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Modification at the Lipophilic Domain of RXR Agonists Differentially Influences Activation of RXR Heterodimers

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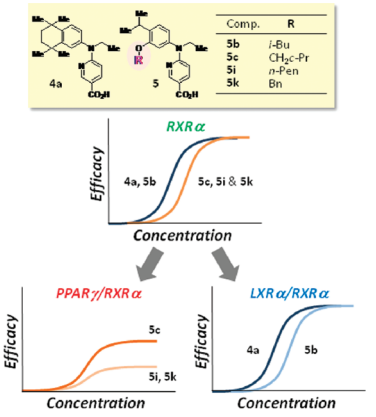
ABSTRACT RXR permissive heterodimers are reported to be activated differently depending upon the chemical structure of RXR agonists, but the relationship of agonist structure to differential heterodimer activation has not been explored in detail. In this study, we performed systematic conversion of the alkoxy side chain of **5a** (6-[ethyl-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid, NEt-3IP) and evaluated the RXR-, PPAR/RXR-, and LXR/RXR-agonist activities of the products. The cyclopropylmethoxy analogue (**5c**) showed similar RXR- and LXR/RXR-agonistic activities to the benzyloxy analogue (**5i**) and *n*-propoxy analogue (**5k**) but exhibited more potent PPAR/RXR-agonistic activity than **5i** or **5k**. Differential modulation of RXR heterodimer-activating ability by conversion of the alkoxy group located in the lipophilic domain of the RXR-agonist common structure is expected be a useful approach in the design of new RXR agonists for the treatment of hyperlipidemia or type 2 diabetes.

KEYWORDS RXR agonists, permissive heterodimers, PPAR, LXR, heterodimer activation, reporter gene assay



Retinoid X receptors (RXRs) are nuclear receptors that act as ligand-dependent transcription factors, functioning as homodimers or as heterodimers with peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptors (FXRs), and retinoic acid receptors (RARs).[1−3] Among RXR heterodimers, PPARγ/RXR is known to be a target of thiazolidinediones (TZDs), which are used for the treatment of insulin resistance,[4] and LXR/RXR is reported to be involved in glucose/lipid metabolism.[5,6] Although these heterodimers can be activated by PPAR agonists or LXR agonists, respectively, synergistic activation with RXR agonists[7] and activation by RXR agonists alone[8] are also known to occur. The RXR-heterodimer partners, which can be activated by RXR agonists alone, are called permissive heterodimer partners.[8] Because the permissive mechanism is thought to be relevant to several RXR heterodimers associated with glucose/lipid metabolism, RXR agonists are considered to be candidate therapeutic agents for the treatment of chronic disorders such as metabolic syndrome. In addition, because PPARγ or LXRα may influence insulin resistance in type 2 diabetes and autoimmune disease, including rheumatism (RA),[9−12] modulation of RXR heterodimers containing PPARγ or LXRα with RXR agonists may be a promising approach for the treatment of these diseases.

LGD1069 (**1**) (Targretin; Figure 1), an RXR agonist, is used clinically in the United States for the treatment of skin disorders associated with cutaneous T-cell lymphoma (CTCL).[13−15] Moreover, several RXR agonists, including **1**, are under investigation for the treatment of metabolic syndrome.[16] However, repeated administration of RXR agonists can elevate blood triglycerides (TGs) and induce hypothyroidism.[17−19] However, recently, PA024 (**2**) and HX630 (**3**) were reported to show differential transcriptional activation activities,[20] raising the possibility that RXR agonists without the above side effects may be obtainable by means of appropriate structural modification.

