Ligand dependent switch from RXR homo- to RXR-NURR1 heterodimerization

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**INTRODUCTION**

The retinoid X receptor (RXR) plays a key role as a transcriptional regulator through formation of heterodimers with other nuclear receptor partners. Activation of RXR heterodimers exerts neuroprotective effects in animal models of neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and multiple sclerosis. The activity of RXRs is influenced by a class of compounds related to the naturally occurring 9-cis-13,14-dihydroretinoic acid. The L-shaped ligand binding pocket is unique to RXRs, and structural information derived from X-ray crystallographic data of the RXR ligand binding domains in the apo or holo state has aided the design of specific ligands for this receptor.

Comprehensive overviews of the structure, biology, and therapeutic implications of targeting RXRs with small molecule ligands are available. However, the chemical diversity of these ligands is limited by the structural constraints placed by the RXR ligand binding pocket and the availability of synthetic methodologies to access designed ligands. Controlled RXR heterodimerization and RXR partial agonism are contemporary biomedical challenges, both of which could in principle be addressed via exploration of an appropriate structural class.

Three RXR subtypes are known, RXRα (subject of this study), RXRβ, and RXRγ (NR2B1, NR2B2, and NR2B3), and all three interact similarly with many coregulator proteins, and with several nuclear receptors to form heterodimers. Ligand binding to RXR can induce the transcriptional activity of some of its heterodimeric partner receptors (NURR1, NGFIB, FXR, LXR, CAR, and PPAR). Thus, RXR-selective ligands that only activate specific RXR heterodimers may have greater therapeutic potential, because they would be expected to cause fewer side effects compared to ligands that cause general activation of RXR−NR heterodimers. In this respect, RXR−PPAR and RXR−LXR heterodimers have gained a lot of attention, as the clearance of Apβ through apoE in Alzheimer’s mouse models is believed to be facilitated by the activation of these heterodimers. For example, the ligand LG101506 was...
identified as the most potent of a series of selective RXR–
PPAR heterodimer activators, whereas it did not activate
the RXR-LXR heterodimer. The potential of this selective RXR
modulator as a treatment for type 2 diabetes with reduced side
effects was shown in mice. Activation of RXR–nuclear receptor
heterodimers with RXR receptor ligands also provides an
important strategy for activating orphan nuclear receptors
which themselves do not readily bind ligands (e.g., NURR1).

The nuclear receptor related 1 protein or NURR1 (also
referred to as NR4A2) controls the development, function,
and survival of dopaminergic neurons.20 NURR1 modulators, both for use as research tools, to address
modulation of NURR1 activity via RXRs
was subsequently evaluated as RXR agonists, using a fluorescence-
based polarization assay and cellular mammalian two-hybrid
assay. In addition, their effectiveness in modulating RXRα–
NURR1 heterodimers over RXRα–RXRα homodimers was tested using cell-based bioluminescence resonance energy
transfer (BRET2) assays.2,33 To corroborate these results and
to elucidate the binding mode and conformational changes in
the protein induced by these compounds, the X-ray structures of five of these novel ligands bound to RXRα were solved.

Results and Discussion

Synthesis. The synthesis and cocrystal structure of ligand 1
were described previously.35 The allyl side chain of 1 partly
occupies the lipophilic pocket in the ligand binding domain of
RXRα, analogously to the tetramethyl-cyclohexene unit found
in typical RXR ligands, leading to closure of the ligand binding
pocket via repositioning of helix 12 in an agonist conformation
and subsequent increased binding toward coactivators. The
high binding affinity and low molecular weight of 1 makes it an
ideal scaffold to explore modifications targeting the lipophilic
pocket (Table 1). Ligands 2–5 were therefore synthesized
bearing structural variations in the hydrophobic side chain. The
synthesis of compounds 2–5 relied on efficient palladium-
catalyzed cross coupling reactions (Scheme 1). The cinnamic
acid derivative 11 was treated with thionyl chloride in methanol
to obtain the methyl cinnamate derivative 12. Four boronic
acids or esters were then reacted with

![Figure 1. Designed biaryl-based RXR ligands 1–10 and established potent RXR agonist LG100268.](image-url)

Table 1. Summary of Fluorescence Polarization (FP) and Mammalian Two-Hybrid (M2H) Data for the RXRα

<table>
<thead>
<tr>
<th>compd</th>
<th>FP/EC50 (nM)</th>
<th>M2H (luciferase)/EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG100268</td>
<td>150 ± 40</td>
<td>5.4 ± 2.0</td>
</tr>
<tr>
<td>1 (R = CH3CH=CH2)</td>
<td>260 ± 110</td>
<td>6.3 ± 4.0</td>
</tr>
<tr>
<td>2 (R = Ph)</td>
<td>140 ± 23</td>
<td>85 ± 9</td>
</tr>
<tr>
<td>3 (R = Bn)</td>
<td>142 ± 9</td>
<td>92 ± 36</td>
</tr>
<tr>
<td>4 (R = iPr)</td>
<td>89 ± 7</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>5 (R = nPr)</td>
<td>170 ± 80</td>
<td>18 ± 10</td>
</tr>
<tr>
<td>6 (R = H)</td>
<td>9000 ± 2500</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>7 (R = H)</td>
<td>1020 ± 60</td>
<td>14 600 ± 1800</td>
</tr>
</tbody>
</table>

