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Identification of potent and selective retinoic acid receptor gamma (RARγ) antagonists for the treatment of osteoarthritis pain using structure based drug design

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ABSTRACT

A series of triaryl pyrazoles were identified as potent pan antagonists for the retinoic acid receptors (RARs) α, β and γ. X-ray crystallography and structure-based drug design were used to improve selectivity for RARγ by targeting residue differences in the ligand binding pockets of these receptors. This resulted in the discovery of novel antagonists which maintained RARγ potency but were greater than 500-fold selective versus RARα and RARβ. The potent and selective RARγ antagonist LY2955303 demonstrated good pharmacokinetic properties and was efficacious in the MIA model of osteoarthritis-like joint pain. This compound demonstrated an improved margin to RARα-mediated adverse effects.

Retinoic acid receptors (RARα, β and γ) are part of a superfamily of nuclear receptors (NRs) that behave as ligand-activated transcription factors and operate as part of a complex signaling network.[1] It has been shown that the natural ligand for the RARs, All Trans Retinoic Acid (ATRA), is deleterious to articular cartilage health and is associated with the breakdown of cartilage in osteoarthritis.[2] Both natural and synthetic retinoids (RAR agonists) are catabolic to cartilage, block early chondrogenesis and promote chondrocyte hypertrophy via RAR-mediated signaling.[3] It was also demonstrated that retinoid levels are increased in the synovial fluid of OA patients as a function of disease severity.[4] Finally, ATRA has been shown to cause nociceptive pain in rodents and this effect can be blocked by a pan-RAR antagonist.[5,6] It has been postulated that RAR antagonists may prevent or reverse retinoid-mediated cartilage destruction and mitigate OA pain.

A RAR pan antagonist (BMS-189453) was previously shown to improve clinical scores in rodent models of joint pain and inflammation,[7] albeit with unacceptable adverse effects on testes.[8] Based on the severe testicular effects observed in RARα knock-out mice,[9] this effect is largely believed to be RARα-mediated. In contrast, we recently showed that a selective RARγ agonist was sufficient to cause an increase in markers of articular cartilage catabolism, such as the proteolytic enzyme ADAMTS-5, in rats and a selective RARγ antagonist could reverse those effects.[10] Thus, due to the apparent importance of RARγ in the joint, and the clear safety issues associated with RARα antagonism in other tissues, we sought to identify RARγ antagonists with very high selectivity versus RARα for potential use in the treatment of OA and OA pain.

Our lead generation began with the identification of **1** (Fig. 1), which contained the tetramethyl-tetrahydronaphthalene substructure (in red), common to many synthetic retinoids. This compound was a potent RARγ binder, but with only modest (~10×) selectivity versus RARα and β. Additionally, **1** demonstrated full RARγ antagonist function in a cellular co-transfection assay (K_B = 2.2 nM, max inhibition >100%).[11,12] Removal of the tetramethyl-tetrahydronaphthalene substructure resulted in **2**, which maintained similar potency, but diminished selectivity.

Crystallography and structure-based drug design were used to improve selectivity of this scaffold. With sufficient potency for

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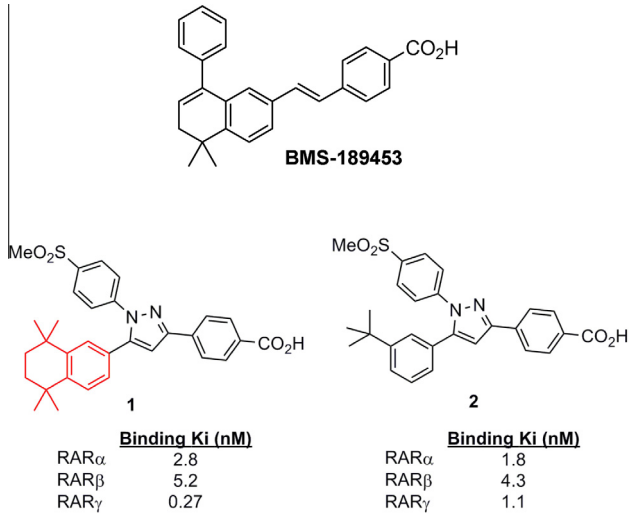


Figure 1. RAR pan antagonist hits.

RARγ, we focused on the solution of a structure bound to RARα. Our goal was to disrupt RARα binding in a way that could potentially maintain RARγ binding. Thus, we solved the structure of **2** bound to the ligand binding domain of RARα (Fig. 2). Based on previously reported RAR structures,[13] we were not surprised by the binding pose of **2** with the benzoic acid functionality anchored to the subtype conserved arginine and the methylsulfonyl-phenyl group bound near helix 12.[14] Similar to other NHR antagonists, this aromatic group perturbs helix 12 in a way that prevents coactivator binding, which is necessary for antagonist function.

