ACS Medicinal Chemistry Letters

Letter

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Dual RXR Agonists and RAR Antagonists Based on the Stilbene Retinoid Scaffold

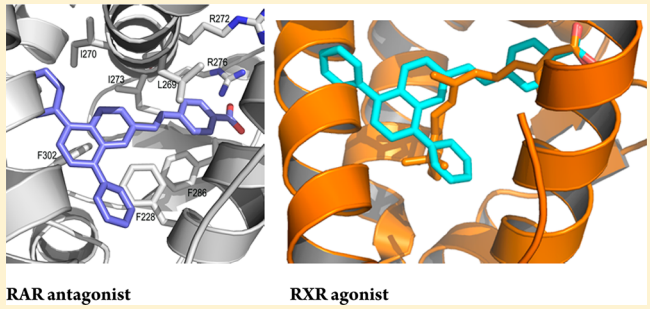
Claudio Martínez,[†] Michele Lieb,[‡] Susana Álvarez,[†] Fátima Rodríguez-Barrios,[†] Rosana Álvarez,[†] Harshal Khanwalkar,[‡] Hinrich Gronemeyer,*[,‡] and Angel R. de Lera*[,†]

[†]Departamento de Química Orgánica, Facultade de Química, CINBIO, Universidade de Vigo, and Instituto de Investigación Biomédica de Vigo (IBIV), 36310 Vigo, Spain

[‡]Equipe Labellisée Ligue Contre le Cancer, Department of Functional Genomics and Cancer, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)/CNRS/INSERM/UdS, BP 10142, 67404 Illkirch, Cedex, C. U. de Strasbourg, France

Supporting Information

ABSTRACT: Arotinoids containing a C5,C8-diphenylnaphthalene-2-yl ring linked to a (C3-halogenated) benzoic acid via an ethenyl connector (but not the corresponding naphthamides), which are prepared by Horner−Wadsworth−Emmons reaction of naphthaldehydes and benzylphosphonates, display the rather unusual property of being RXR agonists (15-fold induction of the RXR reporter cell line was achieved at 3- to 10-fold lower concentration than 9-*cis*-retinoic acid) and RAR antagonists as shown by transient transactivation studies. The binding of such bulky ligands suggests that the RXR ligand-binding domain is endowed with some degree of structural elasticity.



KEYWORDS: *Retinoid receptor subtypes, agonists, antagonists, arotinoids, transactivation, molecular modeling*

All-*trans*-retinoic acid **1**, its isomer 9-*cis*-retinoic acid **2**, and other natural retinoids are important signaling molecules that act in the embryo[1] and throughout adult life.[2−4] At the genomic level, retinoids bind the retinoid receptors (RARs, subtypes RARα,β,γ)[5] and retinoic X receptors (RXRs, subtypes RXRα,β,γ)[6] to regulate many important biological activities. The binding of these native retinoids and also of synthetic analogues to their receptors affect cell growth, proliferation, apoptosis, development, and homeostasis.[2,7−9] For the direct regulation of gene transcription, the functional unit is a RAR−RXR heterodimeric structure, which provides a binding interface with other recruited protein complexes that sense the ligand-modulated activation status of the receptor(s) and bind to DNA regulatory elements in the promoter regions of target genes to control transcription.[10,11] Within the series of heterodimeric complexes with RXR as a partner, RAR/RXR heterodimers are nonpermissive since their functional activation requires agonist binding to RAR, but not to RXR, in a phenomenon called RXR subordination.[12,13]

With the exception of 9-*cis*-retinoic acid (**2**) and a few other flexible analogues, which can bind both RXRs and RARs,[14−16] most of the more than 2500 synthetic retinoids so far reported show selectivity for either of them.[17−19] This is due to the different architecture of the ligand binding pockets (LBPs) in RAR and RXR. Whereas the former is an I-shaped pocket that binds elongated ligands,[20−22] the latter is L-shaped,[23,24] and RXR ligands (so-called rexinoids) must adopt a bent conformation to effectively bind the receptor.