The general chemical structure of RXR agonists can be divided into a lipophilic domain based on 1,1,4,4-tetramethyltetralin structure, an acidic domain comprising benzoic acid or nicotinic acid, and a linking domain connecting the other two domains. So far, to create RXR agonists without the side effects described above and to examine the influence of the carboxylic acid moiety in the acidic domain on RXR-heterodimer activation, we have synthesized compounds whose acidic domains contain carboxylic acid bioisosters, such as a 5-tetrazolyl or hydroxamic acid group, and evaluated their RXR-, PPAR/RXR-, and LXR/RXR-agonist

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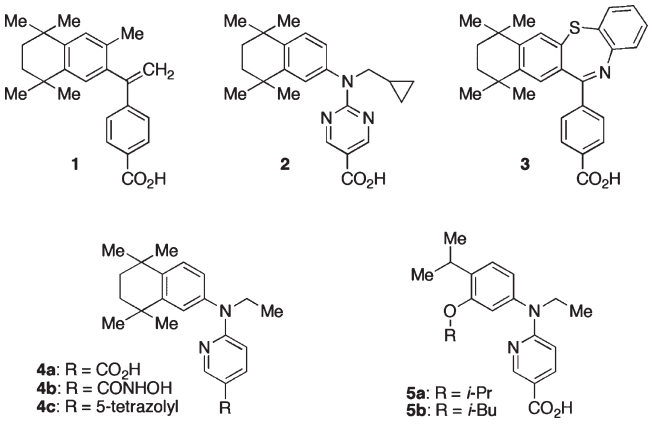


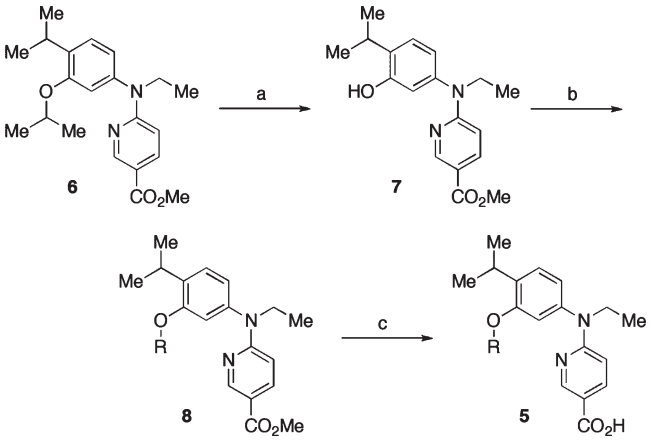
Figure 1. Chemical structures of known RXR ligands **1**−**5**.

activities.[21] We found that structural modification at this location does not influence RXR-heterodimer activation, and the RXR-heterodimer-agonist activities correspond well with the RXR-agonist activities.[21] On the other hand, to create less lipophilic RXR agonists, NEt-3IP (**5a**) and NEt-3IB (**5b**) (Figure 1), which possess an alkoxy group in the lipophilic domain, were synthesized.[22] Because known RXR agonists have a 1,1,4,4-tetramethyltetralin structure, structural conversion of this domain is of interest from the viewpoint of not only RXR-agonistic activity but also RXR-heterodimer activation. Therefore, we converted the isopropoxy group of **5a** to a hydroxyl group by treatment with aluminum chloride and alkylated the resulting intermediate. The RXR- and RXR-heterodimer-agonist activities of the products were examined. We found that while **1**, **4a**, and **5b** show similarly potent RXR-agonistic activities, their patterns of PPAR/RXR- and LXR/RXR-agonist activities were different. Compound **5c** (cyclopropylmethoxy) showed similar RXR- and LXR/RXR-agonist activities to **5i** (*n*-pentyloxy group) and **5k** (benzyloxy), but it was a significantly more potent activator of PPAR/RXR, as compared with **5i** and **5k**. In this letter, we report the patterns of RXR- and RXR-heterodimer-agonist activities of modified RXR agonists bearing an alkoxy group in the lipophilic domain.

Various alkoxy derivatives were obtained from **6** as shown in Scheme 1. Deisopropylation of intermediate **6**, the synthesis of which has already been reported,[22] was performed by using aluminum chloride in methylene chloride at room temperature. Compound **7** thus obtained was alkylated with various alkyl halides in the presence of potassium carbonate and potassium iodide in DMF. Then, de-esterification under alkaline conditions afforded the desired products **5**.