The ligand binding domain of RARs are highly conserved with differences occurring in only 3 residues, as noted in Table 1.[15] Our attention was drawn to the residues in helix 5. Specifically, we were interested in the single residue difference between RARα and RARβ versus RARγ in this region. We hypothesized that the branched sidechain of Ile270 (RARα) and Ile263 (RARβ) would occupy more three dimensional space and be less tolerant to ligand-associated steric volume, than the linear sidechain of Met272 found in RARγ. Thus, to potentially disrupt RARα and RARβ

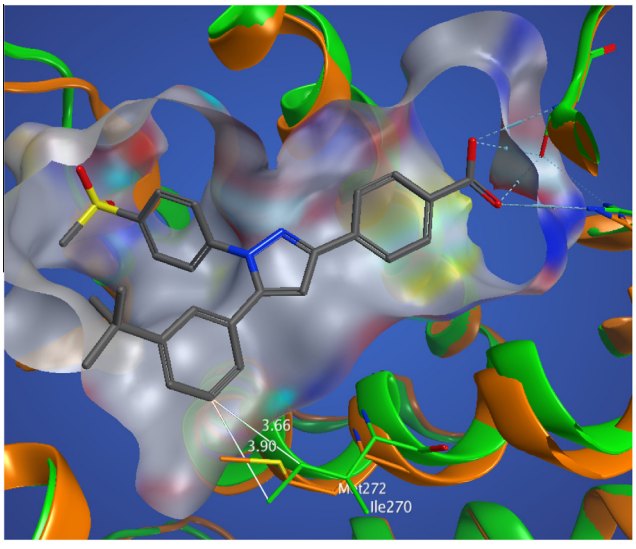


Figure 2. 1.85 Å structure of **2** (grey) bound to the RARα ligand binding pocket (green, PDB: 5K13), aligned and overlaid with the RARγ ligand binding pocket (orange) from the agonist structure (PDB: 1EXA) with helix 12 truncated.

Table 1
Residue differences in RAR subtype ligand binding domains

	Helix 3	Helix 5	Helix 11
RARα	S232	I270	V395
RARβ	A225	I263	V388
RARγ	A234	M272	A397

binding, but maintain RARγ affinity, we added functional groups to the C5 position of this aryl ring, which, as shown in Figure 2, was less than 4 Å away from the sidechain atoms of Ile270 in RARα.

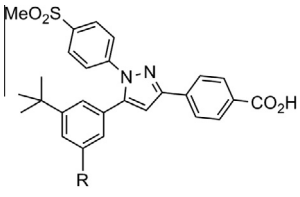
The impact of C5-substitution is shown in Table 2. Increasing the size of this substituent led to progressively poorer affinity towards RARα and RARβ, but maintained high affinity to RARγ. In addition to maintaining good RARγ binding affinity and >100× selectivity versus RARα and RARβ, **5** also demonstrated potent antagonist function in the cellular co-transfection assay (K_B = 35 nM, Max. inhibition >100%).

While we had optimized the selectivity via substitutions near helix 5, the resulting antagonists, such as **5**, demonstrated poor solubility (<100 ng/mL) in simulated intestinal fluid. To mitigate the potential for solubility limiting absorption, we explored amides and found that they were suitable sulfone replacements, as shown in Table 3. In fact, compared to sulfone **5**, substituted amides maintained RARγ affinity and showed even higher selectivities versus RARα and RARβ, in contrast to pan antagonist BMS-189453, which, as expected, was not selective in our hands. Furthermore, compounds in this series were similarly potent and selective in the RARγ co-transfection (CTF) functional assay in HEK293 cells (LY2955303: RARα K_B >4440 nM; RARβ K_B = 1510 nM; RARγ K_B = 7.11 nM). Additionally, being a zwitterion, LY2955303 showed dramatic solubility improvement (>1.0 mg/mL in simulated intestinal fluid).

Having identified a potent and selective RARγ antagonist, with suitable properties, we sought to test our hypotheses around (a) improved pharmacokinetic profile in rats, (b) efficacy in a rat model of OA-like joint pain and (c) improved safety profile versus RARα mediated testicular effects. The pharmacokinetic profile for **10** (Fig. 3) showed proportional increases in C_{max} and AUC at 10, 30 and 100 mg/kg after oral administration, with no accumulation upon chronic dosing up to 14 days.[16] Thus, we were ready to evaluate this compound in efficacy and safety studies.