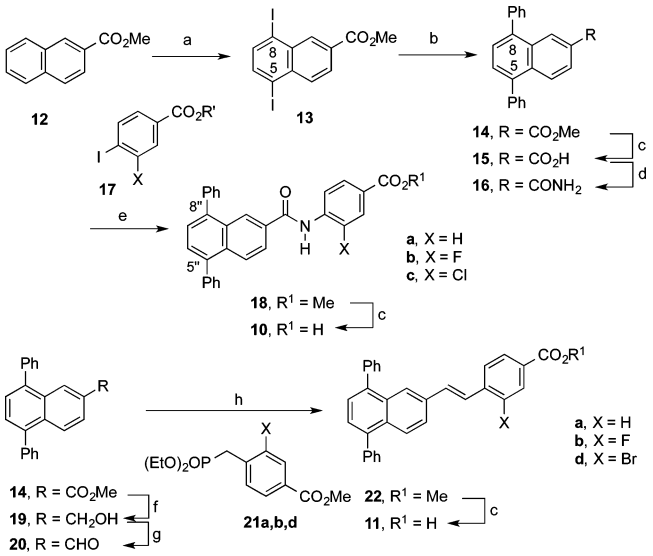
Ligands that act as agonists of RXR and antagonists of RAR are interesting chemical probes to help understand the signaling options of the RAR/RXR and other heterodimers.[25] We wish to report a new retinoid scaffold that displays this unusual property, as it acts in transactivation assays as a potent RXR agonist and RAR antagonist.

The structure of the new retinoid receptor modulator is inspired in the parent RAR pan-agonist TTNPB (**3**).[26−30] Numerous modifications of the basic scaffold have been systematically carried out in order to correlate structure and functional consequences of retinoid ligand modifications on receptor activation.[17−19] Substitution at the naphthalene C8 position afford in general ligands with RAR antagonist/inverse agonist activities,[28−30] and this is modulated[31] by synergy with halogen atoms at the C3 position[32] of the benzoic acid terminus. In these structures, the LBP volume around the naphthalene C5 position is filled with a hydrophobic gem-dimethyl group. Manual docking experiments carried out with RARα suggested that further extension of the substituents was possible, and this increase in bulk could impact on H12 positioning and thus endow the ligand with antagonist properties. Moreover, for synthetic purposes the naphthalene core was considered as a more suitable starting material than

Received: December 18, 2013
Accepted: March 19, 2014
Published: March 19, 2014

the dihydroderivative due to the easy functionalization with halogen atoms using S_EAr reactions, and these halogens could in turn be replaced with aryl groups employing Pd-catalyzed cross-coupling reactions. Both an olefin and an amide connector (compounds **10** and **11**, Scheme 1) were included in the design, as they are present in many of the well-characterized modulators in this series of compounds.[17−19,31]

Scheme 1[a]



[a]Reagents and reaction conditions: a. Ipy_2BF_4, TfOH, CH_2Cl_2, 25 °C, 20 h, 71%. b. $Pd(PPh_3)_4$, $PhB(OH)_2$, Na_2CO_3, 1:1 THF/H_2O, microwaves, 110 °C, 5 min, 91%. c. 2 M KOH, MeOH, 50 °C, 3 h, then H_3O^+ (**15**, 99%; **10a**, 72%; **10b**, 51%; **10c**, 43%; **11a**, 89%; **11b**, 87%; **11d**, 81%). d. EDCI, HOBt, NH_3, DMF, 25 °C, 3 h, 60%. e. CuI, (1*S*,2*S*)-cyclohexane-1,2-diamine, K_2CO_3, dioxane, 140 °C, 17 h (**18a**, 74%; **18b**, 75%; **18c**, 64%). f. DIBAL, THF, −78 °C, 4 h, then H_3O^+, 78%. g. MnO_2, CH_2Cl_2, 25 °C, 3 h, 84%. h. **21a−d**, *n*-BuLi, THF, DMPU, −78 °C (**22a**, 67%; **22b**, 70%; **22d**, 73%).

Using excess bis-(pyridine)iodonium(I) tetrafluoroborate (Ipy2BF4)[33] and triflic acid, the diiodo derivative **13** was obtained from methyl 2-naphthoate **12** by substitution at both activated positions (C5 and C8, Scheme 1). Cross-coupling of the diiodide **13** with phenylboronic acid [$Pd(PPh_3)_4$, Na_2CO_3, 60 °C, 2 h][34] afforded compound **14**. The preparation of *N*-arylamides was based on the Cu(I)-diamine-catalyzed amidation of the 4-halo- or 3,4-dihaloderivatives (using, in this case, the more reactive iodides at the C4 position) with the corresponding naphthamides.[35,36] Saponification of **14** was followed by primary amide formation using EDCI and HOBt in MeOH in the presence of NH_3[37] to furnish the disubstituted naphthamide **16**. Using CuI as catalyst, (1*S*,2*S*)-cyclohexane-1,2-diamine as ligand, and K_3PO_4 in dioxane,[38−41] the condensation of **16** with halogenated benzoates **17** produced the corresponding amides **18** after CAr-N bond formation at the more reactive position in good yield (64−75%). The sequence was completed with the saponification of the esters using KOH in MeOH at 60 °C, which proceeded in high yield except for the 3-chloro analogue (**10c**; 43%).

