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Design of potent PPARα agonists

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Abstract—Computational analysis of the ligand binding pocket of the three PPAR receptor subtypes was utilized in the design of potent PPARα agonists. Optimum PPARα potency and selectivity were obtained with substituents having van der Waals volume around 260. Compound **6** had a PPARα potency of 0.002 μM and a selectivity ratio to PPARγ and PPARδ of 410 and 2000, respectively.

The peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor super family. The marketed insulin sensitizers (rosiglitazone and pioglitazone) and the lipid lowering fibrates (e.g., fenofibrate and clofibrate) are PPARγ and PPARα agonists, respectively. There is no drug available targeting the third PPARδ receptor, but several reports suggest PPARδ to be involved in lipid metabolism as well as in glucose homeostasis.[1,2]

The currently available fibrates were identified as lipid lowering agents two decades ago using animal models. The mechanism of the fibrates was not known at time. The present knowledge shows us that the marketed fibrates are PPARα agonists with rather low potency, which might limit the clinical efficacy.

The present investigation was aimed at identifying potent and selective PPARα agonists to be used as research tools and potential lipid lowering drugs.

The aim was further to investigate if it was possible to utilize the difference in a single amino acid in the lipophilic pocket of the ligand binding domain (LBD) of the three PPAR receptors to design selective PPARα agonists.

Alignment of the amino acids of the LBDs of the PPAR receptors combined with docking studies into the X-ray structures of the three receptors identified five amino acids in the lipophilic pocket: 264, 266, 281, 284, and 348, Figure 1. Of these five amino acids, position 264 had the largest difference in size, with PPARα being the smallest. The idea was to keep the core-structure of our compounds constant and only change the substituents predicted to interact with position 264.

Our structural starting point was the PPARpan-agonist NNC 61-3058, Figure 2, recently described in Sauerberg et al.[3] In vitro transactivation assays using the LBDs of hPPARα, γ, and δ were used to evaluate the designed and synthesized ligands.[4] NNC 61-3058 had a PPARα potency of 0.4 μM (EC_{50}) compared to the EC_{50} of fenofibric acid and clofibric acid of 32 and 3 μM, respectively, Table 1.

The designed compounds were synthesized as outlined in Scheme 1, starting from commercialy available substituted benzaldehydes, or substituted benzaldehydes synthesized according to Scheme 2. Using Horner–Emmons reaction conditions the aldehydes were converted to the corresponding allylic esters, which were reduced to the alcohols using DIBAL. Mitsunobu coupling to the (*S*)-2-ethoxy-3-(4-hydroxy-phenyl)-propionic acid ethyl ester[5] followed by hydrolysis gave the desired compounds **1**–**14**.[12]

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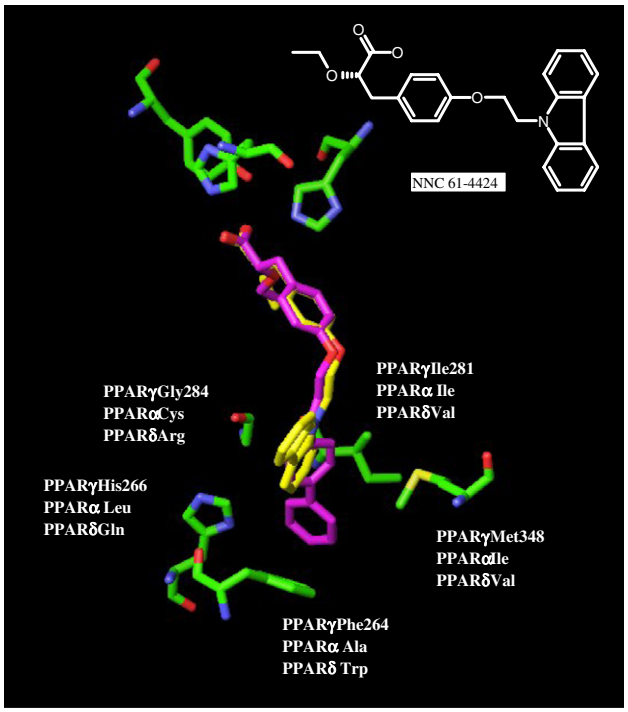


Figure 1. The crystal structure of the ligand binding domain of the PPARγ receptor crystallized with NNC 61-4424 (yellow) and with NNC 61-3058 (magenta) docked into the active site. The amino acids within 5 Å from the biphenyl ring in NNC 61-3058 are listed together with the corresponding amino acids in the PPARα and PPARδ receptors. His323, His449, and Tyr473 are also displayed.