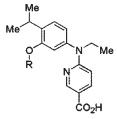
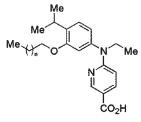
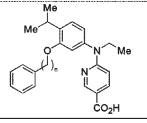
The transactivation assay of the compounds was performed by a reporter gene assay in COS-1 cells. The results for RXRα are shown in Table 1. Because **5b** showed significant RXR-agonistic activity, cyclopropylmethyl derivative **5c** and compounds **5d** and **5e**, which possess a double bond, were evaluated. Although their RXR-agonistic activity was reduced, each compound showed an EC_{50} value of approximately 100 nM. Compound **5e** bearing a 1,1,1-trifluoroethyl group showed similar RXR-agonistic activity to the compounds possessing a double bond, so we next focused on

Scheme 1[a]



[a]Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2. (b) Alkyl halide, K_2CO_3, KI, DMF. (c) NaOH(aq), MeOH, THF.

Table 1. Cotransfection Data for **1**, **4a**, and **5a**−**m** in COS-1 Cells

				RXRα	
Compound	Skeleton	R	n	EC_{50} (nM)	E_{max} (%)
1		-	-	20 ± 3	100
4a		-	-	5.3 ± 1.0	94 ± 1
5a		*i*-Pr	-	66 ± 9	97 ± 6
5b		*i*-Bu	-	19 ± 6	114 ± 0
5c		CH_2*c*-Pr	-	290 ± 40	95 ± 4
5d			-	110 ± 20	110 ± 0
5e			-	160 ± 80	91 ± 3
5f		CH_2CF_3	-	160 ± 30	100 ± 0
5g		-	1	450 ± 80	120 ± 0
5h		-	2	180 ± 30	100 ± 10
5i		-	3	160 ± 20	96 ± 7
5j		-	4	280 ± 150	77 ± 4
5k		-	1	160 ± 60	98 ± 2
5l		-	2	330 ± 120	99 ± 4
5m		-	3	820 ± 110	78 ± 6

[a]All values represent the standard error of the mean value of at least three separate experiments with triplicate determinations. [b]EC_{50} values were determined from full dose−response curves ranging from 10^{-8} to 10^{-5} M in COS-1 cells. [c]The luciferase activity of **1** at 1 μM was defined as 100%.

compounds possessing a linear alkyl chain on the alkoxy group. Interestingly, elongation of the alkyl chain to *n*-propyl, *n*-butyl, or *n*-pentyl increased the RXR-agonistic activity. Even *n*-hexyl derivative **5j** showed an EC_{50} value of about 300 nM, indicating that there is a cavity around the so-called lipophilic moiety of RXR agonists in the ligand-binding domain of RXR. Thus, the RXR-agonistic activity of compounds bearing

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a phenyl group was evaluated. All of the compounds examined showed an EC_{50} value of several hundred nanomolar. Although the activity was slightly reduced, these results indicate that the cavity of RXR that contacts the 3-alkoxy group of our RXR agonists is of sufficient size to accommodate a phenyl ring.

Because compounds **5a**−**m** possessing various alkoxy side chains showed rather similar RXR-agonistic activity despite their structural differences, we performed a reporter gene assay with PPAR, LXR, PPAR/RXR, and LXR/RXR, anticipating RXR-permissive/synergistic action. Figure 2 shows the dose-dependent plots of selected compounds for RXRα. Compounds **1**, **4a**, and **5b** (open circles, triangles, and squares) all showed extremely similar RXR-agonistic activities, as did **5c**, **5i**, and **5k** (closed circles, triangles, and squares).