The stilbenoids **22** were prepared by Horner−Wadsworth−Emmons condensation of naphthaldehyde **20** and the corresponding benzylphosphonates **21a,b,d** (X = H, F, Br) as previously described.[31] Reduction of naphthoate **14** with DIBAL at −78 °C followed by oxidation of **19** provided aldehyde **20**. Reaction of the phosphonate-stabilized anions, obtained by treatment of **21a,b,d** with *n*-BuLi in THF in the presence of DMPU at −78 °C with aldehyde **20** produced the *E*-isomers ($^3J_{H-H}$ between 16 and 18 Hz for the vinyl protons in the ^1H NMR spectra) of stilbenoids **22a,b,d** in good yield. These esters were used in the next saponification step without further purification, due to their ease of isomerization to the *Z* isomers, and furnished the final arotinoids **11a,b,d** (67−73%).

To evaluate the effects of the described retinoids on RARα, RARβ, RARγ, and RXRβ-mediated transactivation, a reporter assay with genetically engineered HeLa cell lines[17,28] was used. A single RXR reporter cell line (expressing RXRβ LBD) is assumed to reveal the ligand responsiveness as a readout for all three RXRs, given the identity of the amino acids constituting the ligand-binding pockets of the RXR subtypes.[6,7] The reporter cell lines are engineered to express a fusion protein, comprising the ligand-binding domain of the corresponding receptor and the DNA-binding domain of the yeast GAL4 transcription factor. In addition, the cells contain a stably integrated luciferase reporter gene, which is controlled by five Gal4 response elements in front of a β-globin promoter; this is termed (17m)5-βG-Luc.[12,42] This reporter system is largely insensitive to endogenous receptors, which cannot recognize the GAL4-binding site. The transcriptional activity of the various compounds was compared with that of the pan-RAR agonists **3** and **2** (Figure 1) as positive controls for RAR and

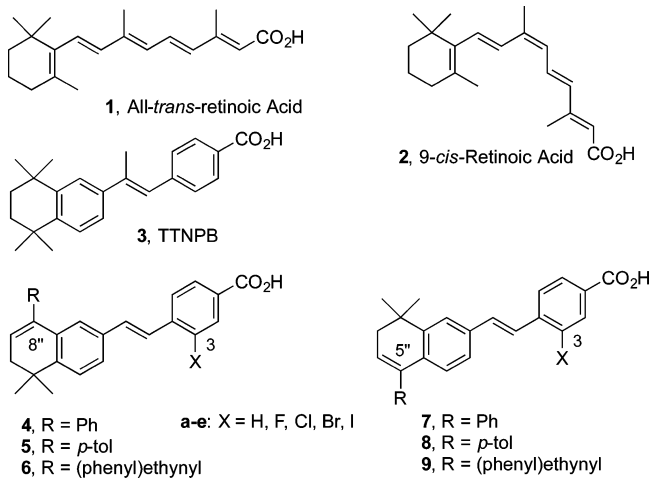


Figure 1. Natural RAR ligands (**1** and **2**), parent arotinoid pan-agonist TTNPB (**3**), and dihydronaphthalene analogues substituted at C8″ (**4**−**6**) and C5″ (**7**−**9**).

RXR activation, respectively. In the antagonist assays the positive controls are challenged with increasing amounts of putative antagonists, resulting in decreased transactivation of the reporter. Note that depending on their receptor binding affinities weak agonists can appear in this in vivo competition assay as antagonists, albeit they retain at high concentration their weak agonist activity. The transcriptional data of the naphthamide and stilbene arotinoids with the RAR subtypes is presented in Figures 2 (agonist activity) and 3 (antagonist activity).

Naphthamides showed no (RARα) to modest activities (RARβ and RARγ at 10 μM), and only **10a** was able to considerably activate RARβ at very high concentrations (>1 μM) to levels obtained with 0.1 μM **3** (Figure 2). There is