NNC 61-3058 was docked into the binding domain of PPARγ crystallized with NNC 61-4424 (pdb code 1KNU).[4] In order to design PPARα selective agonists, amino acids with difference in size or charge were identified. Nearly all of the amino acids identified were neutral, Figure 1, so focus was therefore placed on the size of the amino acids. The amino acid in position 264 showed a difference in size with PPARα being the smallest (Ala), then PPARγ (Phe) and PPARδ the largest (Trp). Docking studies with NNC 61-3058[6] suggested that selective PPARα ligands could be obtained by removing the *p*-phenyl in NNC 61-3058 and introducing substituents (R) in meta-positions of compound **1** leading to a series of analogues, Figure 2 and Table 1.[7] Compound **1** was 2–4 times less potent on the PPAR receptors than the lead compound NNC 61-3058, but the PPARα selectivity was retained (see potency ratios Table 1).

At the PPARδ receptor only smaller substituents (**3**–**8**) increased the potency compared to the un-substituted analogue (**1**), Table 1. The most potent compound (**3**) with CF_3 as substituents was approximately 10 times more potent than **1**. Larger side chains led, as expected, to PPARδ inactive compounds (**10**–**14**), probably due to repulsive steric interactions with the tryptophan.

The meta-substituents also improved the PPARγ potency significantly. A potency optimum was observed with isopropoxy (**7**), which was 100-fold more potent than the un-substituted starting point (**1**). Again, larger substituents gave less potent PPARγ agonists, although even phenylethyl (**13**) was more potent than **1**.

PPARα with the smallest amino acid (Ala) in position 264 tolerated substituents the best. The most potent compound was obtained with OCH_2CF_3 substituents (**6**), which was 800 times more potent than **1**. Smaller (**5**) and larger (**7**) substituents were approximately 2–10 times less potent than **6**, suggesting favorable interactions. Compound **6** also displayed the highest PPARα selectivity with a potency ratio to PPARγ and PPARδ of 410 and 2000, respectively.

To further understand the SAR, various physical–chemical properties (MW, van der Waals area, van der Waals volume, and log *P*) of the substituents were mapped against the PPARα potency. The van der Waals volumes turned out to explain the relationship the best, Figure 3. This reflects the difference in size of the amino acid in the part of the binding pocket in which the substituted phenyl ring is predicted to bind. The physical–chemical properties for the di-meta substituted phenyl ring were calculated with MOE.[8] The PPARα potency was improved when the volume of the substituted benzene was increased up to 266 (**6**) which corresponded to two OCH_2CF_3 substituents. As the volume of the substituents increased, the potency decreased.

In conclusion, computational analysis of the ligand binding pocket of the three PPAR receptor subtypes was utilized in the design of potent PPARα agonists. Analogues with substituents of different van der Waals volume gave PPARα agonists with varying potency and selectivity. Optimum PPARα potency and selectivity were obtained with substituents having van der Waals volume around 260.

Compound **6** had a PPARα potency of 0.002 μM and a selectivity ratio to PPARγ and PPARδ of 410 and 2000,

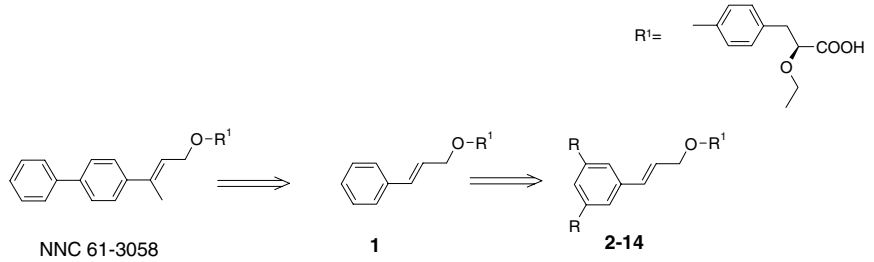
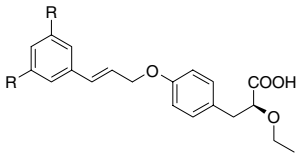


