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LETTER

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Discovery of a Potent Retinoid X Receptor Antagonist Structurally Closely Related to RXR Agonist NEt-3IB

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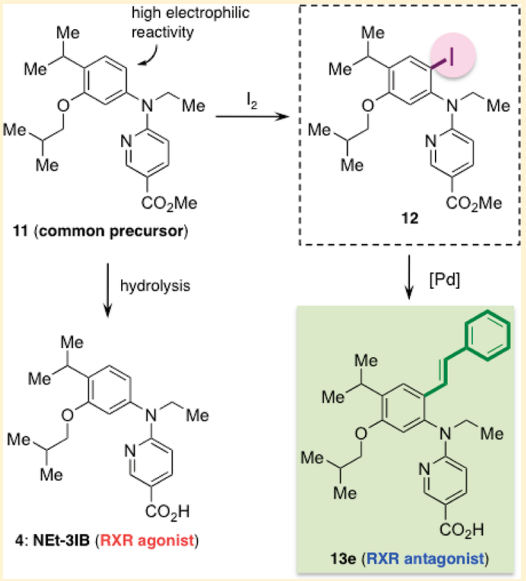
Supporting Information

ABSTRACT: We discovered a potent retinoid X receptor (RXR) antagonist, 6-[*N*-ethyl-*N*-(5-isobutoxy-4-isopropyl-2-(*E*)-styrylphenyl)amino]nicotinic acid (**13e**), that is structurally closely related to the RXR full agonist 6-[*N*-ethyl-*N*-(3-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IB) (**4**). Compound **13e** was synthesized via a simple route from **11**, a methyl ester precursor of **4**. Because **11** possesses high electrophilic reactivity because of the amino and alkoxy groups, it was readily transformed to **12** by iodization, and the iodine atom of **12** was converted to a C−C or C−N bond by means of palladium-catalyzed reaction to afford **13**. Transcriptional activation assay revealed that **13g** (in which the iodine atom was replaced with an amino group) is a weak RXR agonist, while **13d** (a phenyl group), **13e** (a styryl group), and **13f** (an anilino group) are RXR antagonists. Among them, **13e** was found to be more potent than the known RXR antagonist PA452 (**9**).

KEYWORDS: Synthetic route, nuclear receptors, agonists, antagonists, retinoids, RXR



Retinoid X receptors (RXRs) are nuclear receptors that control gene expression in response to ligand binding, and they function as homodimers or heterodimers with other nuclear receptors.[1] Because of their biological activity, RXRs are interesting targets for drug discovery.[2,3] For example, Targretin (bexarotene) (**1**),[4] an RXR full agonist, has already been launched for the treatment of cutaneous T cell lymphoma (CTCL) in the United States.[5] In addition, peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs) function as RXR heterodimers to control lipid/sugar metabolism, and because these heterodimers can be activated even by RXR agonists alone (permissive mechanism),[6] RXR agonists are considered to be candidate drugs for the treatment of metabolic syndrome.[7,8] However, currently known RXR agonists induce hepatomegaly,[9] hyperlipidemia,[10] and hypothyroidism,[11] and other studies have aimed at finding RXR agonists without these side effects.[12] Nevertheless, RXR antagonists have been reported to be effective against diabetes[13] and allergic diseases[14] in animal models and are also useful for analyzing various biological phenomena involving RXRs.[15]

Figure 1 shows the chemical structures of known RXR agonists **1**−**7**[4,16−20] and RXR antagonists **8**−**10**.[15,21−23] Although several agonist/antagonist pairs, such as compounds **5** (agonist)[18] and **8** (antagonist),[21] **6** (agonist)[19] and PA452 (**9**; antagonist),[22] and **7** (agonist)[23] and **10** (antagonist),[15] each possess similar skeletons, the starting materials for their synthesis differ from each other. If RXR agonists and antagonists could be synthesized from common starting materials, this would facilitate systematic RXR ligand production and be both convenient and environmentally friendly.

