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Glycine amides as PPARα agonists

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ABSTRACT

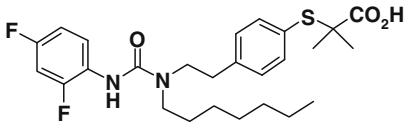
The design, synthesis and pharmacological properties of a novel class of PPARα agonists is described. Compound **2** is a novel, potent and specific glycine amide with oral bioavailability in rodents. The compound is active in vivo and alters plasma lipids in hAPO-A1 transgenic mice after oral administration.

Coronary heart disease (CHD) is the leading cause of death in industrialized countries and coronary events are among the top ranking reasons for working disability in the western world. Lipid disorders are well recognized as one of the leading risk factors for the development of atherosclerosis, cardiovascular morbidity and mortality. Peroxisome proliferator-activated receptors (PPAR's) are a family of nuclear hormone receptors that act as ligand-activated transcription factors and offer the potential to have beneficial effects on cardiovascular disease.

PPARα is one member of this family regulating arteriosclerosis relevant genes by binding to upstream promoter elements. PPARα is highly expressed in liver and its activation leads to plasma triglyceride decrease and high density lipoprotein (HDL) increase in man. Fibrates, which are on the market since many years, have recently been shown to act via PPARα agonism, however, only in the micromolar range and with low specificity. Thus, the idea of identifying potent and specific PPARα agonists has inspired many groups, resulting more recently in new clinical compounds.[1]

In an earlier Letter, we have described a glycine amide lead template that can be utilised to achieve different receptor specificities against the known PPAR subtypes, α, β (δ) and γ.[2] This lead is derived from GW 9578 (**1**), a known PPARα agonist which contains a urea moiety.[3] In this study we explore shifting **1**'s proximal urea NH by one position to furnish glycine amides **A** as novel PPARα agonists. This structural modification leads to zwitterions and offers synthetically convenient options for structural modifications of R^1 and R^2 (Fig. 1).

Due to their zwitterionic nature we were expecting these compounds to display favorable physicochemical properties like higher polarity, lower binding affinity to human serum albumin, better solubility and possibly an improved therapeutic window. We also wanted to evaluate whether such modifications alter the structure–activity relationship (SAR) of the side chains.



1

A

Figure 1. Design of glycine amides (A). Shifting **1**'s urea nitrogen by one position leads to more hydrophilic zwitterions.

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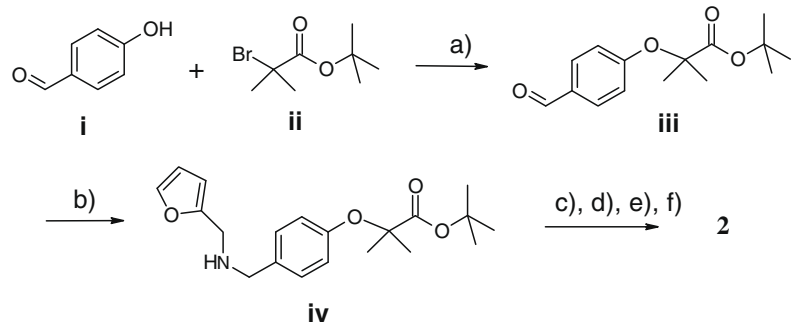


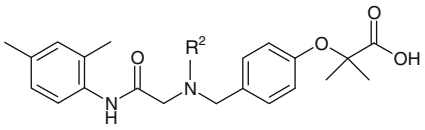
Figure 2. Synthesis of **2**. Reagents and conditions: (a) Cs_2CO_3, DMF, 90 °C, 12 h, 30%; (b) 2-furfurylamine, 1,2-dichloroethane, $NaB(OAc)_3H$, 5 h, rt, 72%; (c) THF/Et_3N (20:1), 0.05 equiv $(tBu)_4N^+I^-$, 1.5 equiv $BrCH_2COOEt$, 3 h 60 °C, 100%; (d) EtOH, 3 equiv NaOH, 1 h 80 °C, 74%; (e) 2,4-dimethyl aniline (1.5 equiv), hydroxy-1*H*-benzotriazole (1.3 equiv), EDC (1.3 equiv), 4-methyl morpholine (3 equiv), DMAP (cat.), 12 h, rt, 78%; (f) CH_2Cl_2/TFA (1:1), 2 h, rt, 82%.

