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Letter

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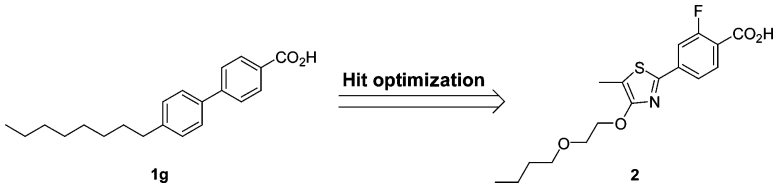

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Discovery of a Potent, Orally Available, and Isoform-Selective Retinoic Acid β2 Receptor Agonist

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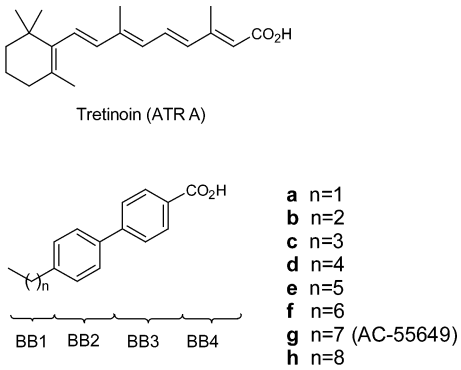
Abstract: 4′-Octyl-4-biphenylcarboxylic acid (**1g**, AC-55649) was identified as a highly isoform-selective agonist at the human RARβ2 receptor in a functional intact cell-based screening assay. The subsequent hit to lead optimization transformed the lipophilic, poorly soluble hit into a more potent and orally available compound (**2**, AC-261066) with retained β2 selectivity and greatly improved physiochemical properties. Being an isoform-selective RARβ2 receptor agonist that discriminates between nuclear receptor isoforms having identical ligand binding domains, **2** will be useful as a pharmacological research tool but also a valuable starting point for drug development.

Isoform-selective retinoic acid receptor (RAR) agonists are of potential use for the treatment of cancer and other hyperproliferative disorders. Currently, several nonselective retinoids are marketed or in clinical trials for cancer therapy (e.g. Tretinoin (*all-trans*-retinoic acid, ATRA) (Chart 1)). However, owing to their nonselective profile, severe side effects have been observed during chronic administration of these compounds.[1]

The biological effects of retinoids are mediated by two classes of nuclear receptors, predominately in the form of heterodimers, RARs and the retinoid X receptors (RXRs), each of which contain three subtypes classified as α, β, and γ.[2] RARγ is associated with skin, bone, and teratogenic toxicity, and RARα with triglyceride elevation. Thus, substantial toxicity may be avoided with a selective RARβ ligand.[3] The RARβ subtype consists of four known isoforms generated from two promoters P1 (RARβ1 and RARβ3) and P2 (RARβ2 and RARβ4).[4] Each of the RARβ isoforms displays unique expression patterns in the developing embryo and the adult, suggesting specific, nonoverlapping physiological functions.[4,5] For example RARβ2 has been suggested to exert an isoform-specific suppressive effect of tumors in humans.[6] Finding RARβ isoform-selective ligands constitutes a challenging endeavor since the ligand binding domains of the isoforms are identical. The variation between the isoforms is located within the proximal N-terminus, which encompasses the ligand-independent activation domain (AF-1).

Chart 1



Here we report on the discovery of the first ligand that discriminates between nuclear receptor isoforms having identical ligand binding domains. We also report on a hit to lead optimization effort transforming a lipophilic, poorly soluble hit into a more potent RAR agonist with retained β2 selectivity and greatly improved physiochemical properties.

The RARβ2 receptor was screened in intact cell uHTS format against a chemical library of over 160 000 small molecule organic compounds using a functional R-SAT assay.[7] The confirmed RARβ2 receptor hits were profiled against RARβ1 and the remaining of RARs (α and γ) and RXRs (α, β, and γ) receptor subtypes. 4′-Octyl-4-biphenylcarboxylic acid, AC-55649 (**1g**), was identified as a novel retinoid-selective agonist with potency in R-SAT of 100 nM at the RARβ2 receptor. Compound **1g** displays 100-fold selectivity for RARβ2 versus the other retinoid receptors (Figure 1).