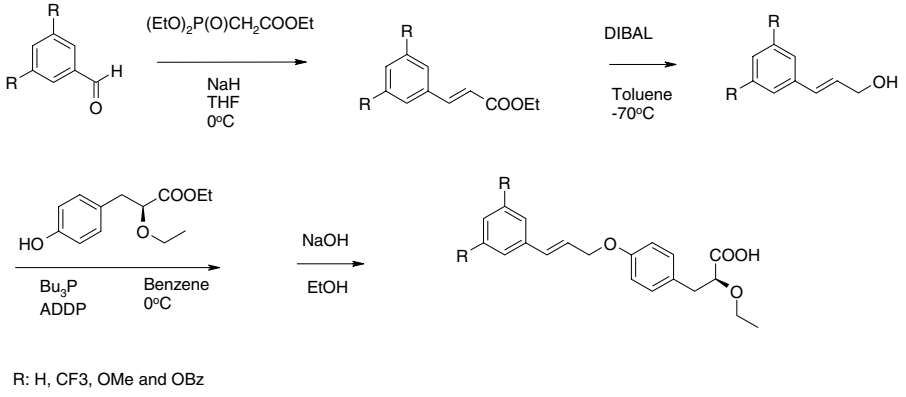
Figure 2. Graphic illustration of the design of selective PPARα agonists.

Table 1. In vitro transactivation data for compounds **1**–**14** and standard compounds

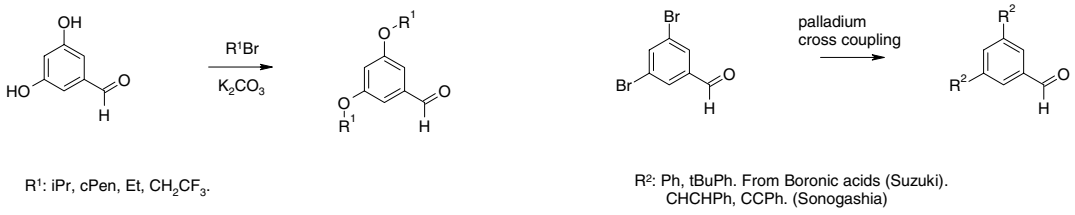


Compound	Substituent R	hPPARα		hPPARγ		hPPARδ		Potency ratio	
		EC_{50}, μM[a]	% max[b]	EC_{50}, μM	% max[c]	EC_{50}, μM	% max[d]	$\frac{\text{hPPARγ}}{\text{hPPARα}}$	$\frac{\text{hPPARδ}}{\text{hPPARα}}$
NNC 61-3058		0.5	158	2.0	118	9.6	235	4	19
1	H	1.6	169	8.0	96	18	177	5	11
2	Br	0.3	121	4.1	58	20	139	14	67
3	CF_3	0.02	195	0.4	70	1.4	74	21	70
4	OMe	0.06	186	1.2	100	13	171	2	217
5	OEt	0.02	197	0.2	79	7.9	124	9	395
6	OCH_2CF_3	0.002	149	0.8	81	4.0	171	410	2000
7	OiPr	0.004	207	0.08	90	6.0	116	20	1500
8	Ph	0.02	30	0.2	80	4.0	117	12[e]	200
9	OcPen	0.02	135	0.3	107	15	71	14	750
10	OBz	0.05	121	0.19	104	3	30	4	60
11	C≡CPh	0.5	108	3.1	84	—	18	6	—
12	CH=CHPh	0.09	66	3.0	91	—	19	33	—
13	CH_2CH_2Ph	0.03	118	5.1	90	—	14	182	—
14	Ph-4-tBu	14	110	1.0	44	—	0	0.07[e]	—
WY14.643		13	100	29	22	—	6	2[e]	—
Rosiglitazone		4.1	43	0.2	100	—	7	0.05	—

[a]Compounds were tested in five concentrations ranging from 0.01 to 30 μM in at least two independent experiments. Data represent mean, with SEM ±15%. EC_{50}'s were not calculated for compounds producing transactivation lower than 25% at 30 μM. [b]Fold activation relative to maximum activation obtained with WY14643 (approx. 20-fold corresponded to 100%), with [c]rosiglitazone (approx. 120-fold corresponded to 100%), and with [d]carbacyclin (approx. 250-fold corresponded to 100%). [e]These potency ratios may not be comparable to the rest as partial efficacy was obtained on either PPARα or PPARγ.



Scheme 1. Synthesis of target compounds from commercially available benzaldehydes.



Scheme 2. Synthesis of substituted benzaldehydes.