RXR ligands generally contain common structural features, having a hydrophobic domain such as 1,1,4,4-tetramethyltetralin, an acidic domain such as an aromatic carboxyl group, and a linker domain connecting the two.[2,3] Structure−activity relationship studies have shown that RXR agonists can be changed to antagonists by introducing an alkyl side chain into the ortho position of the hydrophobic domain with respect to the linker.[2,3,13,24] We have

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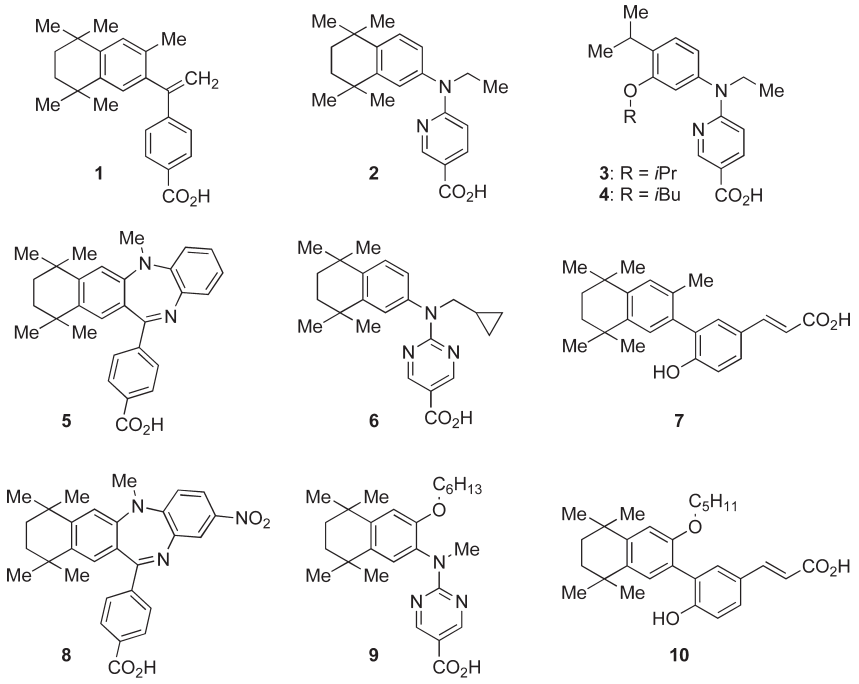
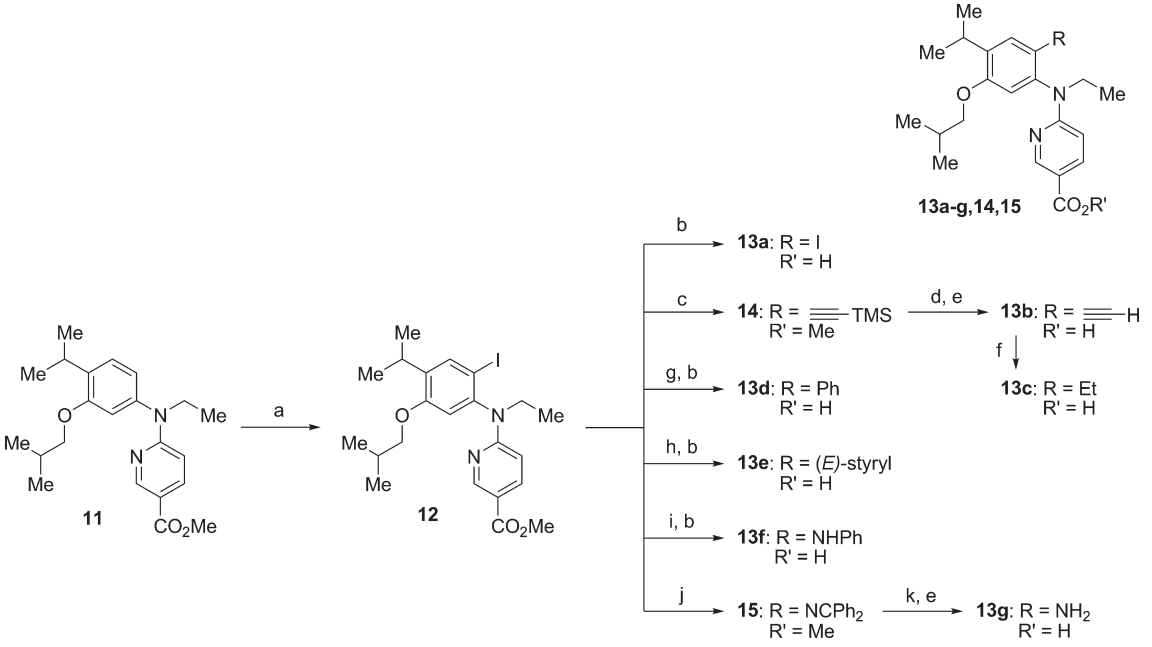


Figure 1. Chemical structures of RXR agonists **1**−**7** and RXR antagonists **8**−**10**.