The compounds were synthesized according to Figure 2 via ether synthesis of **iii** using standard conditions. Reductive amination with furfuryl amine yielded the central building block **iv**. Similar reductive aminations were carried out with a variety of primary amines.

The resulting secondary amines were then coupled with α-bromo ethyl acetate under phase transfer conditions. Selective saponification of the ethyl ester yielded the corresponding acetic acid, which was then coupled with a set of primary amines. For example, the use of 2,4-dimethyl aniline furnished **2** after deprotection of the *tert*-Bu ester.[4,5]

The SAR around the R^2 position displayed clear steric and electronic requirements of the corresponding binding pocket (Table 1). The furanyl methyl moiety in **2** was well tolerated, yielding a potent and full PPARα agonist with up to 1000-fold enhanced potency as compared to marketed fibrates like Gemfibrozil (100 μM), Clofibrate (55 μM), Bezafibrate (30 μM) or Fenofibrate (25 μM). Replacing the furfuryl moiety with an electron rich benzyl system (**3**, **5**) or a thiophene methyl system (**4**) resulted in a significant drop in activity indicating rather tight SAR in this region. We also introduced aliphatic side chains in order to probe whether substituents corresponding to urea **1** would improve receptor affinity. Interestingly, the introduction of such side chains resulted in a significant loss of activity, indicating different SAR for the glycine amide series

Table 1
Structure–activity relationship of glycine amide PPARα agonists. Variation of the tertiary amine residue R^2. K_i values are medians of three dose–response curves.



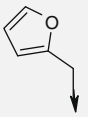
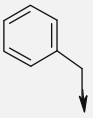
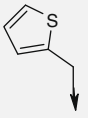
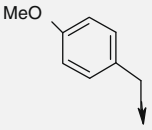
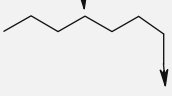
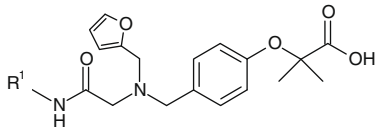
Compound	R^2	h-PPARα EC_{50} (nM)
2		20
3		1000
4		700
5		900
6		1000
7		10,000
8		>25,000

Table 2
Structure–activity relationship of glycine amide PPARα agonists. Variation of the amide part R^1. K_i values are medians of three dose–response curves.



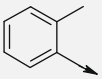
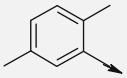
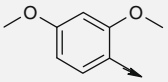
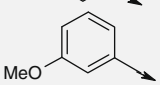
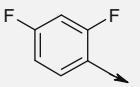
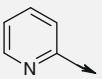
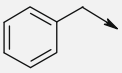
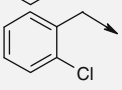
Compound	R^2	h-PPARα EC_{50} (nM)
9		2000
10		600
11		300
12		3000
13		1500
14		>25,000
15		13,000
16		7000

Table 3
Direct comparison of selected in vitro parameters of **1** and **2**

	2	1
h-PPARα (nM)	20	100
h-PPPARγ (nM)	800	1000
h-PPARδ (nM)	6000	2000
m-PPARα (nM)	20	10
m-PPARγ (nM)	300	2500
m-PPARδ (nM)	2000	2000
Membrane affinity	480	13,500
HSA (μM)	10	1,3
Solubility (mg/l)	500	100
F_{max} (rat microsomes)	57%	73%