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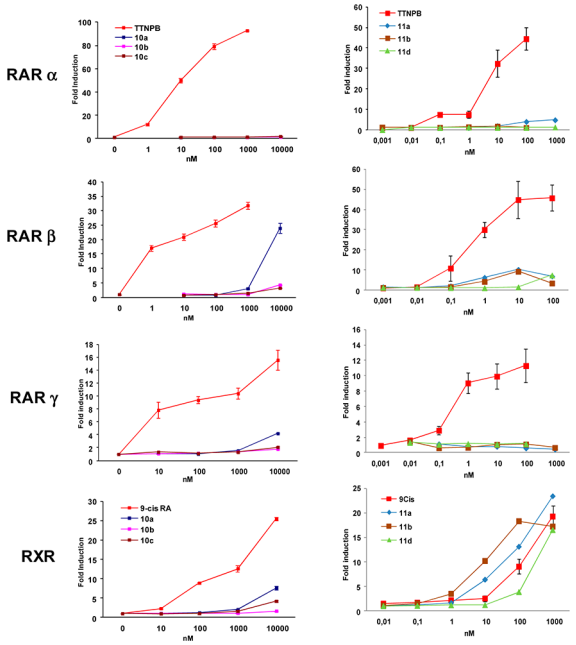

Figure 2. In vitro dose−response luciferase reporter assays revealing the RAR subtype agonist activities of the retinoids described in this study relative to agonists **3** and **2** (Figure 1). For details on the assay system, see the Supporting Information. The data are derived from at least three independent experiments; the standard deviations are indicated.

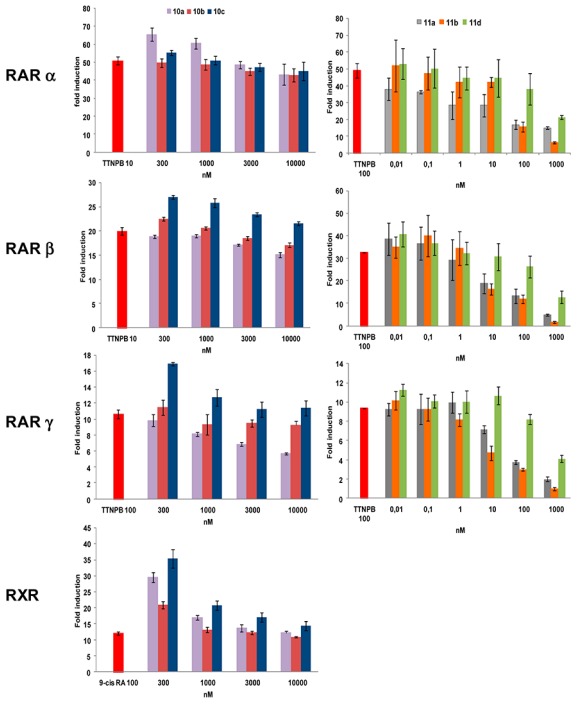

Figure 3. In vitro dose−response luciferase reporter assays revealing the RAR subtype antagonist activities of the retinoids synthesized in this study. The agonists **3** (10 or 100 nM) and **2** (100 nM) are challenged with increasing amounts of the various retinoids, and luciferase activity is determined. The decreased activity relative to **3** or **2** reveals antagonism. For further details on the assay system, see the Supporting Information. The data are derived from at least three independent experiments; the standard deviations are indicated.

some residual activation of RARγ at such high concentrations, indicating that **10a** can bind to RARγ but may not induce a highly active conformation. This likely explains why this ligand acts at the same time as the weak antagonist of RARγ (Figures 2 and 3, left panels). In the case of the stilbenes, however, strong activation of RXRβ was observed, while these compounds were unable to activate RAR subtypes even at 1 μM concentration (Figure 2, right panels). In particular, **11a** and less pronounced **11b** showed a dose−response curve resembling that of **2** but shifted by about 2- and 10-fold, respectively, to higher concentrations ("right shift"); specifically, a 15-fold induction of the RXR reporter cell line was achieved at 51 nM **11b**, 125 nM **11a**, and 875 nM **11d**, compared to 541 nM obtained with **3**. Moreover, competition studies between **3** and the synthesized arotinoids in this series revealed strong antagonism of the RAR subtypes, particularly of **11a** and analogue **11b** (with a fluorine atom at C3) and to a lesser extent of **11d** (with a bromine at C3) (Figure 3, right panels).

The dual modulatory activity of compounds **11a** and **11b** led us to explore the structural basis of their behavior as RXR agonist and RAR antagonist, which is surprising as their structure contains a *trans*-olefin as a connector. Since the structure of RARα bound to antagonist BMS195,614 (Supporting Information) at 2.50 Å resolution (PDB code: 1dkf)[24] is known, the model of RARα-antagonist-**11a** complex was constructed. The structure of human RARβ complexed with **3** (PDB code: 1xap)[22] provided the template for the highly homologous 210−216 region of RARα, which is severely disordered in the antagonist-bound crystal structure of RARα. Ligand **11a** was positioned in the active site on the basis of the canonical positions revealed by the crystal structures.[20−22,32,43,44] Preferred docking sites for functional groups were evaluated with the program GRID,[45] which assisted in the selection of binding modes.[31] The resulting model showed the two phenyl moieties at C5 and C8 positions running almost perpendicular to the ethenylbenzoic acid buried in a predominantly hydrophobic pocket (Figure 4). Unrestrained molecular dynamics simulations[31] showed a notably stable behavior reflecting that the overall architecture of the protein was preserved for the whole length of the simulation, including the ionic bridge anchor. The evolution of the root-mean-square deviation (rmsd) of **11a** with respect to the initial structure shows rmsd lower than 0.5 Å (see Supporting Information) and maintains the ionic bridge of the carboxylate to Arg276 (Figure 4).

