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Modification at the acidic domain of RXR agonists has little effect on permissive RXR-heterodimer activation

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ABSTRACT

Retinoid X receptors (RXRs) function as homo- or heterodimers with other nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs), which are targets for treatment of hyperlipidemia and type 2 diabetes, or liver X receptors (LXRs), which are involved in glucose/lipid metabolism. PPAR/RXR or LXR/RXR are known as permissive RXR-heterodimers because they are activated by RXR agonists alone. Interestingly, the pattern of RXR-heterodimer activation is different depending on the RXR agonist structure, but the structure–activity relationship has not been reported. Here we show that modification or replacement of the carboxyl group in the acidic domain of RXR agonists has little or no effect on permissive RXR-heterodimer activation. Phosphonic acid (**9**), tetrazole (**10**), and hydroxamic acid (**12**) analogues were synthesized from the common bromo intermediate **7**. Except for **9**, these compounds showed RXR full-agonistic activities in the concentration range of 1–10 μM. The order of agonistic activity toward both PPARγ/RXRα and LXRα/RXRα was the same as it was for RXR, that is, **11** > **10** > **12**. These results should be useful for the development of RXR agonists with improved bioavailability.

Retinoid X receptors (RXRs)[1,2] function as transcription factors either alone or as heterodimers with receptors such as PPARγ (the molecular target of thiazolidinediones, which are clinically used to treat insulin resistance),[3] and liver X receptors (LXRs; involved in glucose/lipid metabolism).[4,5] PPAR/RXR and LXR/RXR are activated by PPAR and LXR agonists, respectively, but the agonistic potency can be synergistically activated by RXR agonists.[6] Interestingly, RXR agonists alone can also activate these heterodimers (permissive heterodimers).[7] Focusing on this permissive mechanism, RXR agonists are expected to show comprehensive therapeutic efficacy against insulin resistance or impaired glucose metabolism in type 2 diabetes by modulating various RXR heterodimers, such as PPAR/RXR and LXR/RXR. Thus, RXR agonists, including LGD1069 (**1**) (Targretin®) (Fig. 1),[8–10] which is used in the USA to treat cutaneous T-cell lymphoma, are candidates for the treatment of complex disorders such as metabolic syndrome.[11]

However, RXR agonists often cause side effects, such as weight gain, plasma triglyceride elevation, and hypothyroidism.[12–14] Among them, triglyceride elevation is reported to be based on the transactivation of SREBP-1c by LXR/RXR.[15] It is important to note that the RXR agonists HX630 (**2**) and PA024 (**3**) (Fig. 1) show different patterns of activation of PPAR/RXR and LXR/RXR.[16] Therefore, we hypothesized that appropriate structural modification of RXRs would eliminate the side effects. In addition, most RXR agonists are lipophilic molecules, and their absorption, distribution, metabolism, and excretion (ADME) characteristics are not optimal. But, structural modification at the carboxylic moiety might alter the pattern of permissive RXR-heterodimer activation.

Structural modifications at the carboxylic moiety of RAR agonists have been reported.[17,18] However, no structure–activity relationship (SAR) study of RXR agonists for activity toward permissive heterodimers has been reported. RXR agonists generally consist of a lipophilic domain, such as a 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthyl moiety, an acidic domain, such as benzoic acid or nicotinic acid, and a linking domain. Although there have been SAR studies on the linking domain (**2** and **3**) and on the lipophilic domain (NEt-3IP, **4a**) (Fig. 1),[19] modification of the acidic domain has been limited to TZ335 (**5**) (Fig. 1), which has a thiazolidinedione acidic group.[20] Thus, modification of the acidic domain in RXR agonists seemed worthy of investigation.

Therefore, we synthesized RXR agonists possessing tetrazole, hydroxamic acid, and phosphonic acid at the acidic domain from a common bromo intermediate, and evaluated their RXR agonistic activity and pattern of RXR-heterodimer activation activities toward PPARγ/RXR and LXRα/RXR.