**EC50 values for LG100268 and ligands 1–7. See experimental
section for details of the assays. The 20- to 30-fold difference
between the FP and M2H data for the more potent compounds is a common
phenomenon, which can be explained by intrinsic differences
between the two different assay formats, in particular, the different
protein and peptide concentrations used.

![Diagram](image-url)
Scheme 1. Synthesis of Novel Biaryl RXRα Ligands

The syntheses of 1 and 8 have been previously reported. The biaryls 13–16 were thereafter demethylated using boron tribromide and hydrolyzed using sodium hydroxide, yielding ligands 2−5 in reasonable yields with high purities after a preparative HPLC purification.

Nahoum and co-workers for inducing RXR antagonism. Compound 8 (Figure 1) was previously described by us and found to lack significant RXRα activity, because of the additional polar phenolic functionality, which points toward a lipophilic environment. X-ray crystallographic data have demonstrated that alkylation of the appropriate phenol displaces the position of helix 12 toward a (partial) antagonistic fold, influencing the position of L436, which plays a determining role in the communication with helix 12. Therefore, using the biaryl scaffold, agonist 8 was modified with two different length alkoxy chains. The length of the alkoxy chain was hypothesized to be directly correlated with the displacement of helix 12 and therefore its antagonist properties. The antagonists 9 and 10 were synthesized using intermediate 18 for the Suzuki couplings, using the ligands introduced by Buchwald, with either 23 or 24. Intermediates 23 and 24 were each made in two steps in excellent yields via sp²−sp³ Pd-catalyzed cross couplings on the bromide group to introduce the allyl-substituted group. Finally,
intermediates 25 and 26 were treated with hydrochloric acid in THF for the deprotection of the MOM-group and subsequently with sodium hydroxide for the hydrolysis of the methyl ester to provide the antagonists 9 and 10.

**Pharmacological Evaluation.** The activity of the ligands on RXRα was initially profiled using a fluorescence-based coactivator recruitment polarization (FP) assay and then in a more biologically relevant mammalian two-hybrid (M2H) assay. The FP and M2H assays revealed an EC_{50(FP)} = 260 nM and EC_{50(M2H)} = 6.3 nM for 1, which compares favorably to the established, but more bulky, full agonist LG100268: EC_{50(FP)} = 150 nM and EC_{50(M2H)} = 5.1 nM (Table 1). The differences in measured EC_{50} affinities between the FP assay and the M2H assay are a common phenomenon because of intrinsic differences between the two assay formats, the protein concentrations, and the coregulator peptide.35,39 Besides 1, ligands 2–5 also displayed full agonism in both assays with the measured affinities (EC_{50}) in the nanomolar range (Table 1). Replacing the allyl group with the closely related i-propyl (4) or n-propyl (5) did not strongly affect the ligand affinity for RXRα. The aromatic phenyl (2) and benzyl (3) substituents displayed a 10-fold decrease in potency in the M2H cell-based assay compared to the smaller propyl substituents. Nevertheless, also these ligands still activate RXRα with nanomolar potencies. The RXRα binding of 1–5 is thus dominated by the hydrophilic portion of the biaryl ligands, i.e., the hydroxyl-cinnamic acid moiety. The hydrophobic substituents tune the ligand affinity but are not crucial for high ligand affinity.

The terphenyl ligand 2, provides an interesting platform to study the importance and effects of the rotation around the two phenyl–phenyl bonds. Addition of a single methyl group at the central phenyl (6 and 7) was thought to direct the rotation to preferred orientations in complex with RXRα. Ligands 6 and 7 both displayed full agonism in the FP assay and the M2H assay, albeit with potencies in the 1–10 μM range (Table 1). Comparison with the nanomolar affinities observed for ligand 2, demonstrates that the addition of the single methyl groups strongly impacts affinity for RXRα. This very strict SAR is typical for this class of biphenyls, as in our previous studies the placement of a hydroxyl functionality, such as in 8, similarly impacted affinity by changes over 100-fold.35 In the case of 6 and 7, the decrease in affinity might be caused by a suboptimal conformational match of the ligand for binding to the protein in the conformation befitting the binding pocket (vide infra).