Scheme 1[a]



[a] Reagents and conditions. (a) I_2, Ag_2SO_4, MeOH. (b) (i) 2 N NaOH, THF, MeOH; (ii) 2 N HCl. (c) TMS acetylene, $PdCl_2(PPh_3)_2$, CuI, triethylamine, THF. (d) K_2CO_3, MeOH. (e) (i) 2 N NaOH, MeOH; (ii) 2 N HCl. (f) H_2, Pd−C, MeOH. (g) Phenylboronic acid, Pd−C, Na_2CO_3, EtOH. (h) Styrene, $Pd(OAc)_2$, tris(*o*-tolyl)phosphine, triethylamine, acetonitrile. (i) Aniline, $Pd(OAc)_2$, (±)-BINAP, Cs_2CO_3, toluene. (j) Benzophenoneimine, $Pd(OAc)_2$, (±)-BINAP, Cs_2CO_3, toluene. (k) 2 N HCl, THF.

reported RXR full agonists NEt-3IP (**3**) and NEt-3IB (**4**), which contain a phenyl group.[17] Because these compounds have an amino group at the linker domain and an alkoxy group at the meta position (position-3 carbon) of the hydrophobic ring with respect to the amino group, the position-6 carbon (C-6) is electron-rich and reactive to electrophilic reagents. Therefore, we considered that it should be possible to introduce an iodine atom at C-6. On the basis of this idea, we designed a new method to synthesize RXR ligands, not only agonists but also antagonists, via iodine derivative **12** obtained from precursor **11** (previously used in the synthesis of potent RXR agonist **4**), by substitution of the iodine atom with the aid of palladium catalyst.

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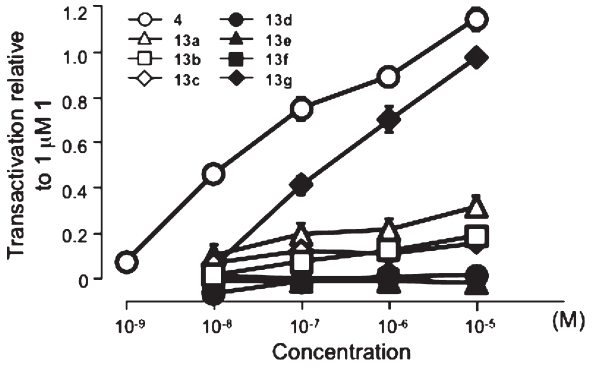

Figure 2. Dose-dependent RXR agonistic activities of compounds **13a**−**g** against RXRα. The relative transactivation activity is based on the luciferase activity of 1 μM **1**, taken as 1.0.

Scheme 1 shows the synthetic scheme using **12** as a common intermediate. Compound **12** was synthesized by iodinating methyl ester **11** with silver sulfate. Hydrolysis of the methyl ester of **12** gave **13a**. Coupling reaction of **12** and TMS-acetylene with palladium catalyst afforded intermediate **14**,[25] and deprotection of the TMS and methyl ester moieties gave **13b**. The acetylene moiety of **13b** was catalytically reduced to afford **13c**. Moreover, **12** was reacted with phenylboronic acid by Suzuki−Miyaura coupling,[26] styrene by Heck reaction,[27] and aniline or benzophenoneimine by Buckwald coupling reaction,[28] followed by deprotection to give **13d**−**g**. The trans structure of **13e** was supported by the ^{1}H NMR coupling constant between the ethylene protons.[29]

The compounds obtained were assessed by reporter gene assay using COS-1 cells. Compound **13g** shows RXR full agonistic activity, although it was less potent than **4** (Figure 2). Compounds **13d**, **13e**, and **13f**, which showed no RXR agonistic activity, were examined for RXR antagonistic activity. Figure 3a shows dose−response curves of NEt-TMN (**2**), a RXR full agonist,[16] in the absence and in the presence of these compounds or **9**, an RXR antagonist, at 1 μM. The presence of **9** shifted the response curve of **2** to a higher concentration (to the right), showing that **9** acts as an RXR antagonist. Although **13d** did not shift the dose−response curve, compounds **13e** and **13f** did do so, in the same way as did **9**. Among them, compound **13e**, possessing a stilbene structure, shifted the dose−response curve of **2** more strongly than **9**, indicating that **13e** is a more potent RXR antagonist than **9**. Thus, to assess the RXR-antagonistic activity of **13e** in detail, the pA_2 value (common logarithm of the concentration of an antagonist that doubles the EC_{50} value of an agonist; used as an antagonistic activity index) was obtained from Schild plots.[30] Figure 3b−d shows the dose−response curves of **2** or **4** in the absence or presence of each concentration of **9** or **13e**, respectively. The Schild plot using **2** indicated that the pA_2 values of **9** and **13e** were 7.11 and 8.23, respectively, indicating that **13e** is 1 order of magnitude more potent than **9**. The pA_2 value of **13e** against **4** was 8.26 (see the Supporting Information, S17).

