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Letters

Novel Retinoid X Receptor Antagonists: Specific Inhibition of Retinoid Synergism in RXR−RAR Heterodimer Actions

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Abstract: Several 2-(arylamino)pyrimidine-5-carboxylic acids were designed as novel retinoid X receptor (RXR) antagonists. Compound **6a** or **6b** alone did not exhibit differentiation-inducing activity toward HL-60 cells and did not affect the activity of a retinoic acid receptor (RAR) agonist, Am80, but did inhibit the synergistic activity of an RXR agonist, PA024 (**3**), in the presence of Am80. The activity of **6** was ascribed to selective antagonism at the RXR site of RXR−RAR heterodimers.

Introduction. Retinoid X receptors (RXRs) exist in three subtypes (α, *β*, and *γ* forms),[1] and their endogenous ligand has been identified as 9-*cis*-retinoic acid (**1**, Chart 1), which also has high affinities for retinoic acid receptors (RARs).[2] While RXRs act as retinoid receptors, their key role is in heterodimer formation with various nuclear receptors, including RARs, vitamin D_3 receptors, thyroid hormone receptors, and peroxisome proliferator-activated receptors (PPARs). Studies using RXR-selective agonists, such as LGD1069 (**2**) and PA024 (**3**), have shown that RXR ligands act in different ways, depending on the heterodimer partner.[1] For example, RXR agonists alone cannot activate RXR−RAR heterodimers but enhance the potency of RAR agonists,[3] while they can activate PPAR−RXR heterodimers as well as PPAR agonists.[4] In contrast to the various RXR-selective agonists reported so far,[5] only a few RXR antagonists have been reported. Further, LG100754 (**4**), the first reported RXR antagonist, is an RXR homodimer-selective antagonist[6] and acts as an agonist of several RXR heterodimers.[7] Previously, we reported that diazepine derivatives HX531 (**5a**) and HX603 (**5b**) are RXR antagonists, which can inhibit RXR heterodimers.[8] Although these diazepines also inhibited activation of RARs induced by RAR agonists at high dose, they exhibited antidiabetic and antiobesity activities by regulating the activities of PPAR-*γ*−RXR heterodimers.[9] In view of the clinical potential of antidiabetic and antiobesity agents, we focused on the development of more potent and/or selective RXR antagonists. Here, we describe novel RXR-selective antagonists, which can inhibit specifically the synergistic activity of an RXR agonist on RXR−RAR heterodimer actions.

Chemistry. Regarding the design of RXR antagonist candidates, we used the RXR agonist−antagonist structure−activity relationship of diazepinylbenzoic acids (**5**) to develop the structure of the potent RXR agonist **3**. As reported before, the introduction of a suitable substituent on the aromatic ring (X in Chart 1) or elongation of the *N*-methyl group of the RXR agonist HX600 (**5c**) resulted in RXR antagonistic activity.[8] Of two antagonists **5a** and **5b**, the latter, having an *N*-*n*-propyl group, is rather RXR-selective, although its potency is weaker. Considering that the *n*-propylamino group of **5b** should correspond to the substituent ortho to the amino group of PA compounds (RO− group in Chart 1), we synthesized several 2-[*N*-(3-alkoxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-*N*-methylamino]pyrimidine-5-carboxylic acids. The synthetic scheme is shown in Scheme 1.[10] 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthol (**7**) was nitrated with HNO_3 in CH_2Cl_2 to give **8** as a major product (71% yield). After O-methylation and subsequent hydrogenation of the nitro group, compound **10** was reacted with ethyl 2-chloropyrimidine-5-carboxylate in the presence of K_2CO_3 at 110 °C to give ethyl 2-(arylamino)pyrimidine-5-carboxylate (**11**) in 94% yield. After N-methylation and

