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Novel retinoid X receptor (RXR) antagonists having a dicarba-*closo*-dodecaborane as a hydrophobic moiety

Kiminori Ohta,[a] Toru Iijima,[b] Emiko Kawachi,[b,†] Hiroyuki Kagechika[b,†] and Yasuyuki Endo[a,*]

[a]*Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1, Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
[b]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

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Abstract—We designed and synthesized novel retinoid X receptor (RXR)-selective antagonists bearing a carborane moiety. Compounds **8a**–**d** or **9a**–**d** themselves have no differentiation-inducing activity toward HL-60 cells and no inhibitory activity towards a retinoic acid receptor (RAR) agonist. However, they inhibit the synergistic activity of an RXR agonist, PA024, in the presence of Am80 on the cell differentiation of HL-60. Transactivation assay using RARs and RXRs suggested that the inhibitory activity of **9b** resulted from the selective antagonism at the RXR site of RXR–RAR heterodimers.

1. Introduction

Carboranes (dicarba-*closo*-dodecaboranes)[1] are a class of carbon-containing boron cluster compounds that exhibit remarkable thermal and chemical stability. Because of their high boron content, they were applied in the field of boron neutron capture therapy (BNCT).[2] Since carboranes are stable in vivo,[2b] they are likely to possess a wide range of possible applications in the field of medicinal chemistry. Therefore, we have focused on the utility of carboranes as a hydrophobic component in biologically active compounds. Recently, we reported the design, synthesis and biological evaluation of a range of receptor modulators containing carboranes as a hydrophobic pharmacophore.[3–5]

Retinoids modulate various biological functions, such as cell differentiation, proliferation and embryonic development in vertebrates,[6,7] through binding to and activating two types of specific nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). RARs and RXRs each have three subtypes α, β, γ and their endogenous ligands are all-*trans*-retinoic acid (**1**) and 9-*cis*-retinoic acid (**2**), respectively.[8,9] RXR is a silent partner of RAR, and RXR–RAR heterodimers can be activated by an RAR agonist, such as Am80 (**3**), but not by an RXR agonist. On the other hand, an RXR agonist such as PA024 (**4**)[10] acts as a retinoid synergist, since it dose-dependently increases the activity of a low concentration of RAR agonist.[11] Besides their retinoidal actions, RXRs play key roles through heterodimer formation with various nuclear receptors, such as thyroid hormone receptors (TRs), vitamin D receptors (VDRs), and peroxisome proliferator-activated receptors (PPARs). Therefore, agents that can alter the behavior of RXRs in these heterodimers may have potent biological effects. For example, an RXR agonist stimulated the differentiation of fatty cells by activation of PPAR–RXR heterodimers to improve insulin resistance.[12] Recently, we reported that the RXR antagonist HX531 (**5**) improved both insulin resistance and obesity through modulation of PPAR–RXR activity.[13] Therefore, specific ligands for RXRs are needed in order to clarify the actions of the agonists and antagonists, though only a few RXR antagonists, including PA452 (**6**), have been reported so far.[14] In this article, we describe the design, synthesis and biological activities

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* Corresponding author. Tel.: +81 22 234 4181; fax: +81 22 275 2013; e-mail: yendo@tohoku-pharm.ac.jp
† Present address: School of Biomedical Science, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan.

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of novel carborane derivatives as specific RXR antagonists.

2. Results and discussion

Our design approach to novel RXR antagonist candidates is illustrated in Scheme 1. Previously, we reported that DA010 is a ligand for both RARs and RXRs, while DA113 is an RXR-specific agonist.[10a] Comparison of their structure–activity relationships indicated that RAR ligands require a planar molecular shape, and RXR ligands require a twisted molecular shape (Fig. 1). Crystal structure of the ligand-binding domain of the RXRα has a more pronounced bending angle than that of the RARγ. The orientation and location of the hydrophobic interaction sites are different between RARγ and RXRα.[15a] On the other hand, we have developed RAR agonists, such as BR503 (**7**),[3a,c] having a carborane as the hydrophobic moiety; this is significant structural feature, since it will interact with the hydrophobic region of the ligand-binding cavity of RAR. Considering these two structural factors, we designed compounds **8** and **9** as RXR modulator candidates. A methyl group on the aromatic nucleus and a substituent on the nitrogen atom may influence the twisting conformation at the phenyl–*N*-phenyl moiety, which is preferred for an RXR ligand. These candidates are expected to be RXR antagonists because the distance between the bulky hydrophobic group (carborane cage) and the nitrogen atom is longer than that of the potent RXR agonist, DA113. In addition, the bulky carborane may cause antagonism by disturbing the helix12 folding.[15b]

