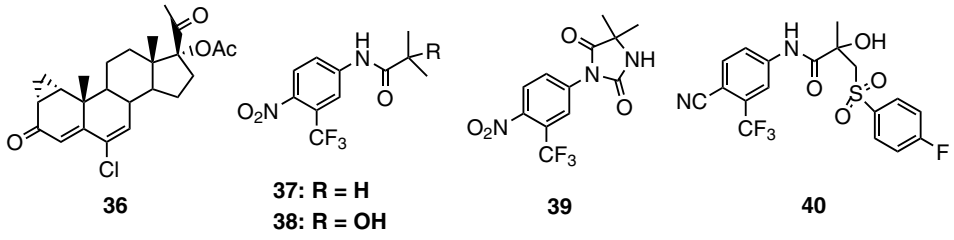

Figure 9. Structures of AR antagonists used therapeutically.

that it requires frequent dosing (three times a day).[38,43] A cyclic derivative of flutamide (**37**), nilutamide (**39**), can be administered once a day, but it elicits side effects including hot flushes, transient night blindness, and alcohol intolerance.[38,44] Bicalutamide (**40**), which is marketed as a racemic mixture with the *R*-isomer being the eutomer, is superior to both flutamide (**37**) and nilutamide (**39**) in terms of its pharmacokinetics and side effect profile.[38,45] Discovery of these non-steroidal AR antagonists stimulated researchers to develop other structural types of androgen antagonists.

We have been engaged in structural development studies of AR antagonists based on the structure of thalidomide (**41**) (Fig. 10).[15,16,46–50] Thalidomide (**41**) is a sedative/hypnotic drug, which was withdrawn from the market in the 1960s because of its severe teratogenicity, but it has been established to be effective for the treatment of various diseases, including leprosy, myeloma, AIDS, tumors including prostate tumor, and others.[46,47,51,52] The drug was approved in 1998 in the United States for the treatment of leprosy, and clinical studies of its use for the treatment of various cancers, including multiple myeloma, colon cancer, prostate tumor, and breast cancer are on-going.[51,52]

Our initial studies yielded phthalimide-type AR antagonists, including compounds **42**–**46** (Fig. 10).[48] These AR antagonists are structurally related to DIMP (**47**), which is a well-known AR antagonist, though it is not used clinically.[53,54] 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.[48] An anilide analog **48**, which was derived from tetrafluorophthalimide analogs **42**, **45**, and **46**, also showed anti-androgenic activity.[48] We also reported azo/azoxybenzene types of AR antagonists, including compounds **49** and **50** (Fig. 10).[55] These AR antagonists shown in Figures 9 and 10 are considered to be misfolding inducer-type AR antagonists (Fig. 4), as judged from CADS data.

The major obstacle in the treatment of prostate tumor with AR antagonists is the sudden appearance of AR antagonist-resistant cells. These cells have generally lost androgen dependency, and further, AR antagonists