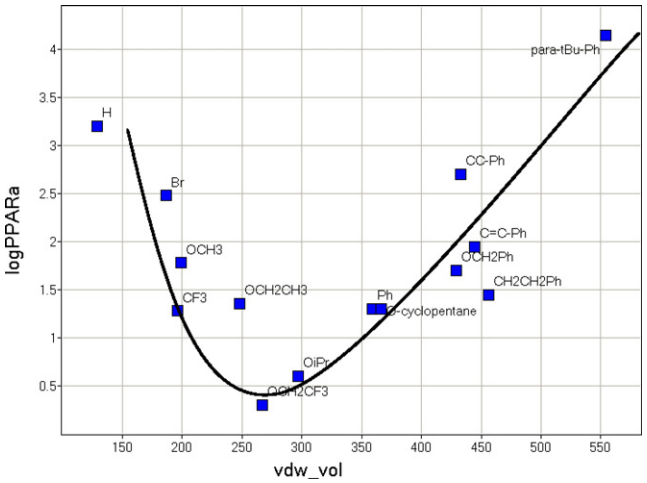


Figure 3. The relationship between in vitro PPARα potency and the van der Waals volume of the di-meta substituted phenyl ring.

respectively. The selective PPARα agonist **6** could be a useful tool to dissect the pharmacology of PPARα mediated effects.[9]

Acknowledgments

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References and notes

1. Oliver, W., Jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *Proc. Natl. Acad. Sci.* **2001**, *98*, 5306.
2. Tanaka, T.; Yamamoto, J.; Iwasaki, S.; Asaba, H.; Hamura, H.; Ikeda, Y.; Watanabe, M.; Magoori, K.; Ioka, R. X.; Tachibana, K.; Watanabe, Y.; Uchiyama, Y.; Sumi, K.; Iguchi, H.; Ito, S.; Dio, T.; Hamakubo, T.; Naito, M.; Auwerx, J.; Yanagisawa, M.; Kodama, T.; Sakai, J. *Proc. Natl. Acad. Sci.* **2003**, *100*, 15924.
3. Sauerberg, P.; Bury, P. S.; Mogensen, J. P.; Deussen, H.-J.; Pettersson, I.; Fleckner, J.; Nehlin, J.; Frederiksen, K. S.; Albrektsen, T.; Din, N.; Svensson, L. S.; Ynddal, L.; Wulff, E. M.; Jeppesen, L. *J. Med. Chem.* **2003**, *46*, 4883.
4. Sauerberg, P.; Pettersson, I.; Jeppesen, L.; Bury, P. S.; Mogensen, J. P.; Wassermann, K.; Brand, C. L.; Sturis, J.; Wöldike, H. F.; Fleckner, J.; Andersen, A.-S. T.; Mortensen, S. B.; Svensson, L. A.; Rasmussen, H. B.; Lehmann, S. V.; Polivka, Z.; Sindelar, K.; Panajotova, V.; Ynddal, L.; Wulff, E. M. *J. Med. Chem.* **2002**, *45*, 789.
5. Ebdrup, S.; Pettersson, I.; Rasmussen, H. B.; Deussen, H.-J.; Jensen, A. F.; Mortensen, S. B.; Fleckner, J.; Pridal, L.; Nygaard, L.; Sauerberg, P. *J. Med. Chem.* **2003**, *46*, 1306.
6. Glide 4.0 docking. The structure manipulations were performed in Maestro version 7.5 (Maestro 7.0, Schrödinger, LLC, New York, NY, 1999–2005). Glide calculations were performed with Impact version 4.0.[10,11] The crystal structure of the PPARγ receptor crystallized with NNC 61-4424 (1KNU) was used for docking.[4] NNC 61-3058 was built in Maestro version 7.5, a formal charge of −1 was assigned and the molecule was minimized with the OPLS_2005 force field. NNC 61-3058 was docked with Glide version 4.0 using the SP mode and the van der Waals radii of the ligand atoms were scaled by 0.8.
7. Simple para-substituted analogues, for example, allyloxy and benzyloxy, were PPARpan-agonists (EC_{50} (% max) α: 0.6 μM (180%), γ: 1.6 μM (117%), δ: 14 μM (186%) and α: 8.4 μM (231%), γ: 1.3 μM (116%), δ: 17 μM (377%), respectively).
8. MOE ver. 2005.06. Chemical Computing Group Inc.; www.chemcomp.com.
9. Two analogues, **5** and **7**, were tested for pharmacokinetic properties in rats. Both compounds exhibited excellent behavior after oral dosing (2.5 mg/kg): F_{po} = 86% and 95%, $T_{1/2po}$ = 130 min. and 145 min., C_{max} = 6155 ng/ml and 1845 ng/ml, respectively.
10. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
11. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750.
12. Experimental procedure for synthesis of (*E*)-(*S*)-3-(4-{3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-allyloxy}-phenyl)-2-ethoxy-propionic acid (**6**): (a) to a solution of 3,5-dihydroxybenzaldehyde (2.0 g, 14.5 mmol) in DMF (35 mL) were added potassium carbonate (11.0 g, 80.0 mmol) and 1,1,1-trifluoro-2-iodoethane (33.3 g, 160 mmol). The reaction mixture was heated in a sealed reactor at 50 °C for 7 days. The mixture was filtered and the filtrate was washed with ethyl acetate. The filtrate was washed with water and the organic phase isolated. The aqueous phase was extracted once more with ethyl acetate. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with toluene to give 906 mg (18%) of 3,5-bis-(2,2,2-trifluoro-ethoxy)-benzaldehyde. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.43 (q, 4H), 6.85 (t, 1H), 7.15 (d, 2H), 9.95 (s, 1H). (b) Sodium hydride (60% in oil, 750 mg, 18 mmol) was added at 0 °C to a solution of triethyl phosphonoacetate (2.67 g, 12 mmol) in dry THF (60 mL). After stirring at 0 °C for 15 min, a solution of 3,5-bis-(2,2,2-trifluoro-ethoxy)-benzaldehyde (1.85 g, 6 mmol) in dry THF (10 mL) was added, and the mixture was stirred for additional 45 min. Water (100 mL) was added and the aqueous phase was extracted with ethyl acetate (100 mL). The combined organic phases were washed with water (3× 100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give 2.2 g of crude (*E*)-3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-acrylic acid ethyl ester. (c) A 1 M solution of DIBAL-H in toluene (30 mL, 30 mmol) was added dropwise at −70 °C to a stirred solution of crude (*E*)-3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-acrylic acid ethyl ester (2.2 g, 6 mmol) in dry THF (100 mL) and the mixture was stirred for 30 min. Methanol (20 mL) was added, and the reaction mixture was poured into 0.1 N HCl (600 mL). The mixture was extracted with ethyl acetate (2× 200 mL), dried (Na_2SO_4) and evaporated to give a crude oil. The crude product was crystallized from ethyl acetate and heptanes to give (*E*)-3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-prop-2-en-1-ol as colorless crystals: 510 mg. (d) Under an atmosphere of nitrogen, 1,1′-(azodicarbonyl) dipiperidine (380 mg, 1.5 mmol) and tributylphosphine (303 mg, 1.6 mmol) were added to a solution of (*E*)-3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-prop-2-en-1-ol (250 mg, 0.75 mmol) and (*S*)-2-ethoxy-3-(4-hydroxy-phenyl)-propionic acid ethyl ester (180 mg, 0.75 mmol) in dry THF (20 mL) at 0 °C. The