To further characterize **1g** retinoid-like activities, its ability to modulate transcriptional properties of retinoid receptors was investigated. **1g** was a potent and selective activator of RARβ2 transcriptional activity (pEC_{50} 7.2, 71% eff).[8] In addition, the activity of **1g** was confirmed by using a well-established in vitro system commonly used to assess retinoid activity (inhibition of proliferation of the breast cancer cell line MCF-7),[8] and nonselective toxic effects were ruled out by a study of DNA synthesis measuring ^{3}H thymidine incorporation.[9]

To establish the SAR of the 4′-alkyl chain length of **1g**, commercially available analogues **1a**-**h** with 2−9 carbons in the 4′-alkyl chain were studied (Table 1). While a number of three carbons or less in the alkyls chain did not result in any activity, the butyl, pentyl, and hexyl analogue were potent but not efficacious. Increasing the number of carbons in the alkyl chain stepwise from 6 to 9 increased the efficacy. Thus, the octyl and the nonyl chain analogues **1g** and **1h** displayed full agonist activity. In addition, **1h** showed an increased potency at the RARβ1 isoform compared to **1g**.

To improve the physicochemical properties of **1g**, a compound developed in the context of liquid crystals,[11] a hit to lead optimization effort was initiated. Four building blocks were combined (Chart 1): branched alkyl and heteroalkyl groups of different chain lengths (BB1); substituted aromatic and hetero aromatic rings (BB2 and BB3); esters, amides, and carboxylic acid bioisosteres, e.g. tetrazoles and sulfonamides (BB4).

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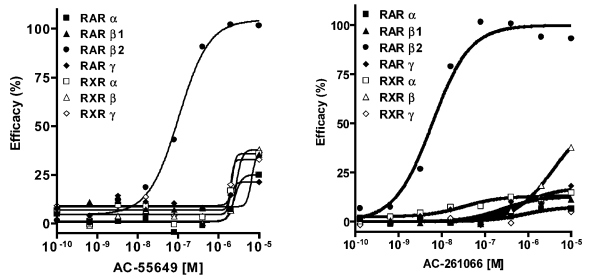


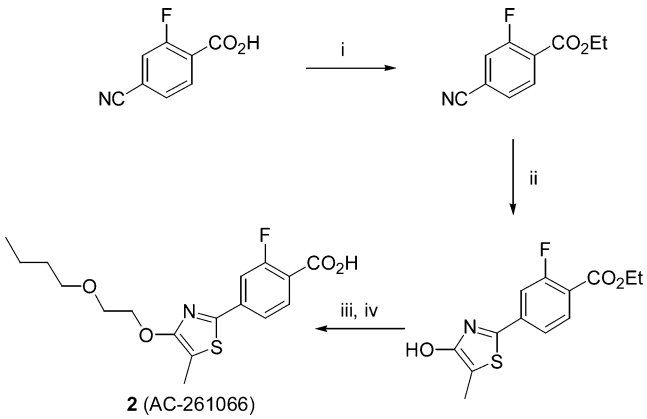
Figure 1. RAR/RXR Activity and selectivity of **1g** and **2** in the functional mammalian cell-based assay R-SAT. R-SAT is based upon the principles of genetic selection and amplification that allows for the monitoring of proliferative responses mediated by receptors in a ligand-dependent manner.

Table 1. Activities of RARβ2-Selective Agonists[a]

	RARβ2		RARβ1	
compd	% eff	pEC$_{50}$	% eff	pEC$_{50}$
Am-580	100	7.7 ± 0.4	103 ± 11	7.3 ± 0.4
ATRA	127 ± 10	8.2 ± 0.4	230 ± 13	7.6 ± 0.4
1a	na		na	
1b	na		na	
1c	36 ± 17	7.2 ± 0.6	na[b]	
1d	38 ± 18	7.8 ± 0.2	na[b]	
1e	37 ± 10	7.3 ± 0.5	na[b]	
1f	64 ± 19	7.6 ± 0.3	na[b]	
1g, AC-55649	92 ± 24	6.9 ± 0.4	31 ± 14[c]	
1h	100 ± 10	7.8 ± 0.4	58 ± 11	6.5 ± 0.2
2, AC-261066	96 ± 33	8.0 ± 0.1	40 ± 10[c]	

[a] Am-580[10] was used as reference and set to 100% eff. [b] na = no activity at pEC$_{50}$ > 5.0. pEC$_{50}$ and efficacy values are the means of at least three experiments ± SD. [c] Max efficacy at concentrations < 10 μM.

Scheme 1[a]



[a] Reagents and conditions: (i) EDCI·HCl, HOBt, DIPEA, EtOH, DMF, r.t., 16 h, 93 yield%; (ii) 2-mercaptopropionic acid, pyridine, 150 °C, 15 min; 67 yield%; (iii) 2-butoxyethyl bromide, Cs_2CO_3, CH_3CN, 180 °C, 25 min; (iv) LiOH, H_2O, THF, 160 °C, 5 min, 68 yield%, two steps.