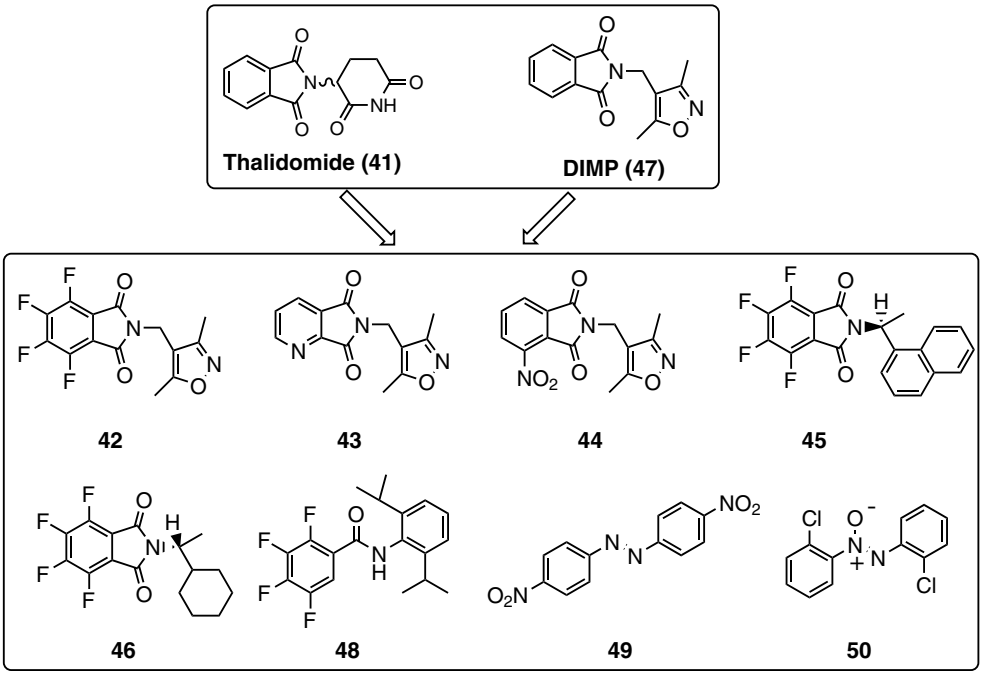

Figure 10. AR antagonists structurally derived from thalidomide (**41**) and DIMP (**47**).

promote their growth, whereas AR antagonists had suppressed cell growth before the acquisition of resistance. One major molecular mechanism of such resistance is point mutation of AR. Some point mutations, including T877A and T874H, are clinically well established.[56,57] ARs which possess such a point mutation are considered to take an H12-folded conformation, and are constitutively active even in the absence of the cognate ligand, androgen (Fig. 11). Of course, these mutated ARs can bind androgens and misfolding inducer-type AR antagonists, which stabilize the active conformation of the AR, leading to super-activation of the mutated ARs. 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 the folding) of H12, would be useful (Fig. 11).

CADS presents only misfolding inducer-type antagonists because the *holo*-form LBD (H12-folded conformation) is used as a template for docking study calculation. Therefore, candidates into which a bulky substituent can be introduced at the region where H12 interacts should be chosen from the group of compounds presented by CADS. One of such compound is the isoxazolone derivative **51** (Fig. 12).[50] Using this

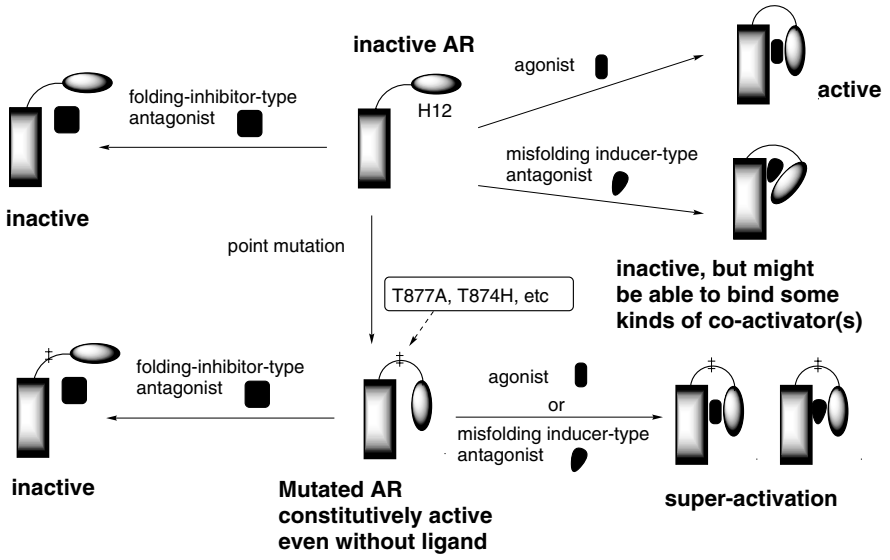

Figure 11. Mechanism of AR antagonist resistance based on point mutation of AR.

