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Bioorganic & Medicinal Chemistry Letters 13 (2003) 257–260

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Novel PPARα/γ/δ Triple Activators Using a Known PPARα/γ Dual Activator as Structural Template

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Received 18 June 2002; revised 19 August 2002; accepted 7 October 2002

Abstract—Using a known dual PPARα/γ activator (**5**) as a structural template, SAR evaluations led to the identification of triple PPARα/γ/δ activators (**18**–**20**) with equal potency and efficacy on all three receptors. These compounds could become useful tools for studying the combined biological effects of PPARα/γ/δ activation.
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The use of Peroxisome Proliferator-Activated Receptor γ (PPARγ) activators, for example rosiglitazone (**1**, Fig. 1) in the treatment of type 2 diabetes or non-insulin dependent diabetes mellitus is well established due to their ability to lower blood glucose and insulin levels and improve insulin sensitivity.[1] Furthermore, the activation of PPARα receptors has been suggested to be the mechanism behind the hypolipidemic effect of the fibrate drugs, for example fenofibrate[2] (**2**). These findings have increased the interest in developing a PPARα/γ dual activators, for example ragaglitazar (NNC 61-0029/DRF2725)[3] (**3**) for the treatment of dyslipidemic type 2 diabetics. In light of the recently published data[4] on the selective PPARδ agonist GW501516 (**4**), which showed increased HDL-cholesterol and decreased triglycerides, along with VLDL and LDL-cholesterol levels effects in obese monkeys, PPARδ activators could also be used in the treatment of dyslipidemia. We report here the design of triple PPARα/γ/δ activators using a dual PPARα/γ activator as structural template.

Results and Discussion

Whereas selectivity for PPARα, PPARγ and PPARδ receptors has normally been obtained by making alterations to both the lipophilic and the acidic part of the molecule (see Fig. 1), we demonstrate here that triple PPARα/γ/δ activators can be designed by modifying only the lipophilic part.

Using the recently published dual PPARα/γ activator **5**[5] as a structural template (Fig. 2), the *N*,*N*-diphenylamine derivative **6** without the bond between the two phenyl rings in the carbazole ring of **5** was synthesised. This resulted in a loss in both potency and efficacy in the in vitro transactivation of both PPARα and PPARγ but it gave no activation of the PPARδ receptor (Table 1). However, by substituting the amino group in **6** with a methine group a more planar lipophilic structure was obtained **7**, which had equal potency on all three PPAR receptor subtypes.

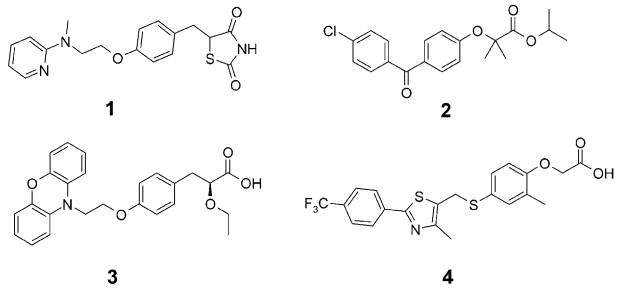

Figure 1. Reference compounds.

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PII: S0960-894X(02)00881-8

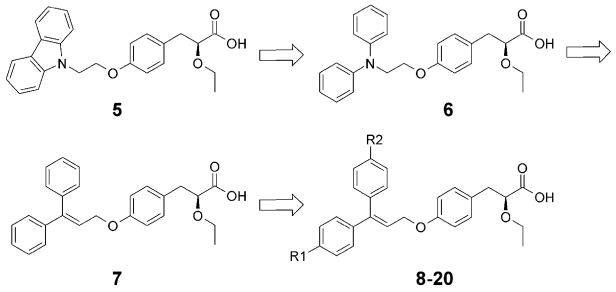


Figure 2. Evolution of the project.

In an attempt to improve the potency of **7** and to understand the SAR of this class of compounds, mono-substituted analogues of **7** were synthesized and tested. The potency was actually improved compared to the non-substituted compound **7**, especially with the more lipophilic substituted analogues **10**–**15**.

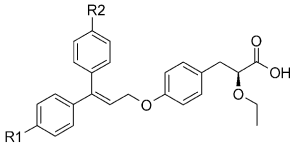
Activation data showed however, only minor potency and efficacy differences between the (*E*) and the (*Z*) isomers (**8** vs **9**; **10** vs **11**; **12** vs **13** and **14** vs **15**). This prompted us to design and synthesize the bis-substituted derivatives **16**–**20**. The bis-substituted analogues were, however, equipotent to the mono-substituted analogues (**10** and **11** vs **19**; **12** and **13** vs **18**; **14** and **15** vs **20**).

The SAR on the PPARδ activity for this class of compounds is in agreement with earlier published data suggesting the presence of two lipophilic pockets in the binding domain of the receptor.[6] However, the fact that both the PPARα and the PPARγ receptors can accommodate such large conformationally constrained and branched lipophilic groups, as in **18**–**20**, has not yet been reported. Although the flexible triple activator GW2433 also supports this finding.[1]

FlexX[7–10] was used to dock compound **18** into PPARα co-crystallized with AZ242,[11] PPARγ co-crystallized with **5**,[5] and PPARδ co-crystallized with GW2433,[6] respectively. Figure 3 shows that compound **18** docks into the active sites with the carboxylic acid in the same position as the co-crystallized ligands. Furthermore, there is good agreement between the position of the hydrophobic portion of compound **18** and the lipophilic regions of the co-crystallized ligands.