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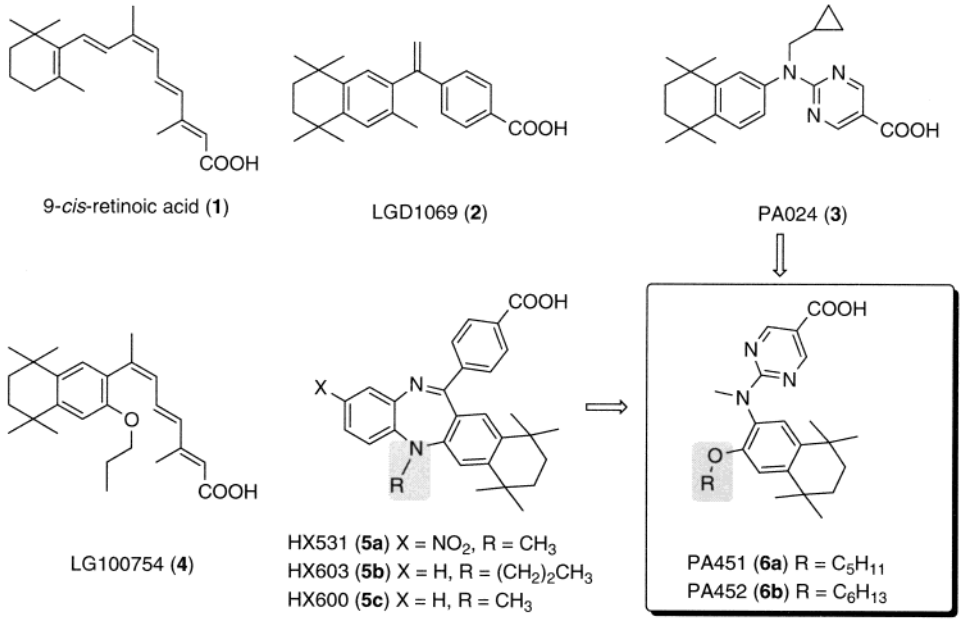
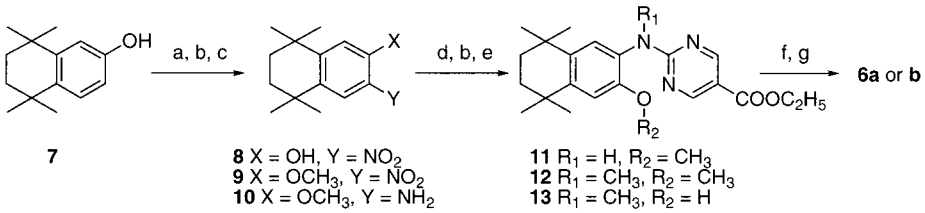
Chart 1. Structures of RXR Ligands



Scheme 1. Synthetic Scheme of PA451 (**6a**) and PA452 (**6b**)[a]



[a] (a) HNO_3, CH_2Cl_2; (b) NaH, DMF; CH_3I; (c) H_2, Pd−C, EtOH; (d) ethyl 2-chloropyrimidine-5-carboxylate, K_2CO_3, △; (e) BBr_3, CH_2Cl_2; (f) NaH, DMF, *n*-$C_5H_{11}I$ or *n*-$C_6H_{13}Br$; (g) KOH, EtOH.

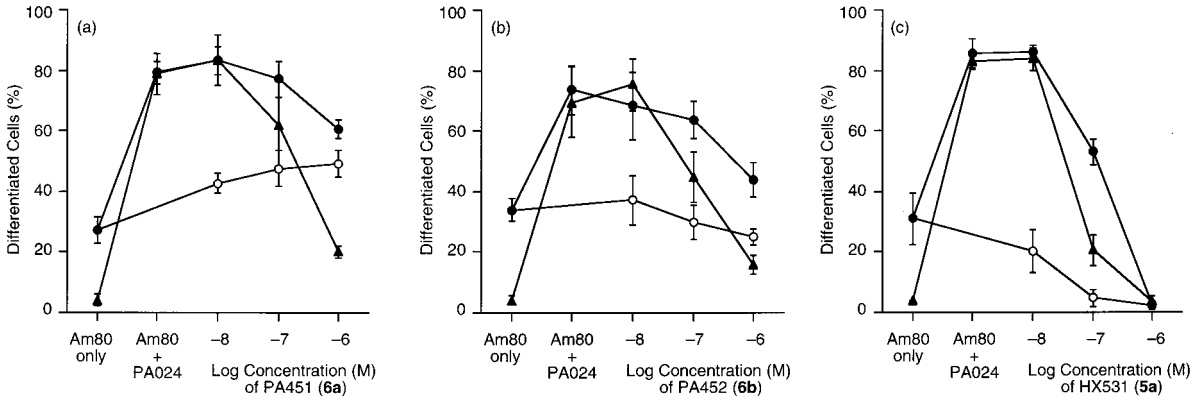
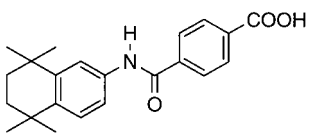