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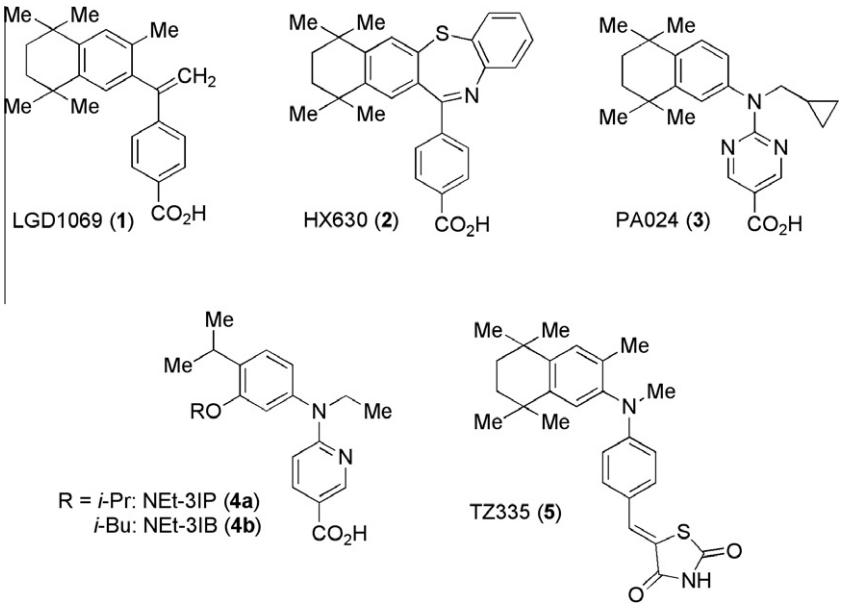
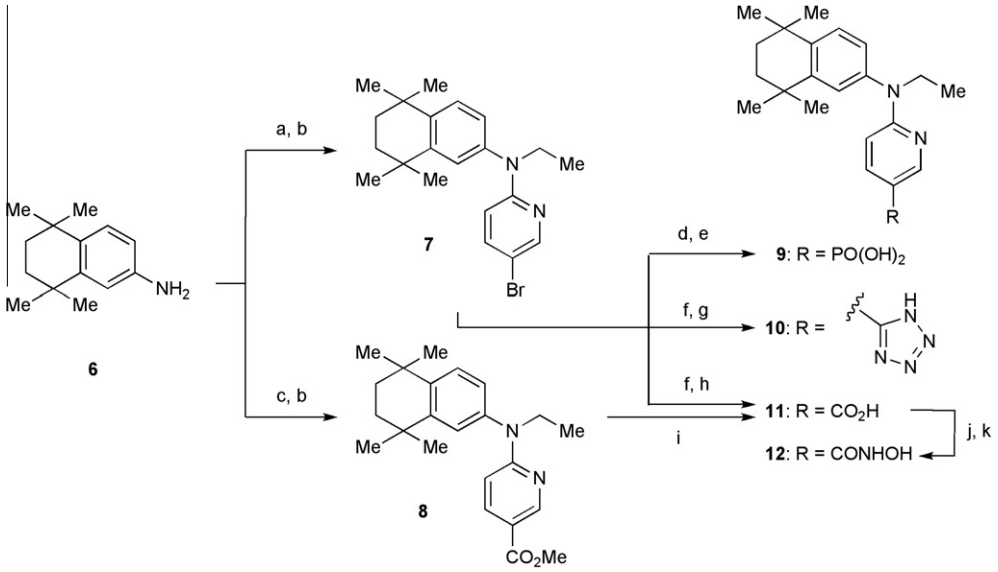


Figure 1. Chemical structures of known RXR agonists.