reaction mixture was stirred for 30 min and then poured into water. The mixture was extracted with ethyl acetate (50 mL), dried, and evaporated in vacuo. The product was purified by column chromatography, eluting with heptane/ethyl acetate (3:1), to give 300 mg (73%) of (*E*)-(*S*)-3-(4-{3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-allyloxy}-phenyl)-2-ethoxy-propionic acid ethyl ester. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.15 (t, 3H), 1.22 (t, 3H), 2.95 (d, 2H), 3.30–3.40 (m,1H), 3.55–3.67 (m, 1H), 3.97 (t, 1H), 4.15 (q, 2H), 4.33 (q, 4H), 4.65 (d, 2H), 6.32–6.48 (m, 2H), 6.55–6.70 (m, 3H), 6.85 (d, 2H), 7.15 (d, 2H). (e) (*E*)-(*S*)-3-(4-{3-[3,5-bis-(2,2,2-trifluoro-ethoxy)-phenyl]-allyloxy}-phenyl)-2-ethoxy-propionic acid ethyl ester (300 mg, 0.54 mmol) was dissolved in ethanol (10 mL) and 1 N sodium hydroxide (1.5 mL, 4.4 mmol) was added. The mixture was heated slightly to obtain a clear solution and then stirred at room temperature for 1.5 h. The ethanol was evaporated in vacuo and the mixture was acidified to pH 1 with 1 N hydrochloric acid. The product was isolated by extraction with ethyl acetate (2× 40 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated to give 130 mg (91%) of the title compound as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.20 (t, 3H), 2.97 (dd, 1H), 3.10 (dd, 1H), 3.41–3.53 (m, 1H), 3.55–3.68 (m, 1H), 4.05 (dd, 1H), 4.35 (q, 4H), 4.67 (d, 2H), 6.35–6.48 (m, 2H), 6.60–6.70 (m, 3H), 6.87 (d, 2H), 7.15 (d, 2H).