The syntheses of the designed molecules **8** are summarized in Schemes 2 and 3. 2-Methyl-5-bromoaniline **10** was coupled with ethyl 4-iodobenzoate in the presence of Pd(0) and *rac*-BINAP by means of the Buckwald amination (43%),[16] followed by *N*-*n*-propylation to give compound **11** in 93% yield. After Sonogashira reaction of **11** with ethynyltrimethylsilane catalyzed by $PdCl_2(PPh_3)_2$ and CuI (quantitative)[17] and desilylation (94%), the acetylene derivative reacted with decaborane (14) in the presence of Et_2S[18] to give the compound bearing *o*-carborane **12** in 54% yield. After introduction of various 2-alkyl groups (79–96%) on the carborane cage, the ester group was hydrolyzed under acidic conditions to give the corresponding carboxylic acids **8a**–**d** in 63–91% yields.

Compound **8e**, which has a phenyl group at the 2-position of *o*-carborane, was obtained by Sonogashira reaction using ethynylbenzene instead of ethynyltrimethylsilane (92%), followed by construction of the *o*-carborane cage (57%) and ester hydrolysis under acidic conditions (95%).

The syntheses of the designed molecules **9** are shown in Scheme 4. 1-Tolyl-*o*-carborane (**14**), prepared from *o*-carborane with 4-iodotoluene in 70% yield,[19] was nitrated with mixed acid, HNO_3 and concentrated H_2SO_4 (87%), followed by reduction of the nitro group (99%) and *C*-ethylation of *o*-carborane (92%) to give **15**. The aniline **15** was reacted with ethyl 4-iodobenzoate under the Buckwald amination conditions to give a key diphenylamine intermediate **16** bearing an *o*-carborane cage in 64% yield. Alkylation on the nitrogen atom of **16** (70–86%), followed by acidic hydrolysis afforded the desired molecules **9a**–**d** (48–89%).

The biological activity of the synthesized compounds, **8** and **9**, was first examined in terms of the ability to induce differentiation of human promyelocytic leukemia cells HL-60.[10b,14c] Differentiated cells were identified by nitro blue tetrazolium (NBT) reduction assay.[10b,14c] Compounds **8** and **9** did not show differentiation-inducing activity alone or in the presence of an RXR agonist, PA024 (**4**). Also, they did not affect the differentiation-inducing activity of an RAR agonist, Am80 (**3**) (data not shown). These data suggest that they act as neither