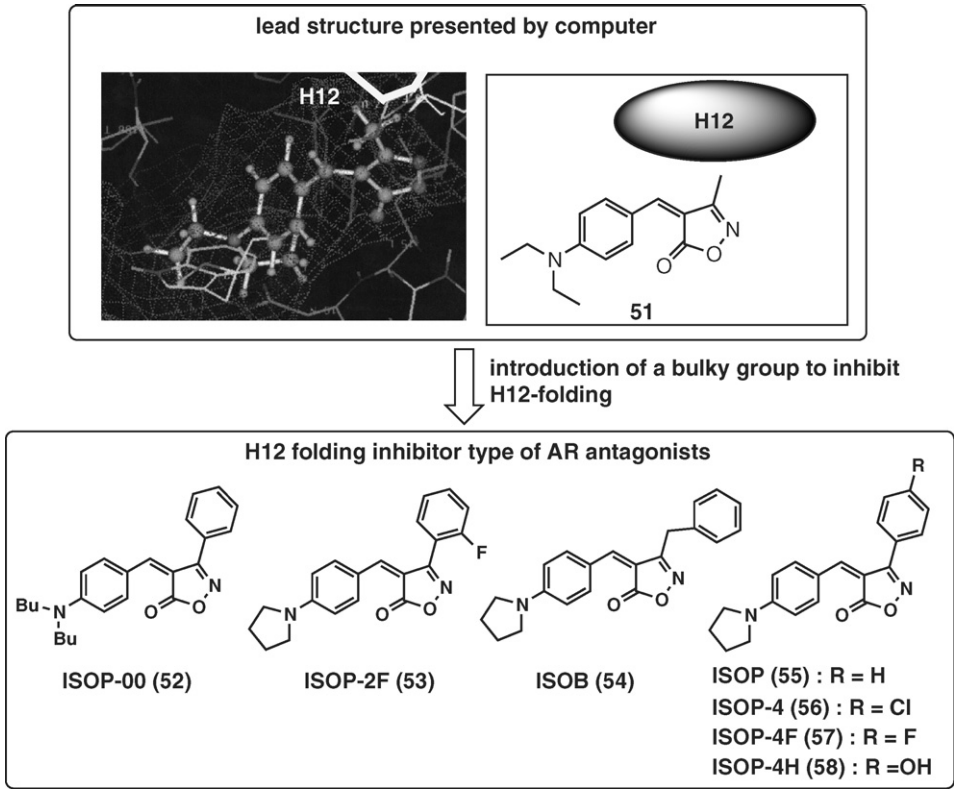

Figure 12. Molecular design of H12 folding inhibitor type of AR antagonists.

skeleton as a scaffold, we introduced various substituents at the position at which H12 interacts, aiming to obtain H12 folding inhibitor-type AR antagonists (**52**–**58**) (Fig. 12).[50]

Among the isoxazolone-type AR antagonists thus created, listed in Figure 12 (**52**–**57**), ISOP-4 (**56**), and ISOB (**54**) possess high binding affinity for AR. In growth promotion/inhibition assay using androgen-dependent SC-3 cells, none of the isoxazolone-type compounds (**52**–**58**) showed growth-promoting activity, suggesting that none is an androgen agonist, and all of them showed dose-dependent growth-inhibitory activity on SC-3 cells cultured in the presence of 10 nM testosterone. The unique feature of these AR antagonists (**52**–**58**) is their activity toward androgen-independent cells, which express point-mutated, constitutively active ARs, that is, human prostate tumor cell lines LNCaP and 22Rv1, which express T877A and T874H point-mutated ARs, respectively.[56,57] ISOB/ISOPs (**52**–**58**) show growth-inhibitory activity on these cells, while known classical AR antagonists, including 2-hydroxyflutamide (**38**), show growth-promoting activity. This result suggests that ISOB/ISOPs (**52**–**58**) do not elicit androgenic activity (i.e., do not super-activate the mutated ARs), but act as AR antagonists on LNCaP and 22Rv1 cells, while 2-hydroxyflutamide (**38**) acts as an AR agonist on these cells.