Similar docking experiments with RXRα failed to provide stable solutions without severe distortion of the protein-binding pocket, and no binding poses could be found due to the bulky phenyl extensions protruding from both sides of the ligand naphthyl core, in particular the C5 phenyl group interacting with H11 (see Supporting Information). Extended pockets in the LBD of nuclear receptors to allow binding of bulky agonists have been noted by X-ray crystalography of ligand-bound estrogen receptor α (ERα).[46] This precedent suggests that the RXR ligand-binding domain is also endowed with some degree of structural elasticity to allow binding of bulky stilbenes such as **11a** or **11b**.

To summarize, diphenylsubstituted (*E*)-4-(2-(naphthalen-2-yl)vinyl)benzoic acids and the corresponding 4-(2-naphthamido)benzoic acids with the same substitution pattern have been prepared and evaluated as retinoid receptor modulators. Transactivation studies in this series revealed

dx.doi.org/10.1021/ml400521f | ACS Med. Chem. Lett. 2014, 5, 533−537

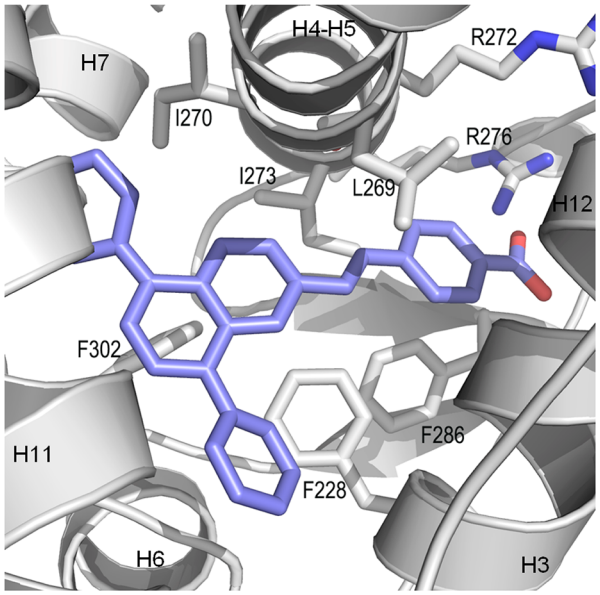

Figure 4. Proposed docking site for **11a** (as blue sticks). The Cα trace of the RARα LBD is displayed as a ribbon, colored in gray. The side chains of Phe228, Leu269, Ile270, Arg272, Ile273, Arg276, Phe286, and Phe302 (which show consistent favorable and large van der Waals interactions with the ligand) are shown as sticks with carbon atoms colored in gray.

that, whereas the amides are poor ligands, the corresponding stilbenes exhibited dual modulatory activities, with both strong activation of RXRβ and strong antagonism of the RAR-subtypes, a profile rarely found in retinoids.[25,47]

■ ASSOCIATED CONTENT

Supporting Information

Experimental and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(A.R.d.L.)Tel: +34-986-812316. Fax +34-986-818622. E-mail: qolera@uvigo.es.

*(H.G.) Tel: +(33)-388653473. Fax: +(33)-388653437. E-mail: hg@igbmc.fr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Spanish MINECO (SAF2010-17935), Xunta de Galicia (INBIOMED-FEDER "Unha maneira de facer Europa"), EU FP7 (BIOCAPS 316265/REGPOT-2012-2013.1), the Ligue National Contre le Cancer (laboratoire labelisé), the Association pour la Recherche sur le Cancer, the Institut National du Cancer (INCa), the Fondation pour la Recherche Médical (FRM), and the AVIESAN/ITMO Cancer for support. We are grateful to Prof. José M. González (Universidad de Oviedo) for his generous gift of Ipy2BF4.

■ ABBREVIATIONS

DIBAL, diisobutylaluminum hydride; DMPU, *N*,*N*-dimethyl propylene urea; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; Ipy, bis-(pyridine)iodonium(I); LBP, ligand binding pocket; PDB, protein data bank

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