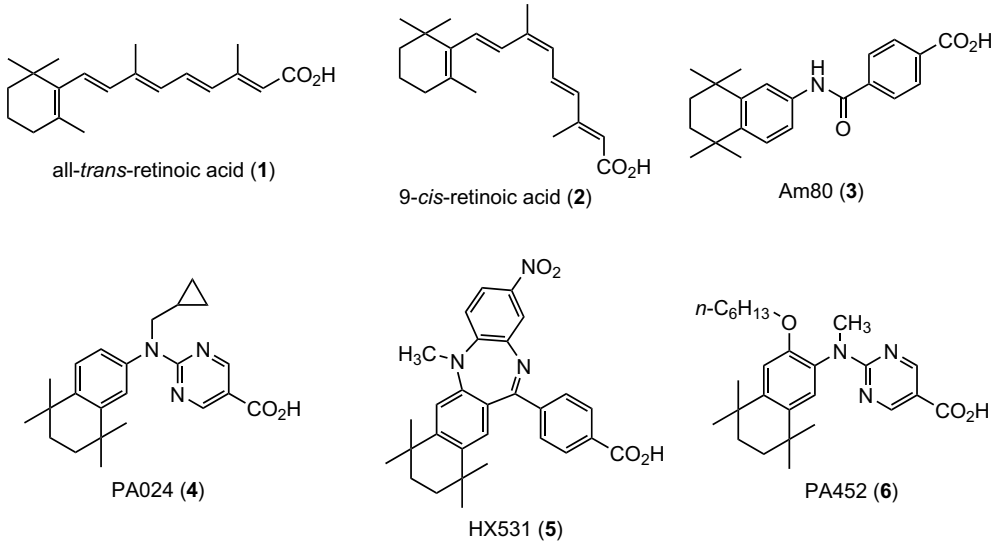
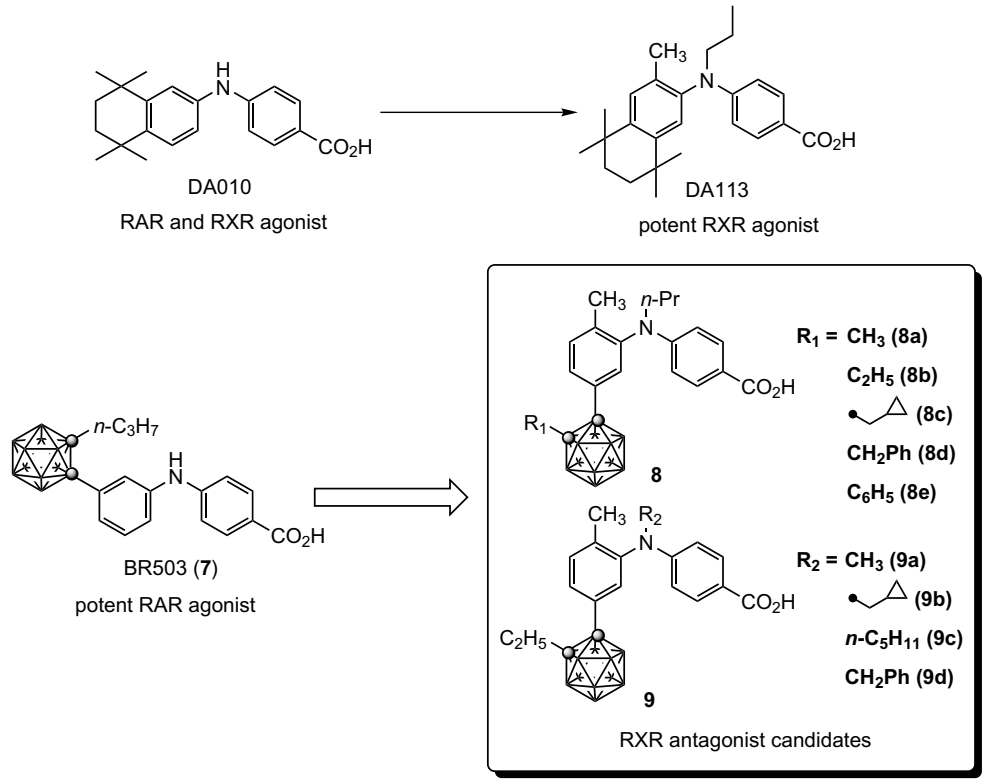
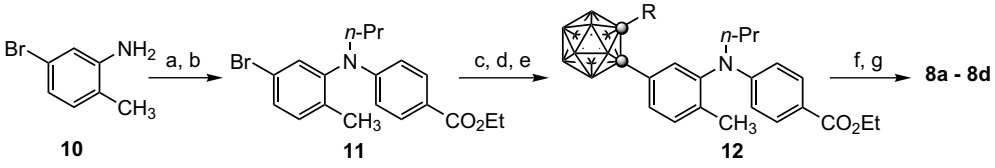


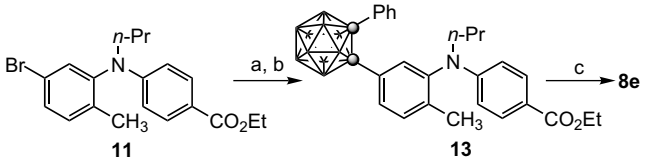
Figure 1. Structure of RAR or RXR ligands.



Scheme 1. Design of novel RXR antagonist containing carboranes.

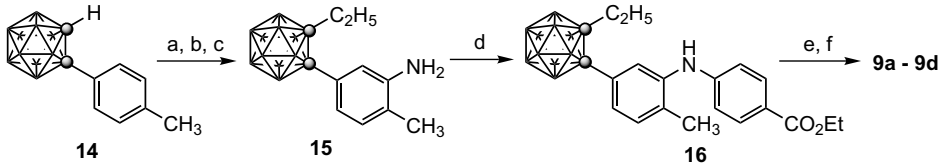


Scheme 2. Synthetic scheme of *C*-substituted derivatives **8a–d**. Reagents and conditions: (a) $Pd_2(dba)_3$, *rac*-BINAP, NaO^{t}Bu, ethyl 4-iodobenzoate, toluene; (b) NaH, *n*-PrI, DMF; (c) $PdCl_2(PPh_3)$, CuI, TMSacetylene, diisopropylamine, THF; (d) TBAF, THF; (e) decaborane(14), Et_2S, toluene; (f) NaH, RX, DMF; (g) concd H_2SO_4, H_2O, dioxane.