Scheme 1. Reagents and conditions: (a) 2,5-dibromopyridine, *p*-TsOH, KI, dioxane, reflux, 60%; (b) EtI, NaH, DMF, 0 °C, q.y; (c) methyl 6-chloronicotinate, *p*-TsOH, dioxane, reflux, 80%; (d) diethyl phosphite, $Pd(PPh_3)_4$, Cs_2CO_3, THF, microwave irradiation (140 °C), 61%; (e) Me_3SiBr, CH_2Cl_2, rt 76%; (f) CuCN, DMF, reflux, 88%; (g) NaN_3, NH_4Cl, DMF, reflux, 43%; (h) NaOH aq, EtOH, reflux, 92%; (i) NaOH aq, MeOH, THF, 60 °C, 98%; (j) *O*-benzylhydroxylamine, HOBt, EDC, TEA, DMF, rt 82%; (k) H_2, 10% Pd-C, EtOAc, rt 76%.

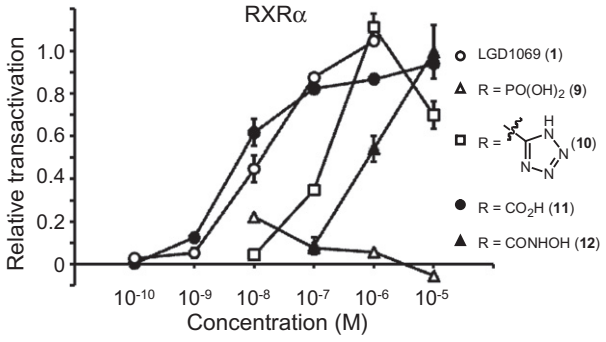


Figure 2. Transactivation dose-response curves of **1** (○), **9** (△), **10** (□), **11** (●), and **12** (▲) against RXRα in COS-1 cells. The vertical scale is normalized with respect to 1 μM **1**, taken as 1.0. The assays were carried out in triplicate at least three times.

As shown in Scheme 1, after coupling reaction of 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthylamine (**6**)[21] and 2,5-dibromopyridine or methyl 6-chloronicotinate in the presence of a catalytic amount of *p*-toluenesulfonic acid, N-ethylation of the linking nitrogen was performed to afford the common intermediates **7** and **8**. Compound **7** was reacted with diethyl phosphite in the presence of a palladium catalyst and cesium carbonate in THF under microwave irradiation at 140 °C,[22] and then deprotection of the esters with trimethylsilyl bromide in methylene chloride gave the phosphonic derivative **9**.[23] The bromo atom of **7** was substituted to a cyano group by coupling reaction of intermediate **7** and copper cyanide in DMF,[24] and then [3 + 2]cycloaddition reaction was performed with sodium azide to give tetrazolyl derivative **10**.[25] Carboxy derivative **11** was synthesized by hydrolysis of the cyanide derivative or ester **8**. Hydroxamic acid **12** was