To examine the reason why **13e** shows potent RXR antagonistic activity, we performed a docking study using AutoDock 4.0 (Figure 4a−c) (see the Supporting Information, S19).[31] First, we were interested in the driving force that causes **13e** to induce the antagonistic form of helix 12 (H12). Bourguet et al. suggested that RXR-full antagonist **10** induces the antagonistic form as a consequence of steric hindrance between leucine 451 (Leu451) in H12 of the ligand binding pocket of RXRα and the

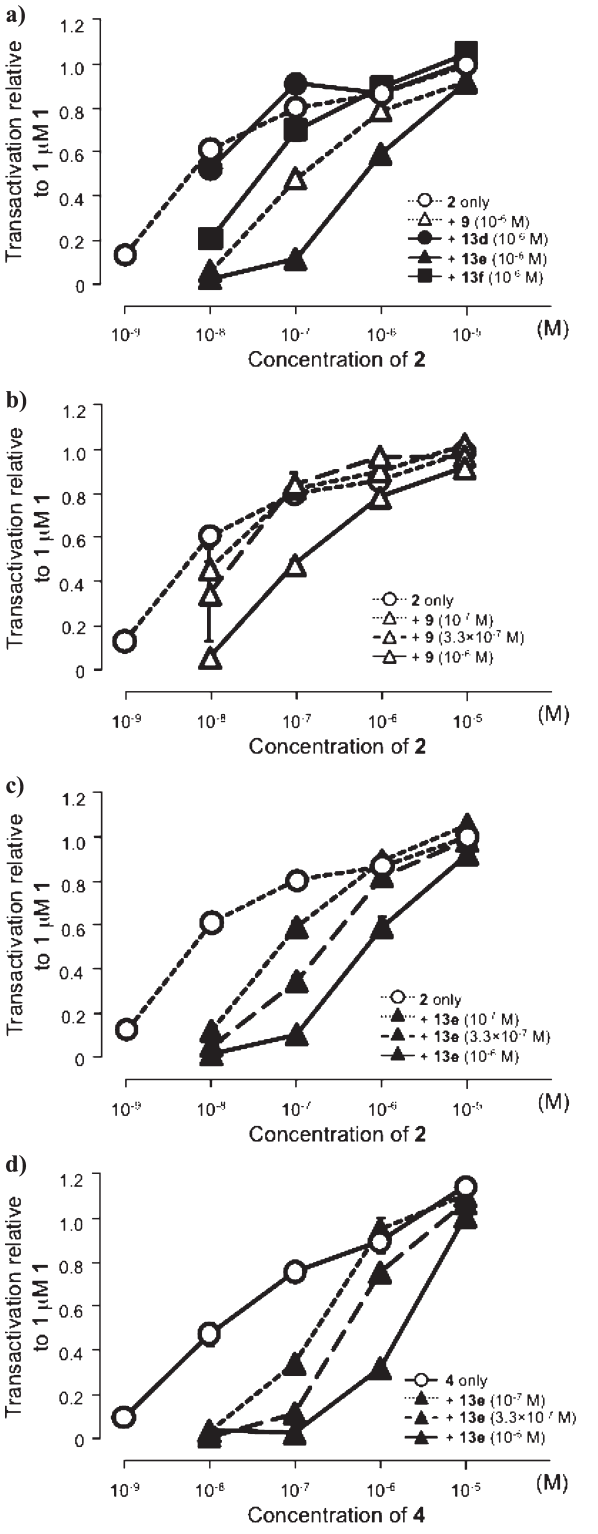

Figure 3. (a) Dose-dependent RXRα agonistic activities of NEt-TMN (**2**), an RXR full agonist, in the absence or presence of **9** or **13d**−**f**. (b) Dose-dependent RXRα agonistic activities of **2** in the absence or presence of compound **9**. (c) Dose-dependent RXRα agonistic activities of **2** in the absence or presence of compound **13e**. (d) Dose-dependent RXRα agonistic activities of **4** in the absence or presence of compound **13e**. The transactivation activity is based on the luciferase activity of 1 μM **1**, taken as 1.0.