Similar results were obtained by quantitative assessment of androgenic and anti-androgenic activities based on measurement of the amount of prostate-specific antigen (PSA) produced by LNCaP cells. PSA is a marker molecule of prostate tumor malignancy and its production is known to be induced by androgens. Therefore, androgenic activity can be detected in terms of enhanced production of PSA, and anti-androgenic activity can be assessed in terms of inhibition of PSA production induced by androgens. LNCaP cells incubated under normal conditions produce a small amount of PSA, and addition of 10 nM testosterone markedly enhances the production. 2-Hydroxyflutamide (**38**) also enhances the production by 4–6 times, indicating that it acts as an AR agonist toward LNCaP cells, which is in accordance with the cell growth promotion/inhibition assay data for LNCaP and 22Rv1 described above. ISOB/ISOPs (**52**–**58**) do not enhance the PSA production at all, indicating that these compounds possess no androgenic activity. In the inhibition assay of testosterone-induced PSA production, 2-hydroxyflutamide (**38**) was almost completely inactive. This result suggests that 2-hydroxyflutamide (**38**) acts as a full AR agonist toward LNCaP cells in the assay system. In contrast, ISOB/ISOPs (**52**–**58**) show inhibitory activity on testosterone-induced PSA production. Above all, ISOB/ISOPs (**52**–**58**) appear to act as full AR antagonists even on LNCaP cells, which constitutively express active mutated AR.

6. Vitamin D_3 receptor (VDR) antagonists

VDR is a receptor of 1,25-dihydroxyvitamin D_3 (**59**) (Fig. 13), which is an active form of vitamin D and plays critical roles in a variety of biological activities, including regulation of calcium homeostasis, bone

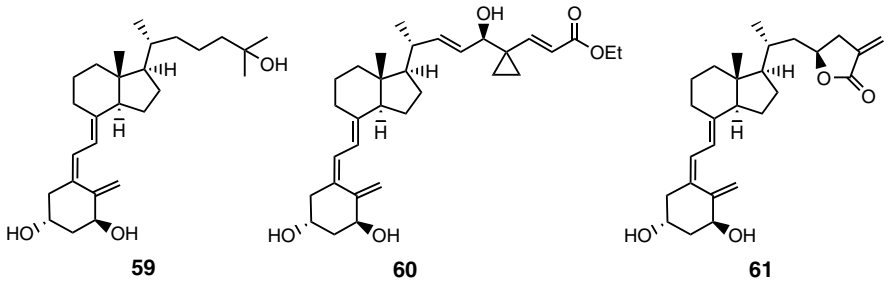

Figure 13. Structures of 1,25-dihydroxyvitamin D3 (**59**) and known VDR antagonists (**60**, **61**).

mineralization, and control of cellular growth, differentiation, and apoptosis. VDR antagonists can be expected to be useful for the treatment of Paget's disease.[58] So far, more than 3000 derivatives of 1,25-dihydroxyvitamin D_3 (**59**) have been synthesized, but only two types of compounds, ZK168281 (**60**) and its analogs from Schering and TEI-9647 (**61**) from Teijin, have so far been reported as VDR antagonists.[59,60]

On the basis of CADS using the *holo*-form LBD of VDR, we designed a novel VDR antagonist possessing a nitrogen atom in its structure, that is, DLAM-1P (**62a**–**d**) (Fig. 14).[61] The nitrogen atom was chosen as a key component to install in the side chain, which is expected to inhibit the folding of H12.