During the hit to lead optimization, compound **2** (Scheme 1) was found to be a more potent RARβ2 receptor agonist than **1g** (Table 1) in an assay based on intact cells. In addition **2** has more traditional druglike properties: CLogD 0.7 (experimental values LogD 1.4, LogP 5.1, and solubility 4.8 mg/mL, phosphate buffer pH 7.4), compared to the hit **1g** CLogD 5.6 (solubility < 0.001 mg/mL, phosphate buffer pH 7.4). Importantly, **2** also displayed a comparable selectivity to **1g** versus the other RAR and RXR receptors. In addition, **2** exhibited good oral bioavailability in rats (F_{oral} = 52%).

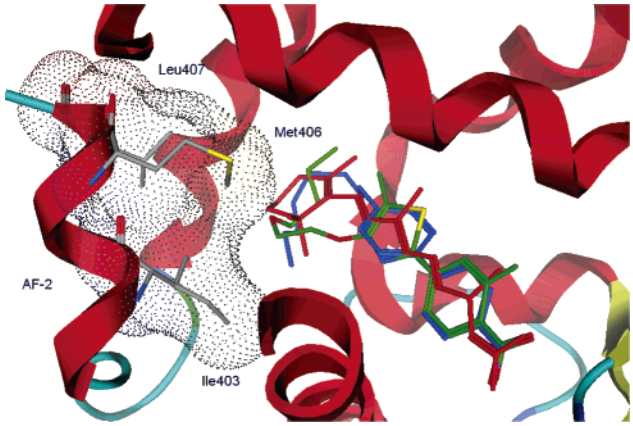


Figure 2. Superposition of **1g** (blue), **2** (green), and tretinoin (red).

The route for the synthesis of **2** is outlined in Scheme 1. After ester formation, the thiazole-4-ol moiety was synthesized under microwave irradiation conditions. The resulting solid was washed with CH_3CN and used without further purification in the O-alkylation step. Distillation of the crude reaction mixture removed the low boiling byproducts, and treating the residue with base afforded carboxylic acid **2** in 68% overall yield (two steps).

Assuming that **1g**, **2**, and tretinoin interact with the classical retinoid acid binding site, these three structures were docked in a model of the ligand binding domain of the RARβ receptor, built by homology modeling using RARγ as a template.[12] As seen in Figure 2, there is a high degree of steric overlap in the carboxy-aromatic part of the molecules, where the negatively charged carboxylate creates a network of hydrogen bonds with Arg278 and Ser289. A lipophilic pocket accommodates the cyclohexenyl ring of tretinoin and the aliphatic chains of both in-house compounds. Our modeling studies suggest that the favorable interactions that **1g** and **2** can form with Leu407, Met406, and Ile403 may keep the important α-helix 12 (AF-2) in the active conformation thereby enabling the recruitment of co-activators and the initiation of the transcription process. Ligands with shorter chains (**1a**−**e**) fail to create a sufficient contact surface with AF2. A modest but still significant ligand-dependent receptor activation is seen with the RARβ2 receptor while the RARβ1 receptor is not activated by ligand **1f**. However, increasing the number of carbons in the alkyl chain (**1h**) caused an activation of the RARβ1 receptor. Hence, experimental and computational data[7] corroborate the hypothesis that the degree of interaction between the alkyl chain of the agonist and AF-2 determines the agonist efficacy.

It has been reported that the ligand binding domain, i.e., AF-2, of a given RAR isotype cooperates with the AF-1 domain in a promoter context manner,[13] and whereas the AF-2 domains are conserved between the isoforms, the AF-1 domains are not.[14] This provides a rationale for the isoform selectivity of **1g** and **2**: The chain fragment of **1g** and **2**, which are interacting with AF-2, differ significantly in structure from that of tretinoin and may induce a beneficial interaction between different AF-1 and the conserved AF-2 regions of the RARβ2 isoform but not of the RARβ1 isoform. The complex between the less selective tretinoin and AF-2,

on the other hand, would not be able to discriminate as powerfully between the AF-1 domains of the two isoforms.

We have discovered a novel class of potent, highly selective RARβ2 receptor agonists using functional high throughput screening. Moreover, the highly lipophilic hit **1g** was developed into a selective and more potent lead compound **2** with druglike properties.

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Supporting Information Available: Synthetic procedures and characterization data for compound **2**. This material is free of charge via the Internet at http://pubs.acs.org.

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