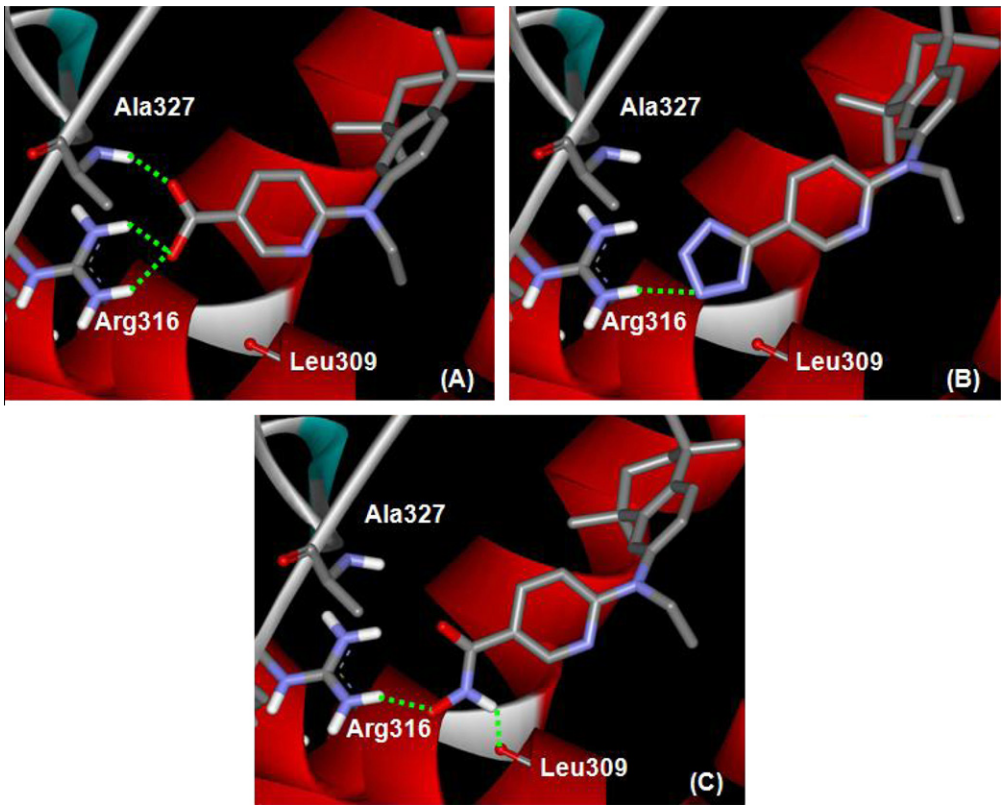


Figure 3. Proposed structures of RXR agonists **11** (A), **10** (B), and **12** (C) bound to the RXRα LBD (pdb code: 1mvc), calculated with AutoDock 4.0.[29] Green broken lines indicate hydrogen bonds.

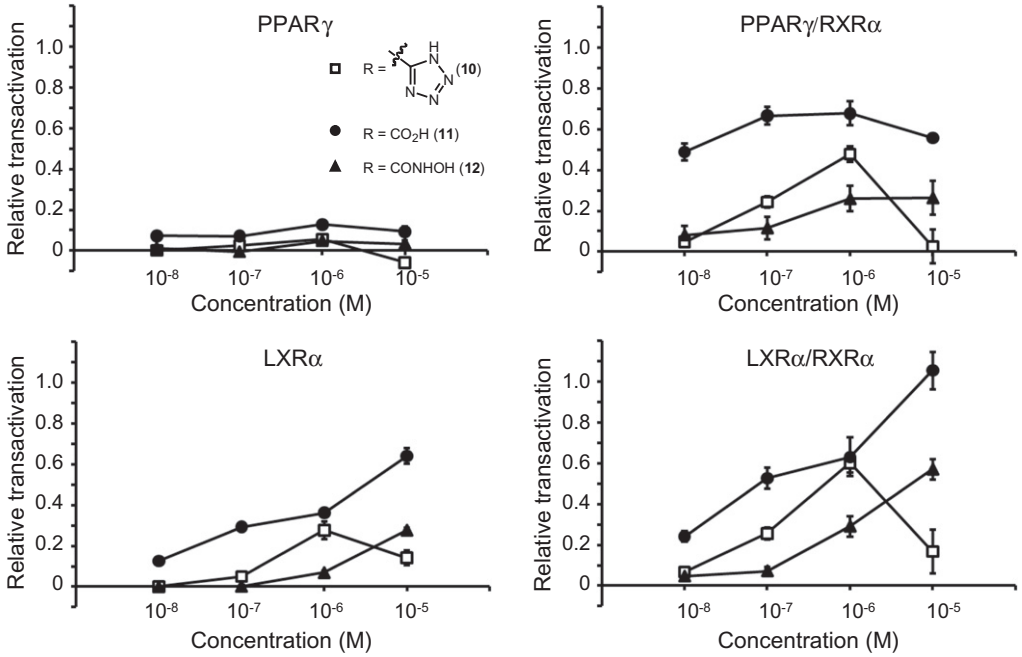


Figure 4. Relative transactivation data of **10** (○), **11** (●), and **12** (▲) toward PPARγ, PPARγ/RXRα, LXRα and LXRα/RXRα. One micromolar TIPP-703 (PPAR-pan agonist) and carba-T0901317 (LXR-pan agonist) were used as positive controls for PPARγ and PPARγ/RXRα, and LXRα and LXRα/RXRα, respectively.