We previously studied ligand 8, which demonstrated full agonism, but with only moderate affinity for RXRα in FP as well as M2H assays.35 The design of 9 and 10 prompted us to study these compounds in a competition format to profile their antagonist characteristics. RXRα was therefore stimulated with the full agonist LG100268 and the subsequent impact of ligands 9 and 10 on coactivator recruitment was studied via fluorescence polarization studies and on transcription via M2H assays. The addition of ligands 9 or 10 to the agonist-stimulated RXRα resulted in decreased fluorescence polarization, indicating lowered coactivator recruitment via displacement of the agonist and stabilization of an inactive RXRα conformation. Ligand 10 demonstrated full antagonism, while ligand 9 showed partial antagonism (Figure 3a). Consistent with its partial antagonist activity, ligand 9 also displayed partial agonism in an agonist assay format. The measured affinities of 9 (IC_{50(FP)} = 48.5 ± 4.6 μM) and 10 (IC_{50(FP)} = 46.9 ± 5.9 μM) at the RXRα receptor were approximately 25-fold lower than that of the known antagonist UVI3003: IC_{50(FP)} = 1.8 ± 0.6 μM. Compounds 9 and 10 were also RXRα antagonists in the cell-based M2H assay. M2H competition experiments showed a clear decrease in luciferase expression upon addition of either ligand in the 10–40 μM range after stimulation with agonist LG100268 (Figure 3b).

The potency and efficacy of the biaryl ligands to induce RXRα–RXRα homodimer and RXRα–NURR1 heterodimer conformational changes was determined using cellular BRET2 assays.2,33 (Table 2). Agonist ligands 1–5 all displayed remarkably strong potencies (single digit and sub-nanomolar EC_{50}’s) and high efficacies, comparable to the chemically optimized agonist LG100268 (Figure 1). Ligands 1–5 all feature preferential affinity for RXRα–NURR1 heterodimers. Ligand 4 displayed an encouraging 25-fold higher potency at RXRα–NURR1 over RXRα–RXRα, with a pEC_{50} of 9.1. It should be noted that in these same assays both the well-studied XR ligand bexarotene and the recently developed dihydrobenzofuran-based ligands only showed 2–7-fold selectivity.2,33 In contrast, the methylated terphenyl ligands 6 and 7 showed higher potency at RXRα–RXRα homodimers. The addition of the single methyl group to 2 ortho to the biphenyl bonds, resulting in 6 and 7, thus leads to a reversal in homo- vs heterodimer affinity.

The potency and efficacy of the antagonistic biaryl ligands 9 and 10 to selectively induce RXRα–RXRα homodimers over RXRα–NURR1 heterodimers toward antagonistic conformational changes was also determined. Partial antagonist 9 shows a profile similar to 6 and 7, but with further biased interactions.
toward RXRα homodimers over RXRα–NURR1 heterodimers by a factor of 10. Additionally, ligand 9 featured a lower efficacy consistent with its partial antagonist character. The full antagonist 10 did not show appreciable activity in the BRET2 assays.

Structural Evaluation. The cocrystallization of ligands 1, 3, 4, 6, and 7 with RXRα showed the canonical interactions of the carboxylate group of the ligands with Arg316, the backbone nitrogen of Ala327, and a conserved water molecule (Figure 2a). The free hydroxyl group on the ligands makes a hydrogen bond with Asn306. This hydrogen bonding network is
conserved for all the ligands and directs the positioning of the hydrophobic part of the molecules. The hydrophobic component of 1, 3, and 4 occupies the lipophilic region of the ligand binding pocket. In this region, ligand-dependent RXRα amino acid reorientations can be observed. Especially ligand 4 (i-propyl substitution) repositions Ile324, Val332, Ser336, and Val342 compared to ligands 1 and 3, creating a smaller ligand binding pocket (Figure 2a, zoom and Supporting Information Figure S60). This tighter packing of helices is less pronounced for the region around Ile 324, but mostly affects helices 6 and 7, showing amino acid displacements up to 2.8 Å, and the end of RXRα helix 11, and with that the loop between helix 11 and 12. The carboxy-terminal part of helix 11 has been identified to play a pivotal role in the dimerization of RXRs,42−44 such as via polar contacts between the C-terminal carboxylic acid of PPAR Helix 12 and lysine 431 of RXR helix 10/11.45 NURR1 features an atypical, longer, helix 1227 which, following a modeled RXR-NURR1 heterodimer46 and published RXR-PPAR crystal structures,45,47 probably points toward the RXRα LBD, notably RXRα helices 7 and 11. RXRα–NURR1 heterodimerization thus implies repositioning of RXRα structural elements in this region to accommodate binding of the NURR1 helix 12. Ligand 4 shows the strongest bias toward RXRα−RXRα homodimerization (Table 2). The repositioning of RXRα helices 7 and 11 by the compact ligand structure might therefore explain its strong selectivity for heterodimerization.