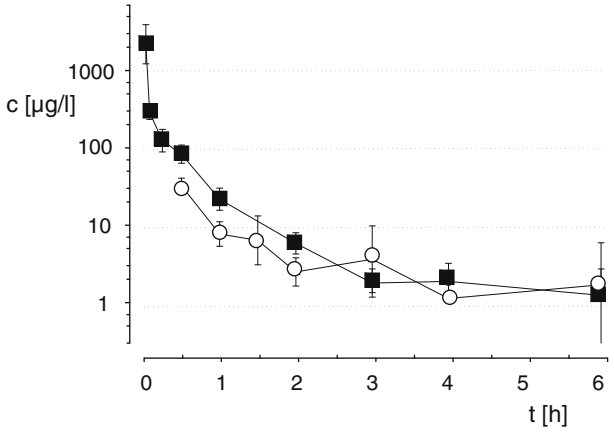


Figure 3. Pharmacokinetic profile of **2**. Dose-normalised (1 mg/kg) plasma concentrations in male NMRI mice after oral (10 mg/kg, $n = 3$, ○) and iv administration (3 mg/kg, $n = 3$, ■) in 20% PEG400.

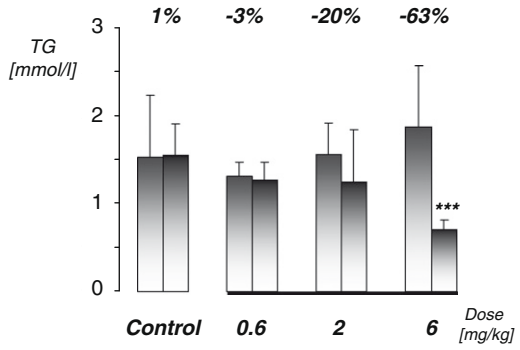


Figure 4. Investigation of **2** in the human APO-A1 transgenic mouse. The left hand column of each pair indicates triglyceride (TG) levels at day 1 (control), the right hand column indicates triglyceride levels after 7 days of oral treatment.

and may be suggesting an overall different orientation of the molecule in the binding site (**6**, **7** and **8**).

Keeping the furan substituent constant, we investigated a series of amide substitutions in the R^1 position (Table 2). Removal of **2**'s *para* methyl group resulted in a 100-fold loss of activity (**9**). Similarly, shifting it into the *meta*-position (**10**) resulted a 30-fold loss, highlighting the steric requirements of the binding pocket. Interestingly, the 2,4-difluoro substitution pattern that confers potency and seems essential in urea **1** doesn't seem to have a beneficial effect in the glycine amide series (**13**). Changing the electronic nature of **13**'s substituents renders a fivefold improvement (**11**). Neither the heterocyclic pyridine **14** nor the benzylamides **15**, **16** seemed to be effective.

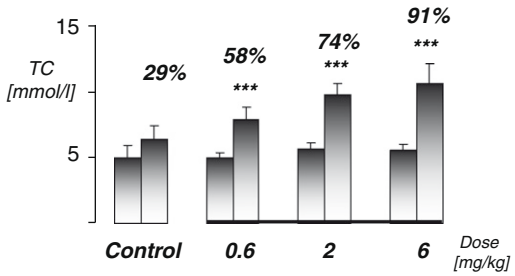


Figure 5. Investigation of **2** in the human APO-A1 transgenic mouse. The left hand column indicates total cholesterol (TC) levels at day 1 (control), the right hand column indicates total cholesterol levels after 7 days of oral treatment.

As the most potent compound in this series, compound **2** has been selected for further characterization and direct comparison to **1** in vitro (Table 3). In mice and humans, **2** displays improved selectivity to PPARγ and δ subtypes over **1**.[5] As expected, the zwitterion **2** is more polar than urea **1**, as demonstrated by its lower affinity to membranes,[6] human serum albumin (HSA)[7] and its enhanced solubility.