DLAM-1P (**62a**–**d**) was synthesized using 1,3-dipolar cycloaddition reaction of the nitrone derivative **63**, which was prepared via reaction of vitamin D_2 with methyl methacrylate (**64**) as a key step (Fig. 15).[61,62] In this 1,3-dipolar cycloaddition reaction, four configurational isomers are obtained with the (*S*,*S*)- and (*R*,*R*)-isomers at the newly created asymmetric carbons being major products.

Among the four configurational isomers of DLAM-1P (**62 a**–**d**), the (23*S*,25*S*)-isomer (**62a**) showed the highest affinity toward VDR and only this isomer showed VDR antagonistic activity with similar potency to that of TEI-9647 (**61**) for inhibition of 1,25-dihydroxyvitamin D_3 (**59**)-induced cell differentiation of HL-60 cells and in a VDR transcriptional activation reporter gene assay system.[61]

In the VDR transcriptional activation reporter gene assay, TEI-9647 (**61**) showed slight activation activity at a very high concentration (10 μM), while (23*S*,25*S*)-DLAM-1P (**62a**) did not show any VDR activation activity. This result suggests that (23*S*,25*S*)-DLAM-1P (**62a**) is a full VDR antagonist, that is, an H12-folding inhibitor. In agreement with this, CADS of (23*S*,25*S*)-DLAM-1P (**62a**) using the *holo*-form LBD of VDR suggested collision of the *N*-benzyl group of (23*S*,25*S*)-DLAM-1P (**62a**) with phenylalanine 422 existing in the H12 of VDR's LBD (Fig. 16).

Collision between the phenyl ring of the *N*-benzyl moiety of (24*S*,25*S*)-DLAM-1P (**62a**) and the phenyl ring of Phe422 in H12 is predicted.

7. Peroxisome proliferator-activated receptor (PPAR) antagonists

Peroxisome proliferator-activated receptors (PPARs) are also members of the NR superfamily, and are

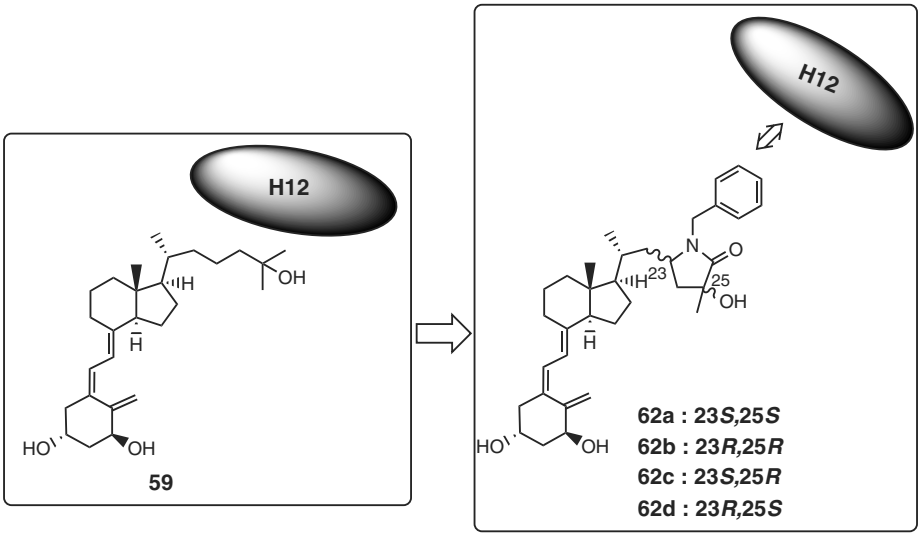

Figure 14. Design of VDR antagonists based on the H12-folding inhibition hypothesis.