Terphenyl ligands 6 and 7 were provided with a methyl functionality at the ortho-position at either of the two biphenyl bonds. Compared to nonmethylated 2, ligands 6 and 7 featured decreased affinity for RXRα and bias toward RXRα−RXRα homodimerization. The X-ray cocrystallization of ligands 6 and
Despite the fact that RXR receptors play major roles in many biological processes through heterodimerization with other nuclear receptors, only a small number of small molecule RXR-heterodimer selective modulators are available, with limited chemical diversity and biophysical properties. This study has delivered a compact and focussed selection of RXRα–NURR1 agonists based on a versatile biaryl scaffold, structurally different than previously reported molecules. In earlier work using chiral dihydrobenzofuran acids, we demonstrated a 3-fold biased interaction with RXRα–NURR1. The biaryl scaffold presented here provides a 25-fold selectivity bias for RXRα–NURR1 in the case of analogue 4, and a >100-fold switch in homo- vs heterodimer selectivity when comparing analogs 4 and 9 in Table 2. The structural elucidation of five of these novel RXRα ligands, in our view, provides a first rationale toward understanding how to generate RXRα–NURR1 heterodimer selective ligands. Key ligand–protein interactions and correlated side-chain displacements were identified, modulating both selective dimerization and coregulator recruitment. Interactions of the ligand with key amino acid chains such as Ile324, Val332, Ser336, and Val342 on helices 6 and 7 tune the size of the ligand binding pocket. These compact ligands bind RXRα in a manner that allows movement of helix 7 and 11 to generate a compact ligand binding pocket conformation which arguably is more suited for heterodimerization with NURR1, potentially by enabling the accommodation of the long NURR1 helix 12. Interactions of substituents on the biaryl scaffold with RXRα amino acids involved in formation of the AF2, such as Leu436, induce helix 12 repositioning and translate into lower ligand affinity or, alternatively, into (partial) antagonist properties. These interactions with helices 11 and 12 are matched by the expansion of the RXRα ligand binding pocket via helices 6 and 7, leading to a selectivity of the biaryl scaffold for RXRα–RXRα heterodimers.

This novel series of ligands allows addressing a wide range of RXRα receptor conformations and associated functional outcomes via substitution patterns on the same biaryl scaffold, expanding the current RXR modulator repertoire with agonist as well as antagonist ligands. The data provide a rationale for the design of RXR ligands comprised of a unique hydrophilic region with a conserved hydrogen bonding network contributing to the binding affinity, and a hydrophobic region to probe the other parts of the receptor influencing dimerization properties or coregulator recruitment. These findings justify further exploration of the ligand-controlled homo- vs heterodimerization of RXR and its interaction partners, for activation of the NURR1:RXRα heterodimer as monotherapy for Parkinson’s disease, for delineating the resulting physiological effects on other RXR heterodimers, and also for potentially revealing conserved mechanisms for other nuclear receptors.

\section*{METHODS}

\textbf{Synthesis.} All the solvents employed were commercially available and used without purification unless stated otherwise. Water was purified using a Millipore purification train. All the reagents are commercially available and used without purification. All the NMR data were recorded on a Varian Gemini 400 MHz NMR, a Bruker Cryomagnet 400 MHz, a Bruker UltraShield Magnet 400 MHz, or a Varian 200 MHz (400 or 200 MHz for $^1$H NMR and 100 or 50 MHz for $^{13}$C NMR). Proton experiments are reported in parts per million.
(ppm) downfield of TMS. All 13C spectra were reported in ppm relative to residual chloroform (77 ppm). Analytical LC-MS was performed on a C4, Jupiter SuC4300A, 150 × 2.00 mm column with a gradient 5%−100% acetonitrile in H2O supplemented with 0.1% v/v formic acid (FA) in 15 min. Silica column chromatography was performed manually using silica with particle size 60−200 μm. Preparative HPLC was performed on a Gemini S4 110A 150 × 21.20 mm column using H2O and acetonitrile supplemented with 0.1% v/v F.A. Purity and exact mass of the compounds were determined using a High Resolution LC-MS system consisting of a Waters ACQUITY UPLC I-Class system coupled to a Xevo G2 quadrupole time of flight (Q-tof) system. The system comprised a Binary Solvent Manager and a Sample Manager with Fixed-Loop (SM-FL). Compounds were treated in vacuo. The crude product was purified by preparative reversed-phase HPLC by UV detection and freeze-dried.

(E)-Methyl 3-(6-Methoxy-[1,1′-terphenyl]-3-yl)acrylate (13). The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (120 mg, 0.529 mmol), [1,1′-biphenyl]-3-ylboronic acid (130 mg, 0.656 mmol), KF (155 mg, 2.67 mmol), SPhos (67 mg, 0.16 mmol), and Pd(dba)2 (48 mg, 0.052 mmol) at 110 °C for 18 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound as a colorless oil, 180 mg, 0.52 mmol, 98% yield. Silica gel TLC Rf = 0.25 (17% v/v EtOAc in heptane); LC-MS (ESI): calcd for C17H12O4 [M + H]+: 317.1178, found 317.1179.