First, we took the three compounds in the former group and examined their agonistic activities with PPARγ, LXRα, PPAR/RXR, and LXR/RXR (Figure 3). The reason why PPARγ and LXRα were selected from among the various possible RXR-heterodimer partners is that these receptors have been well studied, and their modulation is associated with improved insulin resistance, improved glucose control, anti-inflammatory activity, and improved autoimmune regulation.[5,9,11,23−26] These compounds did not activate PPARγ but slightly activated LXR. Every compound activated each RXR heterodimer, but interestingly, **1** was not active at a low concentration, differently from **4a** and **5b**. These results are consistent with a previous report[20] showing that the pattern of heterodimeric activation is dependent on the chemical structure of RXR agonists. In the concentration range from 10^{-7} to 10^{-6} M, **4a** and **5b** showed similar RXR-agonistic activity (Figure 2), but the activity toward PPAR/RXR was weaker (Figure 3), indicating that alteration of the lipophilic domain of RXR agonists can differentially modulate the RXR-heterodimer-activating ability.

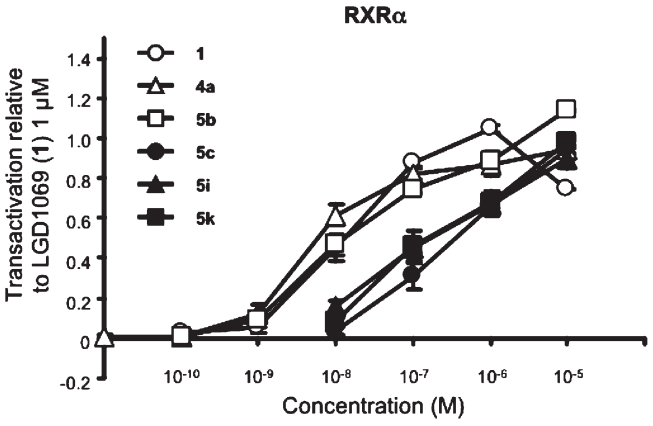

Figure 2. Results of RXRα reporter gene assay for **1** (open circle), **4a** (open triangle), **5b** (open square), **5c** (closed circle), **5i** (closed triangle), and **5k** (closed square).

Next, a similar study was performed on compounds **5c**, **5i**, and **5k**, belonging to the latter group shown in Figure 2. Although LXR/RXR heterodimer was activated similarly by each compound, the PPAR/RXR heterodimer was more potently activated by **5c** than by the other two compounds (Figure 4). Because these compounds differ only in the alkoxy side chain, it appears that conversion of the alkoxy group in the lipophilic domain of RXR agonists can differentially influence the heterodimer-activating ability.

Activation of LXR is reported to improve disordered glucose metabolism in type 2 diabetes.[5] However, excessive activation of LXR induces SREBP-1c expression, resulting in elevation of blood TGs.[27] To avoid side effects of RXR agonists, including TG elevation, moderate activation of LXR/RXR appears to be desirable. Similarly, although activation of PPARγ improves insulin resistance, inflammation, and rheumatoid arthritis,[4,10−12,23−26] excessive activation of PPARγ can also cause edema and obesity.[28] Therefore, RXR