produced by coupling of **11** and *O*-benzylhydroxylamine, followed by deprotection.

RXRα and RARα activities of compounds **9**–**12** were evaluated by reporter gene assay in COS-1 cells. All of them did not show RARα activities even at 10 μM (data shown in Supplementary data). Figure 2 shows dose–response curves of transactivation activity against RXRα compared to that of 1 μM **1** as a positive control.

RXR-agonistic activity of compound **11** possessing a carboxy group reached a plateau at 0.1 μM, and **11** (EC_{50} = 5.28 ± 1.23 nM) was as potent as **1** (EC_{50} = 19.8 ± 3.4 nM). Phosphonic acid **9** did not show RXR-agonist activity up to 10 μM. Tetrazole **10** (EC_{50} = 205 ± 22 nM) and hydroxamic acid **12** showed RXR full-agonistic activity, though they were less potent than **11**. The reason why **10** and **12** show RXR-agonist activities may be the planarity of their acidic moieties. On the other hand, the lower potency of **12** as compared with **10** may reflect the acidic character: the pK_a values of phenyltetrazole and benzohydroxamic acid are 4.5[26] and 8.8,[27,28] respectively. Docking simulation of **10**, **11**, and **12** with RXRα showed that **10** and **12** do not hydrogen bond to Ala327 of RXRα, whereas **11** does form a hydrogen bond (Fig. 3 and 4(B)).

Although there are differences of RXR-agonistic activities between **10, 11**, and **12**, these compounds showed similar permissive RXR- heterodimer activation behavior. Transactivation assay with PPARγ and PPARγ/RXRα showed that while PPARγ was not activated, PPARγ/RXRα was activated by all of the compounds, indicating that the latter activity is based on permissive activation by **10**, **11**, and **12**. Maximum PPARγ/RXRα transactivations of **10**, **11**, and **12** amounted to about 40%, 60%, and 20% of the positive control, respectively.

On the other hand, LXRα was weakly activated by **10**, **11**, and **12**, indicating that their common 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthyl moiety possesses LXRα activity. Maximum LXRα/RXRα heterodimer activation by **10** and **11** was about 60% and that by **12** was about 50% of the positive control. Since each compound also weakly activates LXRα, LXRα/RXRα heterodimer activation by these compounds may be based on both RXR permissive activation and synergistic activation.

The carboxy derivative **11** was the most potent among the compounds examined. At a concentration of more than 1 μM, at which all the compounds showed more than 80% activation of RXR, the order of activity of the compounds was **11** > **10** > **12** for both PPARγ/RXRα and LXRα/RXRα, indicating that the acidic moiety has little influence on RXR permissive heterodimer activation. *c* Log *P* values[30] of **11** and **10** are 5.55 ± 1.07 and 5.06 ± 0.85, respectively. Although the tetrazole derivative **10** is less potent than **11**, tetrazolyl-type RXR agonists seem to be attractive candidate agents from the viewpoint of bioavailability.

RXR agonists possessing tetrazole, hydroxamic acid, and phosphonic acid as the acidic domain were prepared from a common bromo intermediate. Evaluation of their RXR agonistic activity and RXR- heterodimer activation activities toward PPARγ/RXR and LXRα/RXR indicated that modification of the acidic domain of RXR agonists has little influence on permissive RXR-heterodimer activation. This information may be useful for the creation of RXR agonists with superior bioavailability.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.012.

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