(E)-3-(6-Hydroxy-[1,1′-terphenyl]-3-yl)acrylic Acid (2). The described procedure for deprotection, method A was used with (E)-methyl 3-(6-methoxy-[1,1′-terphenyl]-3-yl)acrylate (13) (100 mg, 0.29 mmol) to afford the title compound, 2, as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying (34 mg, 0.107 mmol, 37% over two steps). LC-MS (ESI): calcd for C17H11O3 [M + H]+: 303.1126, found 303.1122.

(E)-3-(3-Benzyl-6-methoxy-[1,1′-biphenyl]-3-yl)acrylate (14). The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (97 mg, 0.444 mmol), 2-(3-benzylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborole (153 mg, 0.520 mmol), KF (125 mg, 2.15 mmol), SPhos (54 mg, 0.13 mmol), and Pd(dba)2 (39 mg, 0.040 mmol) at 110 °C for 18 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound as a colorless oil, 146 mg, 0.41 mmol, 95% yield. Silica gel TLC Rf = 0.28 (17% v/v EtOAc in heptane); LC-MS (ESI): calcd for C21H16O3 [M + H]+: 337.1340, found 337.1341.
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126.50, 116.63, 115.08, 42.09; HRMS (m/z): [M + H]+ calcld 331.1334, found 331.1319.

(E)-Methyl 3-′-(3′-isopropyl-6-methoxy-1′,1′-biphenyl)-3′-yl)-acrylate (15). The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (104 mg, 0.51 mmol) as the substrate after treatment with BBr3. The crude material was purified using gel chromatography eluting with 5%−10% v/v EtOAc in hexane to yield the title compound as a yellow oil, 95 mg, 0.43 mmol, 85% yield; HRMS (m/z): [M + H]+ calcld 331.1334, found 331.1331.

(E)-Methyl 3-′-(3′-isopropyl-6-methoxy-1′,1′-biphenyl)-3′-yl)-acrylate (16). The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(4-methoxy-3′,4′,5,5-tetramethyl-1,3-dioxaborolan-2-yl)phenylacrylate (152 mg, 0.47 mmol), 1-bromo-3-propylbenzene (78 mg, 0.39 mmol), KF (81 mg, 0.20 mmol), and Pd2(dba)3 (61 mg, 0.066 mmol) at 110 °C for 18 h. The eluent used for purification was 15% v/v EtOAc in heptane to yield the title compound as a colorless oil in a quantitative yield. Silica gel TLC Rf = 0.35 (15% v/v EtOAc in heptane); LC-MS (ESI): calcld for C20H17O3: [M + H]+ 331.1334, found 331.1331.

(E)-3′-(6-Hydroxy-3′-isopropyl-1′,1′-biphenyl)-3′-yl)-acrylic Acid (4). The described procedure for Sonogashira coupling conditions method A was used with (E)-methyl 3′-(3′-isopropyl-6-methoxy-1′,1′-biphenyl)-3′-yl)-acrylate (15) (100 mg, 0.32 mmol) to afford the title compound, 4, as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying. 1H NMR (400 MHz, CDCl3): δ 8.05 (d, J = 7.7 Hz, 3H), 7.53 (d, J = 7.0 Hz, 3H), 7.29 (d, J = 7.4 Hz, 3H), 7.14 (d, J = 7.3 Hz, 3H), 7.04 (d, J = 7.2 Hz, 1H), 6.34 (d, J = 15.9 Hz, 1H), 2.98 (d, J = 6.9 Hz, 1H), 1.30 (d, J = 6.9 Hz, 6H); 13C NMR (100 MHz, CDCl3): δ 172.43, 155.03, 150.06, 146.82, 135.97, 130.79, 129.68, 129.65, 129.17, 127.26, 127.14, 126.80, 126.38, 116.57, 115.00, 34.34, 24.14; HRMS (m/z): [M + H]+ calcld 283.1334, found 283.1334.

(E)-Methyl 3′-(4′-Methoxy-3′,4′,5,5-tetramethyl-1,3-dioxaborolan-2-yl)phenylacrylate (12b).1 1-bromo-3-propylbenzene. To a solution of 3-bromopropiophenone (2.98 g, 14.0 mmol) in TFA (30 mL, 0.40 M) was added dropwise triethylsilane (11.5 mL, 72.0 mmol) at 0 °C in 5 min, and the mixture was stirred for additional 20 min. The reaction mixture was heated to 80 °C and stirred overnight. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. Toluene was added, and the mixture was again concentrated in vacuo to obtain the crude material. The product was purified via flash silica gel chromatography eluting with hexane to yield the title compound, 78 mg, 0.39 mmol, 3% yield; HRMS (m/z): [M + H]+ calcld 394.1441, found 394.1444.

