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Structure-guided evolution of a 2-phenyl-4-carboxyquinoline chemotype into PPARα selective agonists: New leads for oculovascular conditions

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ABSTRACT

Small molecule agonism of PPARα represents a promising new avenue for the development of non-invasive treatments for oculovascular diseases like diabetic retinopathy and age-related macular degeneration. Herein we report initial structure–activity relationships for the newly identified quinoline-based PPARα agonist, Y-0452. Preliminary computational studies led to the hypothesis that carboxylic acid transposition and deconstruction of the Y-0452 quinoline system would enhance ligand–protein interactions and better complement the nature of the binding pocket. A focused subset of analogs was designed, synthesized, and assessed for PPARα agonism. Two key observations arose from this work 1) contrary to other PPARα agonists, incorporation of the fibrate "head-group" decreases PPARα selectivity and instead provides *pan*-PPAR agonists and 2) computational models reveal a relatively unexploited amphiphilic pocket in PPARα that provides new opportunities for the development of novel agonists. As an example, compound **10** exhibits more potent PPARα agonism (EC_{50} = ~6 μM) than Y-0452 (EC_{50} = ~50 μM) and manifests >20-fold selectivity for PPARα over the PPARγ and PPARδ isoforms. More detailed biochemical analysis of **10** confirms typical downstream responses of PPARα agonism including PPARα upregulation, induction of target genes, and inhibition of cell migration.

Despite a number of treatment options (e.g., laser photocoagulation, blood-glucose regulation, corticosteroids and anti-vascular endothelial growth factor (VEGF) injections), the ability to address the complex nature of diabetic retinopathy (DR) and related oculovascular diseases (e.g., wet age-related macular degeneration) remains a significant challenge.[1–3] Anti-VEGF has emerged as the primary treatment option, but suffers from the requirement of frequent intraocular injections, high cost, and the need for specialized facilities. Additionally, although effective for most, ~40–50% of patients are refractory to intravitreal injection of anti-VEGF and corticosteroids.[3,4] This implies that auxiliary pathways and factors that remain unaddressed with current interventions are involved in disease causation and progression. A critical need exists to develop new treatment options that are non-invasive and complementary to current approaches.

Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear hormone-activated receptors and transcription factors that consists of three members, PPARα, PPARγ and PPARδ.[5,6] Although PPAR isoforms share significant sequence homologies, they exhibit diverse functions, have different tissue distributions, and can be selectively targeted.[7–10] Within this family, PPARα has garnered the most attention as a therapeutic target. PPARα regulates the expression of genes involved in hyperlipidemia, diabetes, and inflammatory disorders and agonism of PPARα provides pharmacological benefits for these conditions.[10,11] Only recently, however, have the roles of PPARα in regulating inflammation, apoptosis, and neovascularization (NV) in diabetic retinae been revealed, establishing a new avenue for PPARα agonists as therapeutics for oculovascular diseases.[12,13]

Two independent large clinical studies (FIELD and ACCORD) demonstrate that fenofibrate (Fig. 1), a widely used drug in the clinic for the treatment of hyperlipidemia, has robust protective effects against diabetic macular edema (DME) and retinal NV in type 2 diabetic patients.[14,15] Fenofibrate represents the first orally available and safe drug with proven clinical efficacy on retinal NV and DME in humans with DR. This finding has excited clinicians, researchers and pharmaceutical companies who are interested in new drug treatments for oculovascular diseases. The protective

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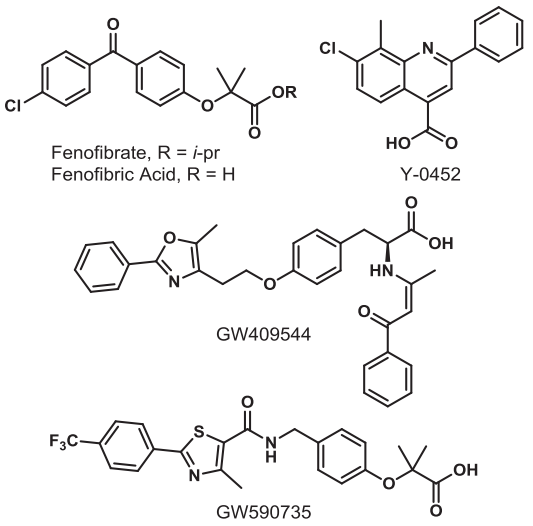

Fig. 1. PPARα agonists referenced in this manuscript.

effects of fenofibrate on retinal NV and DME are unrelated to its lipid-lowering activity, but rather result from its agonism of PPARα.[12,16] To date, fenofibrate is the only PPARα agonist known to cross the blood-ocular barrier and provide protective effects against DME and NV. Fenofibrate however, suffers from low ocular distribution, low affinity for PPARα, lack of selectivity between PPAR isoforms, and dose-limiting toxicities, all of which will limit its use as a DR therapy.[16–21] The clinical results paired with recent biochemical confirmation,[12,22–24] however, demonstrate that small molecule PPARα agonists with improved potency and enhanced ocular distribution have high promise to become non-invasive treatment options for oculovascular conditions.

