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

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ABSTRACT: We discovered a potent retinoid X receptor (RXR) antagonist, 6-[N-ethyl-N-(3-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.† Because of their biological activity, RXRs are interesting targets for drug discovery.‡,§ For example, Targretin (bexarotene) (1), a RXR full agonist, has already been launched for the treatment of cutaneous T cell lymphoma (CTCL) in the United States.§ 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),° RXR agonists are considered to be candidate drugs for the treatment of metabolic syndrome.‡,§ However, currently known RXR agonists induce hepatomegaly,† hyperlipidemia,‡ and hypothyroidism,§ and other studies have aimed at finding RXR agonists without these side effects.‡ Nevertheless, RXR antagonists have been reported to be effective against diabetes,° and allergic diseases,‡ in animal models and are also useful for analyzing various biological phenomena involving RXRs.‡,§ Figure 1 shows the chemical structures of known RXR agonists 1—7,16–20 and RXR antagonists 8—10,15,21–25 Although several agonist/antagonist pairs, such as compounds 5 (agonist)18 and 8 (antagonist), 21 6 (agonist)19 and PA452 (9; antagonist),15 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.‡,§ 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.‡,§ We have

Received: August 12, 2011
Accepted: September 27, 2011
Published: September 27, 2011
reported RXR full agonists NEt-3IP (3) and NEt-3IB (4), which contain a phenyl group. Because these compounds have an amino group at the linker domain and an alkox 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.

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

Scheme 1a

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Reagents and conditions. (a) I2, Ag2SO4, MeOH. (b) (i) 2 N NaOH, THF, MeOH; (ii) 2 N HCl. (c) TMS acetylene, PdCl2(PPh3)2, CuI, triethylamine, THF. (d) K2CO3, MeOH. (e) (i) 2 N NaOH, MeOH; (ii) 2 N HCl. (f) H2, Pd-C, MeOH. (g) Phenylboronic acid, Pd–C, Na2CO3, EtOH. (h) Styrene, Pd(OAc)2, tris(o-tolyl)phosphine, triethylamine, acetonitrile. (i) Aniline, Pd(OAc)2, (±)-BINAP, Cs2CO3, toluene. (j) Benzophenoneimine, Pd(OAc)2, (±)-BINAP, Cs2CO3, toluene. (k) 2 N HCl, THF. 

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Scheme 1a
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. Deprotection of the TMS and methyl ester moieties gave 13b. The acetylene moiety of 13b was catalytically reduced to 13c. Moreover, 12 was reacted with phenylboronic acid by Suzuki–Miyaura coupling, styrene by Heck reaction, and aniline or benzophenoneimine by Buckwald coupling reaction, followed by deprotection to give 13d–g. The trans structure of 13e was supported by the 1H NMR coupling constant between the ethylene protons.

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, 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 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 pA2 value (common logarithm of the concentration of an antagonist that doubles the EC50 value of an agonist; used as an antagonistic activity index) was obtained from Schild plots. 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 pA2 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 pA2 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). 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 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 pocket of RXRα.
between the side chain of white stick molecule is a RXR-partial agonist UVI3002, which binds in the ligand-binding domain of RXR. 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). 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.

**Figure 4.** (a) Antagonist 13e (yellow) modeled into the ligand-binding pocket of RXRα (PDB code: 2P1V) 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). 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.

**ASSOCIATED CONTENT**

**Supporting Information.** General information, synthetic procedures, combustion analysis data, HPLC charts, luciferase reporter gene assay, Schild plot for pA2 determination, and molecular docking procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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