(E)-Methyl 3′-(3′-isopropyl-6-methoxy-1′,1′-biphenyl)-3′-yl)-acrylate (16). The described procedure for Suzuki couplings method A was used with (E)-methyl 3′-(4-methoxy-3′,4′,5,5-tetramethyl-1,3-dioxaborolan-2-yl)phenylacrylate (152 mg, 0.47 mmol), 1-bromo-3-propylbenzene (78 mg, 0.39 mmol), KF (114 mg, 1.96 mmol), SPhos (32 mg, 0.078 mmol), and Pd2(dba)3 (25 mg, 0.027 mmol) at 110 °C for 21 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound as a colorless oil in a quantitative yield. Silica gel TLC Rf = 0.31 (heptane/EtOAc 17% v/v); LC-MS (ESI): calcld for C20H17O3: [M + H]+ 331.1334, found 331.1331.
for purification was 17% v/v EtOAc in heptane to yield the title compound 65 mg, 0.17 mmol, 70% yield. Silica gel TLC Rf = 0.29 (heptane/EtOAc 17% v/v); LC-MS (ESI): calcd for C27H30O5 [M + H]+: 389.18, observed 389.08, LC, Rt = 8.65 min; 1H NMR (400 MHz, CDCl3); δ (ppm) 7.69 (d, J = 16.0 Hz, 1H), 7.63–7.59 (m, 2H), 7.57–7.32 (m, 9H), 6.36 (d, J = 16.0 Hz, 1H), 5.3 (s, 2H), 3.79 (s, 3H), 3.37 (s, 5H), 1.65–1.48 (m, 2H), 1.37–1.08 (m, 6H), 0.85–0.76 (m, 3H); 13C NMR (100 MHz, CDCl3) δ 167.88, 156.92, 155.02, 144.80, 137.86, 131.77, 131.71, 131.67, 131.30, 131.11, 128.33, 127.63, 126.83, 124.71, 115.77, 115.15, 112.61, 94.89, 68.74, 56.15, 57.90, 53.92, 51.58, 29.25, 22.68, 14.11.

**[E]-3-(5′- Allyl-2′-(hexyloxy)-6′-hydroxy-[1,1′-biphenyl]-3-yl)acrylate (10).** The described procedure for deprotection, method B was used at room temperature with methyl ([E]-3-(5′-allyl-2′-(hexyloxy)-6′-hydroxy-[1,1′-biphenyl]-3-yl)acrylate (26) (17 mg, 0.039 mmol) LC-MS (ESI): calcd for C27H30O5 [M + H]+: 381.21, observed 381.00, LC, Rt = 7.83 min; 1H NMR (400 MHz, CDCl3) δ 7.79 (d, J = 15.9 Hz, 1H), 5.89 (dd, J = 8.4, 2.2 Hz, 1H), 7.27 (d, J = 8.3, 2.2 Hz, 1H), 7.16 (d, J = 8.3, 2.2 Hz, 1H), 7.07 (d, J = 8.3, 2.2 Hz, 1H), 7.00 (d, J = 8.3, 2.2 Hz, 1H), 6.35 (d, J = 15.9 Hz, 1H), 5.87 (s, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 1.78–1.56 (m, 2H), 1.51–1.30 (m, 6H), 0.91–0.76 (m, 3H). 1H NMR (400 MHz, CDCl3) δ 167.95, 156.87, 155.08, 144.86, 137.86, 131.77, 131.71, 131.67, 131.30, 131.11, 128.33, 127.63, 126.83, 124.71, 115.77, 115.15, 112.61, 94.89, 68.74, 56.15, 57.90, 53.92, 51.58, 29.25, 22.68, 14.11.

**Brominated Suzuki coupling reaction conditions.** The described general Suzuki coupling conditions A was used with ([E]-ethyl 3-(4′-(5′-methoxymethoxy)-3′,5′-dimethoxyphenyl)benzene. To a solution of 4-bromo-2-chlorophenol (73.5 g, 34.5 mmol, 1.0 equiv) in CHCl3 (50 mL, 0.70 M) was added NaN3-diisopropylamine (18.5 mL, 106 mmol, 3.0 equiv) and MOMCI (53.8 mL, 70.8 mmol, 2.0 equiv). The reaction was stirred at room temperature for 17 h. The reaction mixture was separated between CH2Cl2 and H2O and the aqueous layer was extracted twice with CH2Cl2. The combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated in vacuo. The product was purified via flash silica gel chromatography eluting with 10% v/v CH3OH in heptane to yield the title compound as a colorless oil, 8.9 g, 35 mmol, 99% yield. Silica gel TLC Rf = 0.29 (heptane/EtOAc 17% v/v); LC-MS (ESI): calcd for C27H30O5 [M + H]+: 389.18, observed 389.08, LC, Rt = 8.65 min; 1H NMR (400 MHz, CDCl3) δ 7.69 (d, J = 16.0 Hz, 1H), 6.05–5.66 (m, 3H), 5.76–4.79 (m, 2H), 3.78 (s, 3H), 3.37 (s, 5H), 1.65–1.48 (m, 2H), 1.37–1.08 (m, 6H), 0.85–0.76 (m, 3H); 13C NMR (100 MHz, CDCl3) δ 167.88, 156.92, 155.02, 144.80, 137.86, 131.77, 131.71, 131.67, 131.30, 131.11, 128.33, 127.63, 126.83, 124.71, 115.77, 115.15, 112.61, 94.89, 68.74, 56.15, 57.90, 53.92, 51.58, 29.25, 22.68, 14.11.