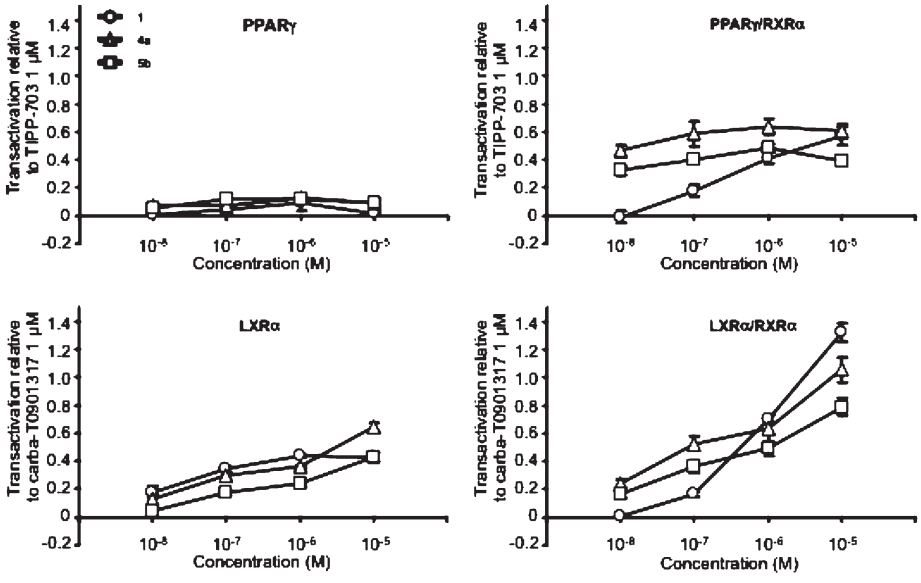

Figure 3. Relative transactivation data of **1** (open circle), **4a** (open triangle), and **5b** (open square) toward PPARγ, PPARγ/RXRα, LXRα, and LXRα/RXRα.

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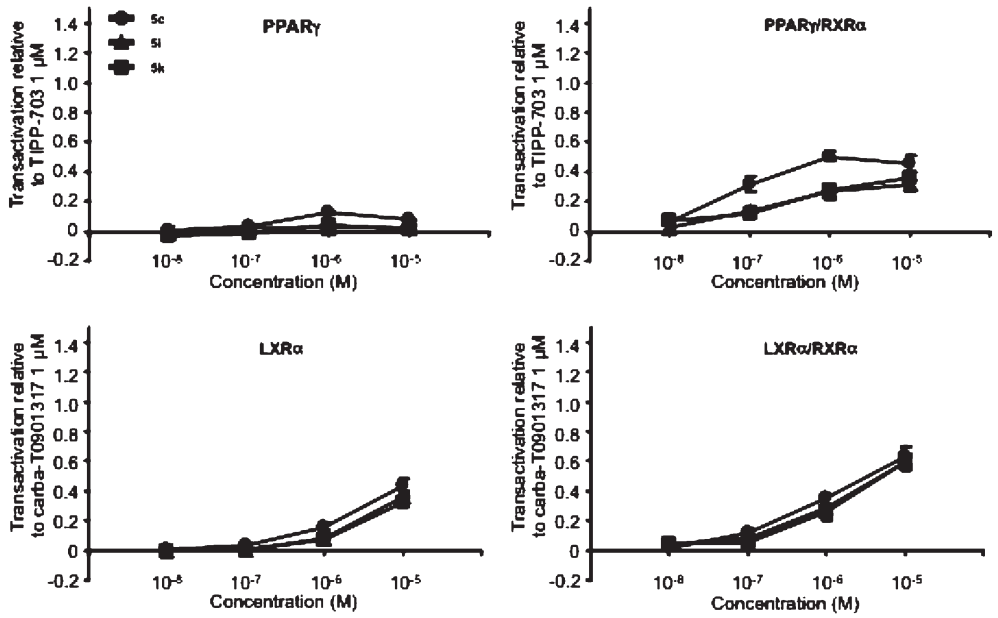

Figure 4. Relative transactivation data of **5c** (closed circle), **5i** (closed triangle), and **5k** (closed square) toward PPARγ, PPARγ/RXRα, LXRα, and LXRα/RXRα.

agonists that activate these heterodimers moderately without causing side effects are attractive candidates for clinical application.

The 1,1,4,4-tetramethyltetralin structure in the common lipophilic domain of general RXR agonists was changed to a phenyl ring bearing an isopropyl moiety and various alkoxy side chains. The RXR- and RXR-heterodimer-agonistic activities of these compounds were examined. RXR agonists showing similar levels of RXR-agonistic activity were found to show different patterns of RXR-heterodimer activation, depending upon the nature of the alkyl side chain in the lipophilic domain. This finding should be helpful in designing new RXR agonists without the side effects described above.

SUPPORTING INFORMATION AVAILABLE

General information, general procedures, combustion analysis, and general biological assay procedures and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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