Figure 1. Effects of (a) PA451 (**6a**), (b) PA452 (**6b**), and (c) HX531 (**5a**) on HL-60 cell differentiation induced by Am80 in the presence or absence of PA024 (**3**). Concentration of Am80 is 3×10^{-10} M (open and closed circles) and 1×10^{-10} M (▲), and that of PA024 (**3**) is zero (○), 3×10^{-10} M (●), and 1×10^{-9} M (▲).

subsequent O-demethylation, various *O*-alkyl groups were introduced into compound **13**. Finally, the ester group was hydrolyzed under basic conditions.

Results and Discussion. The biological activity of the synthesized compounds was first evaluated in terms of the induced differentiation of human promyelocytic leukemia cells HL-60.[8b,10] Among pyrimidinecarboxylic acids with various *N*- and *O*-alkyl groups, PA451 (**6a**) and PA452 (**6b**) exhibited unique activity, as shown in Figure 1, compared with **5a**. These three compounds did not elicit differentiation-inducing activity alone or in the presence of an RXR agonist such as **5c** or **3** (data not shown). When **6a** or **6b** was added together with a

Chart 2



retinoid, Am80 (an RAR agonist, Chart 2), the differentiation-inducing activity of Am80 was not affected (open circles in Figure 1). However, **6a** and **6b** inhibited the differentiation induced by the combination of Am80 and **3**, an RXR agonist, in a dose-dependent manner (closed circles and triangles). **6a** similarly inhibited