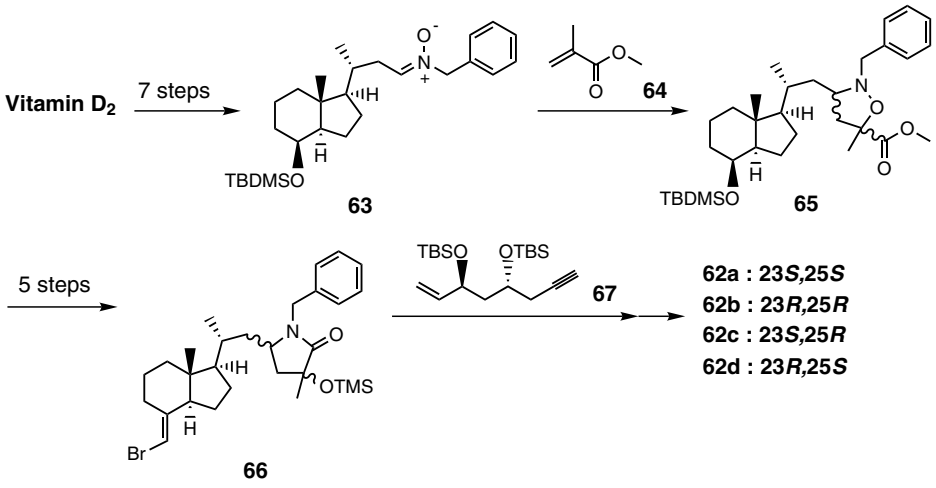

Figure 15. Synthesis of DLAM-1P (**62**).

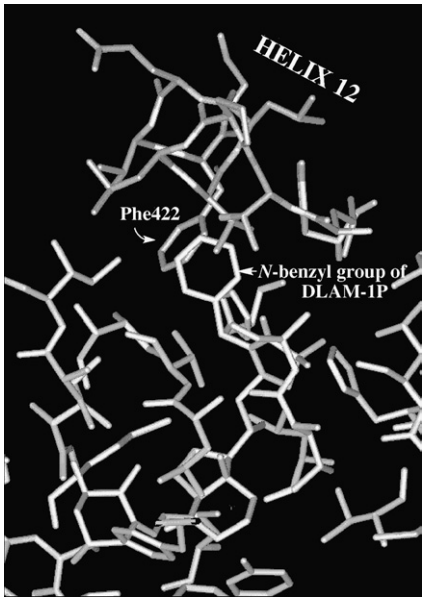

Figure 16. CADS of (24*S*,25*S*)-DLAM-1P (**62a**) with *holo*-LBD of VDR.

activated by endogenous saturated and unsaturated fatty acids, some kinds of metabolites of fatty acids, and various synthetic ligands.[63,64] PPARs are heterogeneous, and three subtypes have been isolated to date; PPARα, PPARδ, and PPARγ. Both PPARα and PPARγ are well defined, probably because of their medicinal interest and/or possible importance for the treatment of Type 2 diabetes and dyslipidemia, metabolic disorders, which affect up to about 20% of the population in industrialized societies.[65–68] Although PPARs were only cloned slightly more than a decade ago, the rapid progress in functional analysis of these receptors has established that they play a central role in regulating the storage and catabolism of glucose and lipid in both animals and humans.

The primary therapy for Type II diabetes is life-style modifications and oral hypoglycemic agents, such as sulfonylureas and biguanides. Sulfonylureas, which stimulate insulin release from pancreatic β-cells, are often only moderately effective and can induce hypoglycemia and weight gain; they are also subject to both primary and secondary failure, so an attractive alternative approach would be to attenuate insulin resistance without stimulating insulin secretion. Following the excellent pioneering work by Takdea Co. Ltd.,[69] the thiazolidine-2,4-dione (TZD) PPARγ full agonists (glitazones) have been recognized as a new class of anti-diabetic agents. Glitazones exhibit their beneficial pharmacological effect(s) by binding to and activating (as full agonists) PPARγ, which is mainly expressed in insulin-sensitive target tissues, such as adipose tissue, liver, and skeletal muscle.[70]

On the other hand, recent molecular pharmacological studies have indicated that a moderate reduction of PPARγ activity observed in heterozygous PPARγ-deficient mice can prevent insulin resistance and obesity induced by a high-fat diet by decreasing serum leptin levels.[71] Therefore, both PPARγ full agonists and PPARγ partial agonists (antagonists) could be useful for the treatment of type 2 diabetes.