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chromatography eluting with 25% v/v EtOAc in heptane to yield the title compound, 524 mg, 1.51 mmol, 80% yield. Silica gel TLC Rf = 0.25 (heptane/EtOAc 25% v/v); LC-MS (ESI): calc for C20H16O3 [M + H]: 349.18, observed 348.92, LC, Rt = 7.42; 1H NMR (400 MHz, CDCl3) δ 7.87 (d, J = 2.3 Hz, 1H), 7.65 (d, J = 16.0 Hz, 1H), 7.53 (dd, J = 8.6, 2.3 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.36 (d, J = 16.0 Hz, 1H), 5.22 (s, 2H), 3.78 (s, 3H), 3.49 (s, 3H), 1.35 (s, 12H); 13C NMR (100 MHz, CDCl3) δ 167.82, 163.36, 144.49, 139.62, 132.38, 128.00, 116.05, 94.78, 83.89, 56.35, 51.71, 24.97.

4-Bromo-2-chloro-1-propoxybenzene. 4-Bromo-2-chloro-1-propoxybenzene (4.0 g, 19 mmol, 1.0 equiv) was dissolved in dry DMF (100 mL, concentration 0.20 M) in an oven-dried round-bottom flask. To this solution, K2CO3 (8.0 g, 58 mmol, 3.1 equiv) and 1-bromopropane (8.8 mL, 96 mmol, 5.1 equiv) were added, and the reaction was stirred at 70 °C for 18 h. The reaction was separated between H2O and CH2Cl2 and the aqueous layer was washed with CH2Cl2 three times. The combined organic layers were filtered via ash silica gel chromatography, eluting with 5% v/v EtOAc in heptane to yield the title compound, 724 mg, 1.52 mmol, 80% yield. Silica gel TLC Rf = 0.25 (heptane/EtOAc 5% v/v); GC-MS (ESI) m/z calc for C19H18BrClO: 249.53, most abundant peaks observed: 250, 210, 168, 133, Rt = 5.04; 1H NMR (400 MHz, CDCl3) δ 7.18 (d, J = 2.3 Hz, 1H), 6.98 (dd, J = 8.4, 2.2 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.04–5.70 (m, 1H), 5.10–5.06 (m, 1H), 5.06–5.01 (m, 1H), 3.95 (t, J = 6.5 Hz, 2H), 3.54–2.99 (m, 2H), 1.91–1.76 (m, 2H), 1.05 (t, J = 7.4 Hz, 3H); 13C NMR (50 MHz, CDCl3) δ 153.04, 137.21, 133.11, 130.36, 130.72, 127.80, 116.11, 113.56, 70.82, 39.18, 22.14, 20.94. 1.40 (m, 2H), 1.40–1.24 (m, 4H), 0.95–0.82 (m, 3H); 13C NMR (100 MHz, CDCl3) δ 154.04, 132.68, 130.53, 124.10, 114.47, 112.26, 69.44, 31.60, 29.08, 25.69, 22.68, 14.11.

4-Allyl-2-chloro-1-(hexyloxy)benzene (24). An oven-dried Schlenk flask was charged with 4-bromo-2-chloro-1-(hexyloxy)benzene (1.01 g, 3.47 mmol, 1.0 equiv), CsF (1.12 g, 7.37 mmol, 2.1 equiv), and Pd(PPh3)4 (0.379 mg, 0.344 mmol, 0.05 equiv). The flask was evacuated and backfilled with argon three times, and THF (22 mL, aryl halide concentration 0.16 M) was added. The mixture was stirred for 30 min at room temperature, and then 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.16 mL, 6.17 mmol, 1.8 equiv) and THF (7.5 mL) were added. The reaction was stirred at 78 °C for 21 h. Another portion of CsF (1.12 g, 7.37 mmol, 2.1 equiv), Pd(PPh3)4 (0.401 mg, 0.347 mmol, 0.01 equiv), and THF (30 mL) was added, and the mixture was stirred at 78 °C for another 24 h. The mixture was allowed to cool to room temperature and was separated between pentane and H2O. The aqueous layer was washed with pentane twice and the combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated in vacuo. The product was purified via flash silica gel chromatography eluting with 3% v/v EtOAc in heptane to yield the title compound as a colorless oil, 4.7 g, 19 mmol, 98% yield. Silica gel TLC Rf = 0.46 (heptane/EtOAc 5% v/v); GC-MS (ESI) m/z calc for C20H24BrClO: 379.10, most abundant peaks observed: 350, 312, 295, 255, 219, 183, 147; 1H NMR (400 MHz, CDCl3) δ 7.87 (d, J = 2.3 Hz, 1H), 7.65 (d, J = 16.0 Hz, 1H), 7.53 (dd, J = 8.6, 2.3 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.36 (d, J = 16.0 Hz, 1H), 5.22 (s, 2H), 3.78 (s, 3H), 3.49 (s, 3H), 1.35 (s, 12H); 13C NMR (100 MHz, CDCl3) δ 167.82, 163.36, 144.49, 139.62, 132.38, 128.00, 116.05, 94.78, 83.89, 56.35, 51.71, 24.97.