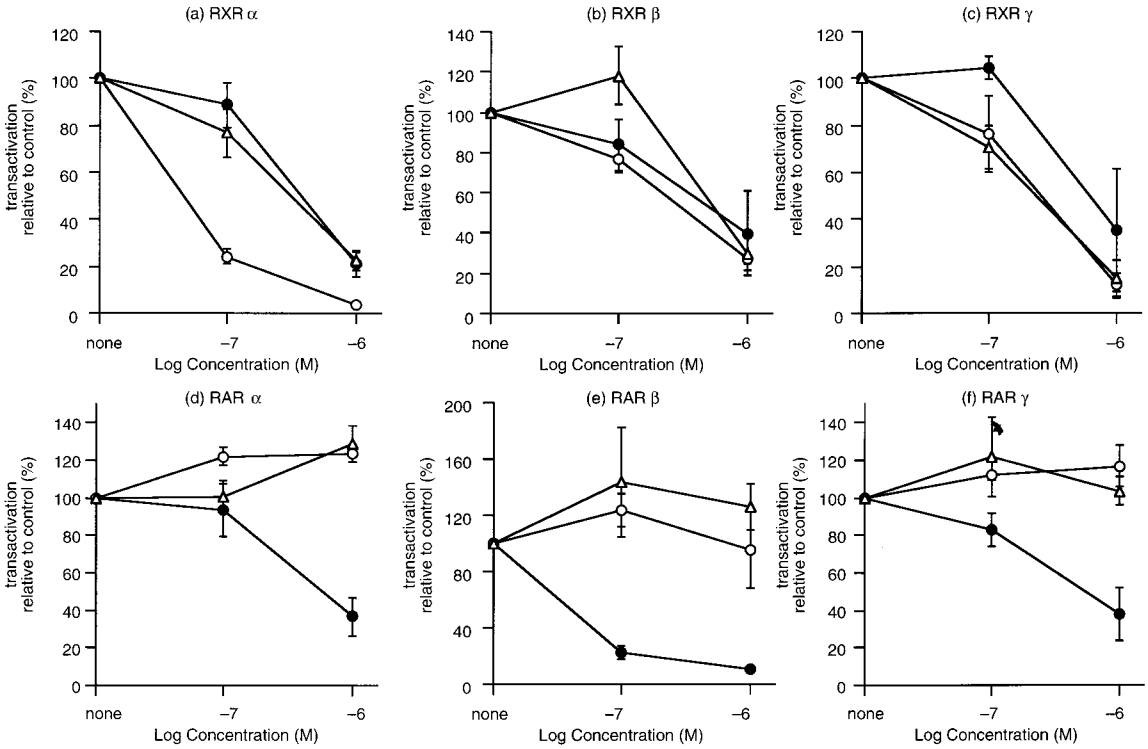


Figure 2. Transactivation assays in Cos-1 cells transiently transfected with mRXR/(DR1)5-pGL-TK (RXRα, -β, and -γ in parts a, b, and c, respectively) and hRAR/(TREpal)3-TKLUC (RARα, -β, and -γ in parts d, e, and f, respectively). The vertical scale is receptor transactivation induced by 1×10^{-8} M Am80 (RARα and -β), 1×10^{-8} M retinoic acid (RARγ), and 1×10^{-8} M PA024 (RXRs). The values were normalized to that obtained when the activator (agonist) alone was added, taken as 100. The horizontal scale is the molar concentration of the added compound. Added compounds were HX531 (**5a**, ●), PA451 (**6a**, ○), and PA452 (**6b**, △).

synergistic combinations of other RAR and RXR agonists (such as Am80 plus **5c**, data not shown). Significantly, the inhibition by **6a** and **6b** did not reach the basal level, and the percentage of differentiated cells in the presence of a high dose of **6a** or **6b** was more than that induced by Am80 alone. For example, addition of 3×10^{-10} M **3** increased the extent of differentiation induced by 3×10^{-10} M Am80 from 34% to 73% of the cells. **6a** inhibited this differentiation to 44% (still higher than 34%, $p < 0.05$), even at 1×10^{-6} M (Figure 1b, closed circles). In contrast, **5a** inhibited the activity of both Am80 alone and the combination of Am80 and **3** to the basal level in both cases.

The inhibitory mechanism by **6a** and **6b** is assumed to be antagonism at the RXR site of RXR−RAR heterodimers. Therefore, their effects on the activation of retinoid receptors, RARs and RXRs, were investigated by transient transactivation assay (Figure 2).[8b,10] None of the test compounds **6a**, **6b**, and **5a**, for comparison, when examined alone, activated any retinoid receptor (data not shown). These three compounds dose-dependently inhibited the transactivation of all subtypes of RXRs induced by 1×10^{-8} M **3** (Figure 1a−c). Among the three compounds, **6a** is the most potent inhibitor, especially for RXRα. Remarkable differences were observed in the effects on RAR transactivations. **5a** inhibited the RAR transactivation induced by 1×10^{-8} M Am80 (α and β subtypes) or by 1×10^{-8} M retinoic acid (γ subtype), while **6a** and **6b** were not inhibitory at concentrations below 1×10^{-6} M.

The results obtained here indicate that **6a** and **6b** do not activate RXR−RAR heterodimers or affect RXR−RAR activation by an RAR agonist alone. When both RAR and RXR agonists bind to the heterodimers, **6a** and **6b** antagonize the RXR agonist at the RXR site, and consequently, the retinoid synergism by the RXR agonist is decreased. If the RAR agonist occupies the RAR site of the RXR−RAR heterodimer, the binding of **6a** or **6b** at the RXR site does not affect the RXR−RAR actions. Recently, retinoid synergism was explained in terms of further stabilization of the complex of RAR agonist-activated RXR−RAR heterodimers and coactivators caused by binding of RXR ligands.[11] In light of this hypothesis, the antagonistic potency of these pyrimidinecarboxylic acids may be ascribed to loss of this further stabilization between holoRXR sites and coactivators.

In conclusion, we have developed the first RXR-selective antagonists that do not affect retinoid actions but selectively inhibit retinoid synergism by RXR agonists in RAR−RXR heterodimer actions. Since RXRs form various kinds of nuclear receptor heterodimers and RXR antagonists improved obesity and insulin resistance through modulation of the PPAR-γ−RXR activity,[9] these RXR-selective antagonists may be useful tools for the elucidation of RXR-related gene networks and for possible clinical application in the fields of diabetes and obesity. Further investigations on the biological potency of RXR-selective antagonists, including the regulatory mechanisms of various RXR-related heterodimers, are in progress.

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Supporting Information Available: Detailed experimental procedures for synthesis and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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