alkoxy side chain of **10**, based on the superposition of **10** with the X-ray structure of RXR-partial agonist UVI3002 in the ligand-binding

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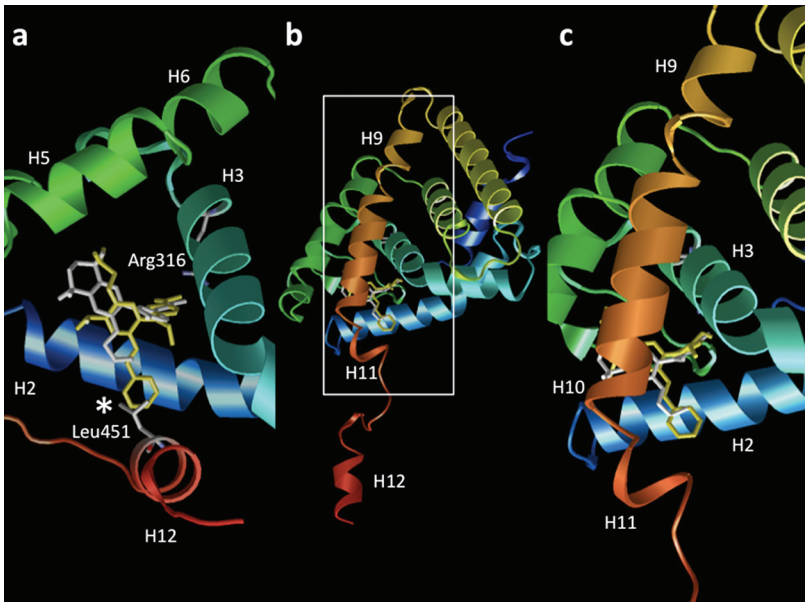

Figure 4. (a) Antagonist **13e** (yellow) modeled into the ligand-binding pocket of RXRa (PDB code: 2P1V)[32] and superposed onto the agonist **4**. The white stick molecule is a RXR-partial agonist UVI3002, which binds in the ligand-binding domain of RXRα (2P1V). The asterisk indicates a steric clash between the side chain of **13e** and Leu451 in helix 12 (H12). (b) Antagonist **13e** (yellow) modeled into the ligand-binding pocket of RXRα (PDB code: 3A9E).[33] The white stick molecule is RXR antagonist LG100754, which binds in the ligand-binding domain of RXRα (3A9E). (c) An enlarged view of the boxed region in b.

pocket (PDB code: 2P1V).[32] Thus, according to their method, we superposed the structure of **13e** on the most stable docked structure of **4** in the ligand binding pocket and examined its interaction with RXRα (Figure 4a). The result reveals that the styryl moiety of **13e** lies closer to Leu451 in H12 than does the alkoxy group of UVI3002 and thus indicates that **13e** exerts its antagonistic effect through the classical mechanism of antagonist action. Next, to examine the binding structure of **13e** in the antagonistic form of the RXR-ligand binding pocket, docking simulations using an X-ray structure of the RXR antagonistic form (PDB code: 2P1V)[33] were performed (Figure 4b,c). The docking energies of **13e** and **13f**, which are less potent than **13e**, were calculated to be −11.60 and −11.07 kcal/mol, respectively, indicating that **13e** affords a more stable complex than **13f**.

In summary, we have discovered a potent RXR antagonist, 6-[*N*-ethyl-*N*-(5-isobutoxy-4-isopropyl-2-(*E*)-styrylphenyl)-amino]nicotinic acid (**13e**), that is structurally closely related to the RXR full agonist 6-[*N*-ethyl-*N*-(3-isobutoxy-4-isopropyl-phenyl)amino]nicotinic acid (NEt-3IB) (**4**). A series of compounds **13** were synthesized from **11**, a methyl ester precursor of **4**, as a key intermediate, thus providing a new methodology to synthesize RXR agonists and antagonists easily via a common precursor. RXR agonistic and antagonistic activity assay revealed that compound **13e** is a more potent RXR antagonist than the known antagonist **9**. This synthetic route provides a convenient approach for RXR ligand synthesis. Compound **13e** is expected to be a useful tool for analyzing biological phenomena involving RXRs.

■ ASSOCIATED CONTENT

Supporting Information. General information, synthetic procedures, combustion analysis data, HPLC charts, luciferase reporter gene assay, Schild plot for pA_2 determination, and molecular docking procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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