Given the in vitro rat microsomal clearance data,[8] we decided to investigate **2**'s pharmacokinetics in male NMRI mice and were pleased to see bioavailability acceptable for oral dosing ($F = 20\%$, 10 mg/kg, Fig. 3).[9]

We therefore investigated **2**'s ability to reduce triglyceride levels and elevate serum total cholesterol levels in human APO-A1 transgenic mice. These mice express the human APO-A1 gene under the control of the natural APO-A1 promotor and have been widely used in the evaluation of PPARα agonists. In contrast to wild-type mice, these transgenic species respond to PPARα agonist treatment with increased HDL levels. As most of the serum cholesterol exists in the form of HDL, we used serum total cholesterol as a surrogate for HDL.[10]

Gratifyingly, **2** altered lipid parameters in this species in a dose-dependent manner after 7 d once daily oral treatment (Figs. 4 and 5). It was also evident that **2** reduced triglyceride levels and enhanced serum total cholesterol levels. This is in line with observations made with other PPARα agonists in this species and confirms **2**'s proposed mechanism of action.[11]

In summary, we have identified novel glycine amides as potent and selective PPARα agonists, starting from urea **1**. Modifying the urea motif to a glycine amide markedly changed structure–activity trends. An optimized derivative **2**[12] is orally bioavailable and alters triglyceride and cholesterol levels in rodents after oral administration.

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5. The primary assay was performed as described.[4] The different PPAR subtypes are fusion proteins containing the ligand binding domain for PPARα (aa 167–468), PPARγ (aa 203–506) and PPARδ (aa 139–442), respectively, fused to the GAL4 DNA-binding domain (aa 1–147).
6. Membrane affinities were determined as described. Briefly, the reduction of compound concentration after incubation with liposomal egg yolk lecithin followed by ultracentrifugation was measured by HPLC: Loidl-Stahlhofen, A.;

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8. The prediction of hepatic clearance was performed according to Houston, J. B.; Carlile, D. J.; *Drug Metab. Rev.* **1997**, *29*, 891. Briefly, compounds were incubated (1 μM, 37 °C) with 0.2 mg/ml rat microsomal protein. Samples were taken at seven time points within 30 min to calculate half-lives and extrapolate clearance.
9. Pharmacokinetics of **2**: A 10 mg/kg dose of **2** (suspension in 20% PEG 400, 5 ml/kg) was given to male NMRI mice and plasma samples were analyzed at 7 different time points up to 6 h. c_{max}: 0.32 mg/ml, AUC: 0.37 mg h/l, t_{max}: 0.5 h. In a second experiment 3 mg/kg were administered intravenously in the same vehicle as a solution and plasma levels determined at nine different time points, up to 6 h: Clearance: 5.35 l/h kg, $t_{1/2}$: 2 h.
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11. Our compound was investigated in vivo according to Ref. 4. Briefly, plasma samples were taken before and after oral u.i.d. 7 d treatment with **2** (vehicle: Solutol HS15 + ethanol + brine (0.9%) = 1 + 1 + 8, 10 ml/kg) of male transgenic hAPO-A1 C57BL/6 mice (n = 10/dose, Jackson Lab., Bar Harbor, ME). Triglycerides and cholesterol levels were determined photometrically. In an independent experiment, we confirmed that LDL plasma levels in these mice were very low (<5%, HPLC) as expected, suggesting that the measurement of total cholesterol can be used as a surrogate for HDL in this species.
12. Characteristic data of **2**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.57 (s, 6H), 2.24 (s, 3H), 2.27 (s, 3H), 3.31 (s, 2H), 3.67 (s, 2H), 3.75 (s, 2H), 6.22–6.36 (m, 2H), 6.88 (m, 2H), 6.93–7.03 (m, 2H), 7.23 (d, 2H), 7.34–7.40 (m, 1H), 7.78 (m, 1H), 8.00 (s, br, 1H), 9,09 (s, 1H). MS (ESI): 451 $[M+H]^+$, 901 $[2\,M+H]^+$.