Scheme 3. Synthetic scheme of *C*-phenyl derivatives **8e**. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, ethynylbenzene, diisopropylamine, THF; (b) decaborane(14), Et_2S, toluene; (c) concd H_2SO_4, H_2O, dioxane.

RAR agonists nor antagonists. However, compounds **8** and **9**, excluding **8e** (inactive), inhibited the differentiation induced by the combination of Am80 (**3**) and PA024 (**4**) in a dose-dependent manner (Fig. 2). The effect of the *C*-substituent of *o*-carborane on the antagonistic activity is shown in Figure 2. When the *C*-substituent was a shorter alkyl unit, such as methyl or ethyl, the antagonist activity increased, and the *C*-ethyl derivative **8b** was a more potent antagonist than the known RXR antagonist, PA452 (**6**). In contrast, the



Scheme 4. Synthetic scheme of *N*-substituted derivatives **9a–d**. Reagents and conditions: (a) HNO_3, H_2SO_4; (b) Zn, AcOH; (c) NaH, EtI, DMF; (d) $Pd_2(dba)_3$, *rac*-NaO^{t}Bu, ethyl 4-iodobenzonate, toluene; (e) NaH, RX, DMF; (f) concd H_2SO_4, H_2O, dioxane.

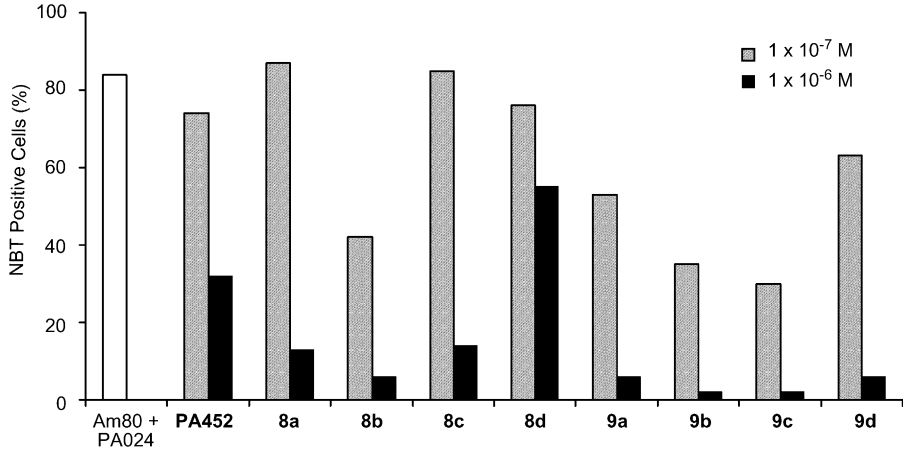


Figure 2. Effect of compounds **8a–d** and **9a–d** on HL-60 cell differentiation induced by 1×10^{-10} M Am80 (**3**) in the presence of 1×10^{-10} M PA024 (**4**).

compound having a benzyl group (**8d**) had almost no antagonist activity. The effect of the *N*-substituent was also investigated among the *C*-ethyl derivatives (Fig. 2). The *N*-cyclopropylmethyl derivative **9b** and *N*-*n*-pentyl derivative **9c** were more potent antagonist than **8b**. Although the inhibition by PA452 (**6**) did not reach the basal level, even at 1×10^{-6} M, the *C*-*n*-ethyl derivatives **9a–d**, including,[8b] inhibited the activity to the basal level. In particular, **9b** and **9c** inhibited the differentiation by the combination of Am80 (**3**) and PA024 (**4**) to 30–40% at the concentration of 1×10^{-7} M.

The results of HL-60 differentiation assay suggest that the antagonist activity of **8** and **9** arises from antagonism at the RXR site of RAR–RXR heterodimers. To confirm the inhibitory mechanism of these compounds, transient transactivation assay using RARs and RXRs was conducted for **9b** (Fig. 3).[10b,14c] When the potent