Recent X-ray crystal structure analysis of the LBD of PPARγ indicated that the LBD is very similar to other nuclear-receptor structures in terms of overall folding from helix 3 (H3) to the C-terminal end.[72] But PPAR LBD is unique in its overall tertiary structure, and contains an extra helix, designated H2′, between the first β-strand and H3. The tertiary alignment of H2 in PPAR is different from that in other nuclear-receptor LBD tertiary structures.[72] PPARs have a large Y-shaped ligand-binding pocket of approximately 1300–1400 $Å^3$. The increased size of the ligand-binding pocket relative to that of other nuclear receptors (by comparison, the binding pocket of RXRα is about 470 $Å^3$) accounts for the ability of the PPARs to accommodate a wide variety of ligands.

Crystal structures of TZD-based (**70**) and carboxylic acid-based (**71**, **74**) (Fig. 17) agonists complexed with PPARγ LBD reveal a common binding mode in which

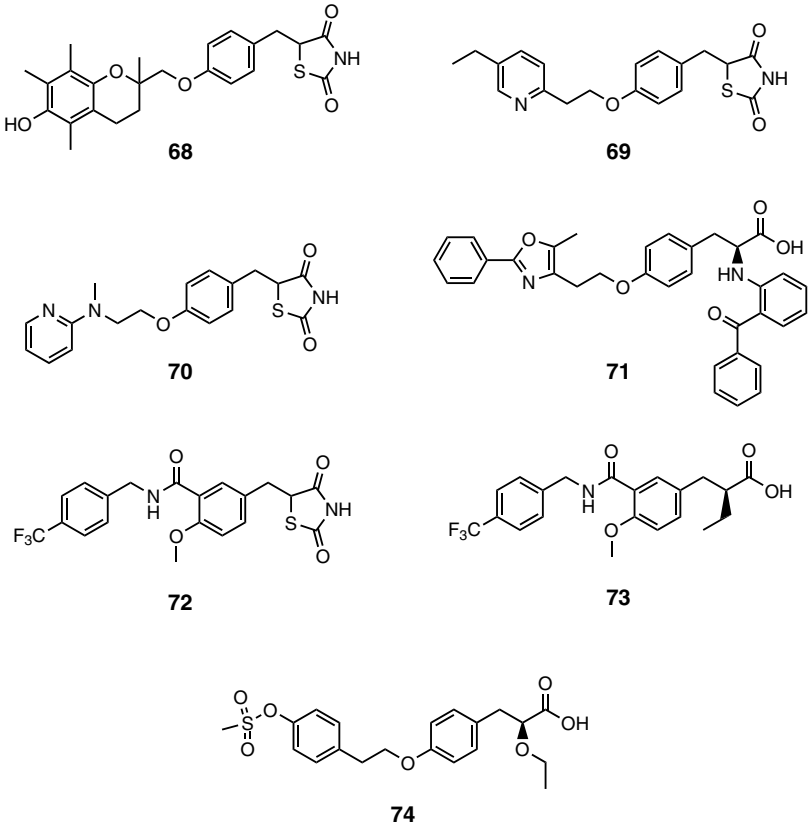

Figure 17. Structures of PPARγ full agonist Troglitazone (**68**), Pioglitazone (**69**), Roziglitazone (**70**), Farglitazar (**71**), PPARγ/α dual-agonist KRP-297 (**72**), Tesaglitazar (**74**), and PPARα agonist KCL (**74**).

the acidic moiety of the ligands forms a conserved hydrogen-bonding network with His449, Tyr473, His323, Ser289, and Gln286.[72–74] Tyr473 is located in the C-terminal AF-2 helix, which appears to be crucial for achieving a conformation conducive to co-activator recruitment and subsequent DNA binding.