To assess the pharmacodynamic effects of selective antagonism of RARγ, we evaluated LY2955303 in the mono-iodoacetate (MIA) model of OA-like joint pain in rats.[17] This model assesses differential weight bearing as a measure of joint pain after a single intra-articular injection of MIA into a hind limb joint. At nine days post

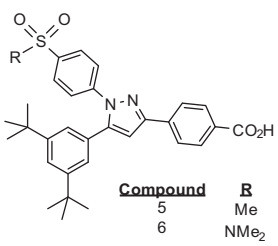
Table 2
Binding affinity and subtype specificity of RARγ antagonists

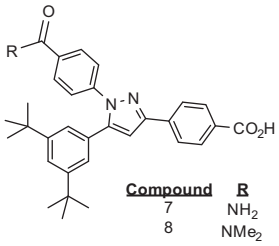


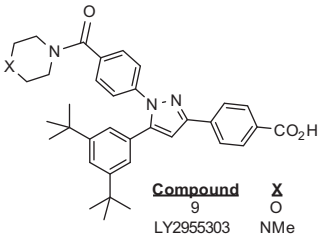
	R	Binding K_i + SEM[a] (nM)			Selectivity
		RARα	RARβ	RARγ	γ versus α
2	H	1.8 ± 0.3	4.3 ± 0.9	1.1 ± 0.5	1.6×
3	Me	6.2 ± 3.3	10.0 ± 2.6	1.1 ± 0.4	5.6×
4	*i*-Pr	87 ± 7.8	131 ± 9.9	1.6 ± 0.2	54×
5	*t*-Bu	461 ± 93	995 ± 196	4.4 ± 0.8	106×

[a] All reported data derived from at least 3 replicates.

Table 3
In vitro profile of compounds **5**–**9** and LY2955303







Compd	Binding K_i + SEM[a] (nM)			Selectivity		CTF functional[a]	
	RARα	RARβ	RARγ	γ versus α	γ versus β	RARγ K_B + SEM (nM)	Max. inhib. (%)
5	461 ± 93	995 ± 196	4.4 ± 0.8	106×	228×	13.5 ± 6.4	>100
6	>1700	>2980	4.1 ± 1.4	>414×	>725×	11.1 ± 5.9	>100
7	>1700	>2980	1.4 ± 0.3	>1259×	>2207×	14.7 ± 8.8	79
8	>1700	>2980	2.2 ± 0.5	>766×	>1342×	21.3 ± 14.6	>100
9	>1700	>2980	2.3 ± 0.1	>752×	>1319×	7.2 ± 5.5	>100
LY2955303	>1700	>2980	1.1 ± 0.3	>1560×	>2734×	7.1 ± 4.9	>100
BMS-189453	7.7 ± 1.1	16.7 ± 4.4	17.3 ± 2.8	Not selective	Not selective	18.5 ± 9.2	>100

[a] All reported data derived from at least 3 replicates.

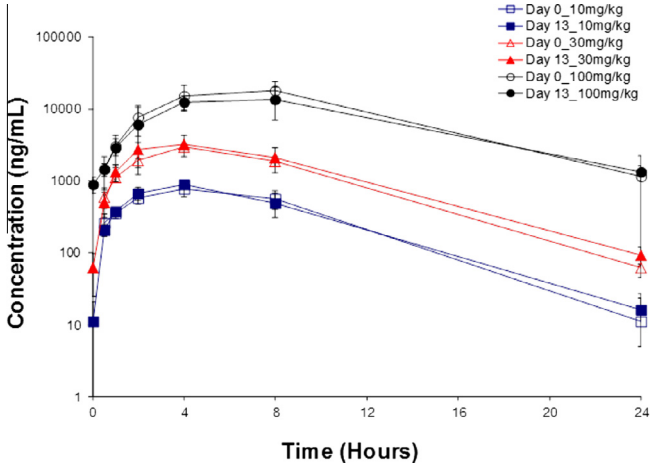


Figure 3. Pharmacokinetic profile of LY2955303.

MIA injection, the differential weight bearing between the contralateral and ipsilateral limbs was about 22 g (Fig. 4). On the same day, a single oral dose of LY2955303 demonstrated a dose responsive effect whereby the rat reduced differential weight bearing (ED_{50} = 0.72 mg/kg). The maximal analgesic response observed was similar to the maximal response observed for other analgesics.[16]

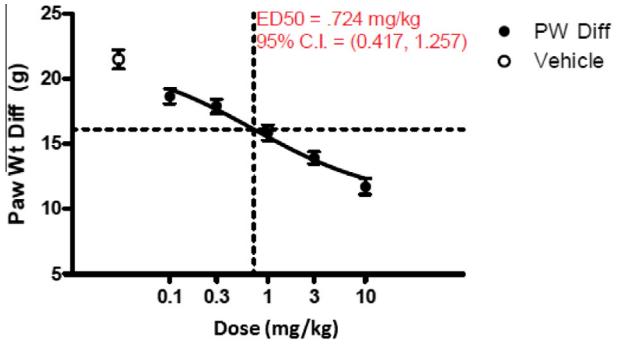


Figure 4. Dose responsive MIA analgesic data for LY2955303.