Mammalian Two-Hybrid (M2H) Assays. Mammalian two-hybrid (M2H) assays were performed as previously described.35

General Considerations for Protein Expression and Purification. All solutions and equipment used in the handling of microbial cultures were autoclaved or sterile filtered. Media, plastic, and glassware were autoclaved at 121 °C for 20 min prior to use. Bacterial cultures were incubated in a New Brunswick Series 25 shaker. Centrifugation was performed in a Beckman Coulter Avanti J-25 centrifuge. Microcentrifugation was performed in an Eppendorf centrifuge 5415R or a Beckman Coulter microfuge 18. All biological laboratory buffers and media were bought from common suppliers and used as purchased. BL21(DE3) and NovaBlue Escherichia coli competent cells were purchased from Novagen, XL-10. DNA and protein concentration was determined using a NanoDrop 1000 spectrometer from Thermo Scientific using a 260 and 280 nm wavelength, respectively. Gel electrophoresis for proteins was performed using 12% SDS-PAGE gels in running buffer and visualized using InstantBlue stain. Protein concentration was determined using a NanoDrop 1000 spectrometer with a wavelength ratio of 280–260 nm. The fluorescent D22 coactivator peptide was purchased from Invitrogen life technologies. RXRα-NURR1 heterodimerization and RXRα homodimerization BRETT2 assays were performed as described.36 Briefly, RXRα and NURR1 receptors were tagged with GFP and renilla luciferase. pEC50 is the negative logarithm of the EC50 in molar, and e is the exponential constant.

Fluorescence Polarization Assay. His6-RXRα-NURR1 expression, Purification, and Crystallization of the RXRα LBD. The histidine-tagged LBD of human RXRα (in a pET15b vector) was expressed in E. coli BL21(DE3). Cells were grown at 37 °C in LB
medium supplemented with 100 mg mL−1 ampicillin until OD600 reached about 0.7. Expression of T7 polymerase was induced by addition of isopropyl-β-D-thiogalactoside (IPTG) to a final concentration of 0.1 mM. After an additional incubation for 15 h at 15 °C, and cell cultures were harvested by centrifugation at 8000g for 20 min. The cell pellet from 2 L of RXRα LBD was resuspended in 50 mL buffer A (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 5 mM imidazole) supplemented with a protease inhibitor (PMSF) and DNase I. The suspension was then lysed by sonication and centrifuged at 35 000g and 4 °C for 45 min. The supernatant was loaded onto a 5 mL Ni2+-affinity column, pre-equilibrated with buffer A. The column was washed with 10 volumes of buffer A and 10 volumes of buffer A supplemented with 50 mM imidazole. Bound proteins were eluted with buffer A containing 200 mM imidazole. The fractions containing RXRα LBD were pooled, concentrated, and desalted to buffer B (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM DTT). To remove the histidine-tag, the protein was incubated for 16 h at 4 °C with threonin (1 unit/mg RXRα). The protein was passed through a Ni2+-column and a superdex gel filtration column. The protein was concentrated and stored at −80 °C until further use.

Before crystallization, the protein was mixed with a 1.5-fold molar excess of ligand and a 3-fold excess of TIF2 NR2 cofactor peptide (686-KHKILHRLLQDSS-698). The complex was incubated for 1h at 4 °C. Drops with a size of 2 μL containing 0.1 mM. After an additional incubation for 15 h at 15 °C, the cell pellet from 2 L of RXRα was collected using a centrifuge at 35 000g and 4 °C. Expression of T7 polymerase was induced by 0.1 mM. After an additional incubation for 15 h at 15 °C.

The manuscript was written through contributions of all authors. M.S. and F.A.M. performed synthesis; M.S., F.A.M., and J.-N.M. performed biochemical and cellular studies; M.S., S.A.A., R.M.J.M., and O. performed structural characterization and analysis; E.S.B., R.O., L.-G.M., and L.B. designed the studies; M.S., L.-G.M., and L.B. wrote the manuscript. All authors have given approval to the final version of the manuscript.

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Notes

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REFERENCES