First-generation glitazones, such as troglitazone (**68**),[75] pioglitazone (**69**),[76] and rosiglitazone (**70**)[77] (Fig. 17) were discovered prior to the cloning of PPARγ and without knowledge of their mechanism of action. Structural optimization of these drugs was carried out mainly by the use of conventional in vivo assays. However, the advent of cloned PPARγ, in addition to recent parallel high-throughput screening methods, with binding assays and/or transactivation assays, prompted a search for more potent and subtype-selective PPARγ agonists, leading to Farglitazar (**71**),[78] PPARγ/α dual-agonists such as KRP-297 (**72**),[79] and PPARα agonists such as KCL (**73**)[80–83] (Fig. 17). The molecular volume of Farglitazar (**71**) is very large as compared to those of traditional nuclear receptor agonists, but Farglitazar (**71**) still exhibited full PPARγ agonistic activity in both in vitro and in vivo assays, presumably due to the extremely large ligand-binding pocket of PPARγ. As far as we know, there has been no report on folding inhibitor-type PPARγ antagonists. So, rational design of folding inhibitor-type antagonists of PPARγ represents an interesting challenge.

Notably, there is a remarkable report from Glaxo Smithkline Inc. about a misfolding-type PPARγ partial agonist (antagonist), GW-0072 (**75**) (Fig. 18).[84] GW-0072 (**75**) is a carboxylic acid derivative, like other PPARγ agonists, but X-ray crystal structure analysis indicated that GW-0072 (**75**) occupies the region of the ligand-binding pocket bounded by helices 3, 6, and 7.[84] Unlike other known PPAR agonists, the carboxylic acid of GW-0072 (**75**) is aligned toward the loop region between H2′ and H3 and does not come into direct contact with the AF-2 helix. It is important to note that the residues Tyr473, His449, and His323 take conformations that are shifted from their agonist-bound positions, but are similar to those in the *apo*-PPAR crystal structure, resulting in a receptor conformation that may accommodate co-activators not stabilized through direct interactions with the ligand.

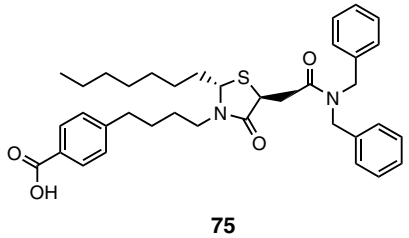

Figure 18. Structure of PPARγ partial agonist, GW-0072 (**75**).

Although GW-0072 (**75**) interacts with the PPARγ ligand binding pocket in a manner distinct from other known agonist ligands, it could not induce conversion of multipotential stem cells into adipocytes and it is a potent antagonist of TZD-induced adipocyte differentiation. Therefore GW-0072 (**75**) will be an important pharmacoprotophore to create partial agonists and/or antagonists for the treatment of human metabolic diseases.

8. Concluding remark

The NRs are attractive targets for drug design to treat various chronic diseases. We have designed and prepared various NR antagonists based on the H12-folding inhibition hypothesis. This simple approach should be generally applicable to NRs in addition to those described in this article. However, the existence of H12-misfolding inducer-type partial antagonists and various agonists, which exhibit relatively specific effects, as well as the existence of various kinds of co-repressors and co-activators, which associate with NRs, suggests that the H12-folding inhibition hypothesis may be an oversimplification, and that different H12-folded conformations induced by ligands with different structures result in selective dissociation of co-repressors and selective binding of co-activators. Therefore, more specific ligands, which can selectively dissociate certain co-repressor(s) and recruit certain co-activator(s), that is, which can discriminate the plural functions of NRs, may be obtainable by more precise conformational control of H12 folding.

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