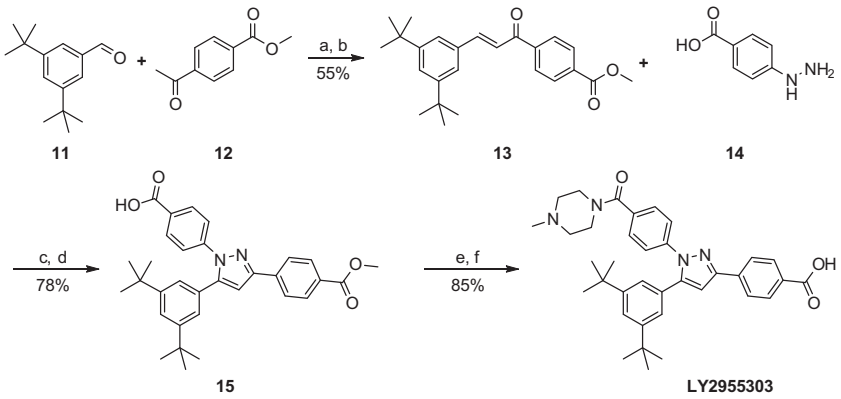
Previously, Beehler reported that RAR pan-antagonist BMS-189453 was efficacious in the mouse collagen induced arthritis (CIA) model and the streptococcyl cell wall induced arthritis (SCWA) model, both at a dose of 15 mg/kg.[7] It was also reported by Schulze that this same compound demonstrated testicular adverse effects in rats at doses as low as 2 mg/kg.[8] Based on the previous genetic studies that showed robust testicular degeneration in RARα,[9] but not RARγ knockout mice,[18] we were eager to learn if a selective RARγ antagonist could avoid the testicular effects noted with a pan antagonist at efficacious exposures. Thus, we tested LY2955303 in a 14 day toxicology study in rats and found adverse testicular effects only at doses significantly exceeding the MIA ED_{50} (Table 4). Based on drug exposure (AUC) in the rat at the efficacious dose of 0.72 mg/kg, the margin of exposure at the no effect dose for testicular effects (10 mg/kg) was 59 fold. Testicular degeneration was observed at the 30 mg/kg dose, which had a margin of exposure equal to 239 fold over the exposure at the MIA ED_{50}.

The methods used to prepare triaryl pyrazoles have been previously described.[11] As a representative example, Scheme 1 outlines the procedure used to prepare compound LY2955303. Methyl 4-acetylbenzoate (**12**) was treated with sodium hydroxide in methanol, followed by the addition of 3,5-di-*tert*-butylbenzaldehyde (**11**), resulting in formation of the intermediate chalcone acid, which was esterified using methanesulfonic acid in methanol to give chalcone ester **13**. **13** was treated with hydrazine **14** and acetic acid in *n*-butanol, condensing and cyclizing to the intermediate pyrazoline, which was oxidized to pyrazole **15** using manganese dioxide in dichloromethane. **15** was treated with *N*-methylpiperazine, HOBT and EDCI in dichloromethane to give the corresponding amide-ester, which was saponified to complete the synthesis of LY2955303.

Table 4
Rat toxicological data for LY2955303

Dose (mg/kg)	AUC (ng h/ml)	MOE[1]	Testes effect
0.72 (ED_{50})	154[2]	1	
10	9100[3]	59×	No adverse effect
30	36,800[3]	239×	Degeneration

[1] Margin of exposure.
[2] Determined from a rat exposure study at the ED_{50} of 0.72 mg/kg.
[3] Determined from toxicokinetic study.



Scheme 1. Synthesis of compound LY2955303. Reagents and conditions: (a) NaOH, CH_3OH; (b) CH_3SO_3H, CH_3OH; (c) HOAc, *n*-BuOH; (d) MnO_2, CH_2Cl_2; (e) *N*-methylpiperazine, HOBT, EDCI, CH_2Cl_2, (f) KOH, THF/water.

In conclusion, we have identified triaryl pyrazoles, a novel RAR antagonist scaffold. We also utilized a ligand–protein x-ray crystal structure to guide a structure-based drug design strategy to improve selectivity. This approach culminated in the identification of LY2955303, which displayed exquisite potency and robust selectivity for RARγ versus the other subtypes RARα and RARβ in vitro. This compound showed good pharmacokinetic properties and efficacy in rodents at exposures significantly lower than the exposures that produced testicular toxicity, consistent with our hypothesis that a selective RARγ antagonist could maintain efficacy with an improved safety profile relative to RAR pan antagonists.

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