Recently, a new PPARα agonist, 7-chloro-8-methyl-2-phenylquinoline-4-carboxylic acid (Y-0452, Fig. 1), was reported.[23] Y-0452 displays protective effects *in vivo* against DR and exhibits anti-inflammatory, anti-angiogenic and neuroprotective effects without signs of toxicity in the retinas of mice and diabetic rats.[23] Y-0452 is structurally distinct from fenofibrate, making it an attractive lead; however, Y-0452 exhibits only weak on-target activity in biochemical PPARα assays ($EC_{50} \approx 25–50$ μM), and manifests a low level of agonism compared to known PPARα agonists.[23] Additionally, the highly-functionalized quinoline core of Y-0452 represents significant synthetic challenges regarding comprehensive structure-activity relationship (SAR) studies. These aspects inspired us to investigate the SAR of Y-0452 through molecular simplification with a goal of enhancing synthetic tractability, target engagement, selectivity, and level of PPARα agonism. Towards this initiative, we utilized structure-based approaches to design a series of derivatives, which were then synthesized and evaluated for PPARα agonism. The results from these studies are reported herein.

To gain insight into the potential binding modes of Y-0452 to PPARα, we conducted docking studies with the Schrödinger Drug Discovery Suite. For these initial computational studies we selected PDB 1K7L, a co-crystal structure of GW409544 (Fig. 1) bound to human PPARα (hPPARα).[25] Although GW409544 exhibits 10-fold higher selectivity for hPPARγ ($EC_{50} = 0.28$ nM) over hPPARα ($EC_{50} = 2.3$ nM)[25] this structure was selected on the basis that detailed structural analyses of this chemotype and its interactions with different hPPAR isoforms are available for comparison and the data have been well-vetted in subsequent studies.[25]

To validate our docking approach, constraints, and parameters, GW409544 was extracted, exposed to MM2 energy-minimization, and re-docked into the hPPARα ligand binding domain to ensure that the results reproduced the bound conformation of the ligand. As shown in Fig. 2A, the overlay of co-crystallized (cyan) and docked (orange) GW409544 shows excellent congruence (RMSD = 0.34 Å). Maintaining the same constraints and parameters, Y-0452 was docked into hPPARα and the results were analyzed for strategies to improve or introduce key interactions. Previous studies have demonstrated the significance of hydrogen bond interactions between hPPARα Ser280, Tyr314, His440, and Tyr464 and the carboxylate motif of ligands.[18,25–31] Interactions with all four of these residues is believed to be responsible for triggering full agonism of hPPARα.[27] Poorer agonists tend to only interact with some of these hydrogen-bonding partners. As can be seen in Fig. 2B, while the quinoline core of Y-0452 provides a π-system for additional beneficial ligand-protein interactions via edge-to-face stacking with His440, the position of the carboxylate group on Y-0452 is predicted to only allow for two of the four possible hydrogen bonds (Tyr464 and His440).

We hypothesized that deconstruction of the quinoline core would 1) provide a more synthetically tractable scaffold amenable to facile assessment of carboxylate location and 2) relieve the rigidity encompassed within the aromatic 2-phenyl-carboxyquinoline chemotype. Although conformational constraint is a common technique used in medicinal chemistry to reduce entropic penalties through conformational bias, we hypothesized that, in this case, the rigidity of Y-0452 may be disadvantageous when the fragment is "grown" to fit the "U-shaped" binding pocket. We anticipated, however, that over simplification of an already modest hit may lead to inactive compounds, simply due to a reduction in surface area, thus limiting beneficial ligand-protein interactions. Indeed, simple *N*-benzylated variants of **i** (Fig. 2**C**) resulted in inactive derivatives (data not included). Docking of the simple *N*-benzylated analogs, however, revealed a 180° rotation of the molecules in the binding pocket, which positioned the substituted benzyl group in the same pocket as the oxazole of GW409544. Taking this into account and recognizing the value in the molecular orientation, we utilized structure-guided design to develop **9**–**14** and **21**–**24** that filled the hydrophobic binding pocket more efficiently. This focused set of analogs allowed us to test our hypothesis that quinoline deconstruction and transposition of the carboxylic acid would provide improved PPARα agonists.

Derivatives **9**–**14** were synthesized as shown in Scheme 1**.** Commercially available 4-hydroxybenzaldehyde was coupled with various benzyl bromides **3**–**8** to afford benzaldehydes **3a-8a**. Treatment of **3a-8a** with 3-aminobenzoic acid produced the respective imines *in situ*, which were then reduced upon the addition of sodium triacetoxyborohydride to provide **9**–**14** in an unoptimized 40–82% yield.

In addition to the benzoic acid derivatives **9**–**14**, we wanted to incorporate the classical fibrate "head-group" with an aim to improve potency and instill selectivity for PPARα over other isoforms.[31] The preparation of these analogs is depicted in Scheme 2. Commercially available 3-nitrophenol was coupled with ethyl α-bromoisobutyrate to afford **15**, which was then reduced to the corresponding aniline (**16**) under catalytic hydrogenation conditions (H_2 and Pd/C in ethanol). Treatment of **16** with **3a**, **4a**, **6a**, or **8a** followed by reduction with sodium triacetoxyborohydride yielded **17**–**20**, respectively. Hydrolysis of the pendant ester gave the desired products **21**–**24** in an unoptimized 46–88% yield.

With the focused subset of Y-0452 analogs in-hand, our efforts shifted to the evaluation of these derivatives for PPARα agonism. Preliminary evaluation utilized a commercially available PPARα luciferase cell reporter assay (Indigo Biosciences). The cell-line employed is engineered to constitutively express high-levels of hPPARα. Upon interaction with an agonist, hPPARα translocates to the nucleus, binds to the PPAR response element (PPRE), and upregulates gene transcription, including the inserted luciferase