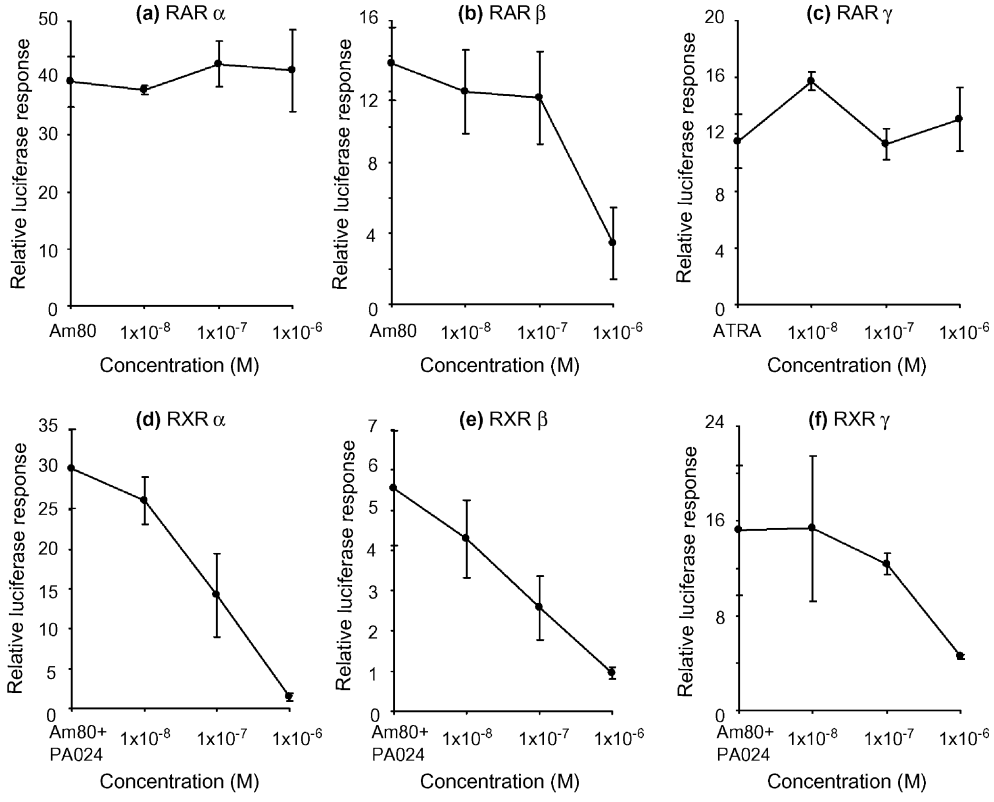


Figure 3. Transactivation assays were carried out using COS-1 cells transfected with hRAR(α, β, or γ) and $(TREpal)_3$-TKLUC or mRXR(α, β, or γ) and $(DR1)_5$-pGL-TK. The receptor transactivation was induced with 3×10^{-9} M Am80 (**3**), 1×10^{-8} M Am80 (**3**), 1×10^{-8} M ATRA (**1**) and 1×10^{-8} M PA024 (**4**) for RARα, RARβ, RARγ and all RXR receptors, respectively. The vertical scale is the transactivation relative to that with EtOH taken as 1.

compound **9b** was examined alone, it did not activate either RAR or RXR subtypes (data not shown). The compound **9b** did not inhibit the transactivation of RARα and RARγ activated by Am80 (**3**) or ATRA (**1**), while **9b** hardly inhibited the transactivation of RARβ activated by Am80 (**3**) in the concentration of 1×10^{-6} M (Fig. 3a–c). On the other hand, the transactivation induced by 1×10^{-8} M PA024 (**4**) with all subtypes of RXRs was dose-dependently inhibited by **9b** (Fig. 3d–f). These results suggest that **8a–d** and **9a–d** antagonize the RXR agonist at the RXR site when both RAR and RXR agonists bind to the heterodimers, and consequently, the retinoid synergism by the RXR agonist is decreased.

The effect of the methyl group on the aromatic ring may arise from changes in the twisting conformation between the two aromatic rings. A carboranyl *C*-substituent may influence the hydrophobic interaction between ligand and receptor, since the structure–activity relationships for the *C*-substituent in derivatives **8** resembled those of BR503 analogues.[3a] A carborane cage may play important part in loss of further stabilization between holoRXR sites and co-activators, because retinoid synergism is closely related to the stabilization of the complex of activated RAR–RXR heterodimer and co-activators caused by binding of RXR ligands.[20]

In conclusion, we have found novel RXR-antagonistic diphenylamines bearing an *o*-carborane moiety, and the RXR antagonistic activity could be separated from retinoid agonistic activity by introduction of a methyl group on the aromatic ring or alkyl groups on the nitrogen atom. These carboranes provide a useful tool to modulate the modes of action of nuclear receptors, owing to their bulky spherical structure and hydrophobic molecular surface. Since an RXR receptor forms heterodimers with various kinds of nuclear receptors, these RXR ligands should have the ability to modulate the functions of other nuclear receptors as well as RAR. These potent RXR modulators are excellent candidates for a new class of therapeutic agents. The biological potency, receptor selectivities and further structure–activity relationships of these carborane-containing RXR antagonists are under investigation.

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