The *bis*-biphenyl compound **19** was tested in our primary diabetes model, the male db/db mouse,[5] primarily reflecting the PPARγ agonist activity. Mice were treated with 1 mg/kg orally once daily for 7 days, after which a non-fasted blood sample was drawn. Compared to vehicle treated animals the blood glucose (BG) and the plasma insulin levels were lowered by 47 and 71%, respectively. For comparison, **3** dosed at 3 mg/kg po

Table 1. In vitro hPPARα, hPPARγ, and hPPARδ activation of reference and test compounds



No.	R1 (E)	R2 (Z)	PPARα[a] EC_{50}, μM (%max)[b]	PPARγ[a] EC_{50}, μM (%max)[c]	PPARδ[d] EC_{50}, μM (%max)[d]
7	H	H	6.4 (107)	2.7 (75)	5.7 (195)
8	F	H	5.3 (121)	8.5 (72)	8.3 (159)
9	H	F	3.2 (201)	4.0 (94)	3.4 (195)
10	Ph	H	0.9 (206)	0.6 (104)	2.2 (274)
11	H	Ph	0.7 (182)	1.7 (129)	2.2 (250)
12	Br	H	1.1 (86)	1.5 (89)	0.9 (312)
13	H	Br	2.2 (161)	4.3 (90)	3.4 (233)
14	2-Furanyl	H	0.3 (160)	0.8 (112)	0.6 (200)
15	H	2-Furanyl	0.4 (198)	2.0 (85)	2.0 (229)
16	Me	Me	0.7 (192)	3.1 (87)	2.0 (214)
17	MeO	MeO	2.2 (133)	2.2 (101)	0.7 (233)
18	Br	Br	0.3 (132)	0.9 (85)	0.4 (239)
19	Ph	Ph	1.1 (77)	0.3 (101)	0.5 (265)
20	2-Furanyl	2-Furanyl	2.0 (103)	2.9 (161)	1.0 (212)
1	Rosiglitazone		4.1 (43)	0.16 (100)	(7)
2[e]	Fenofibric acid		32.1 (265)	(8)	(1)
3	NNC61-0029/ DRF2725		3.21 (97)	0.57 (117)	(7)
5			0.36 (140)	0.17 (108)	(3)
6			4.5 (39)	3.3 (91)	(1)

[a]Values are means of 2–4 experiments in quadruple; SEMs were within 20%. Fold activation relative to maximum activation obtained with:
[b]WY14643 (approximately 20-fold corresponded to 100%), with:
[c]rosiglitazone (approximately 120-fold corresponded to 100%), and with:
[d]carbacyclin (approximately 250-fold corresponded to 100%).
[e]Fenofibric acid was used.

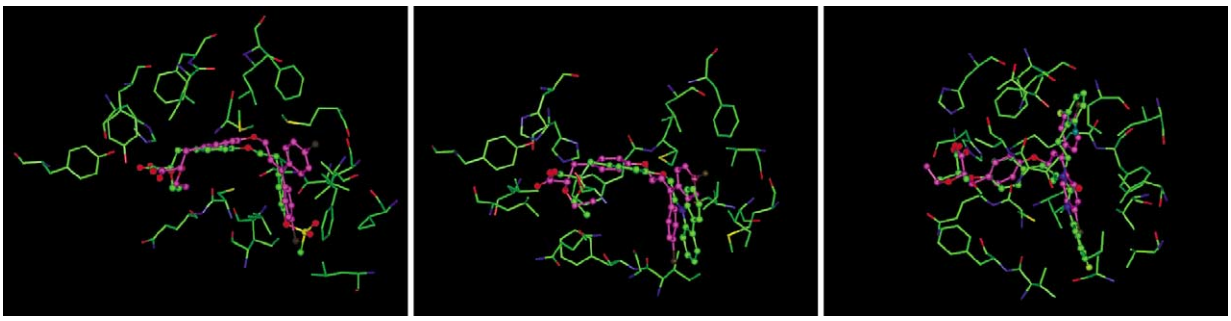


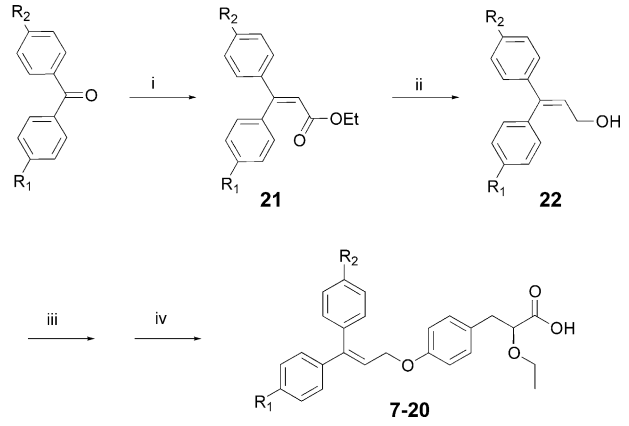
Figure 3. Compound **18** (purple) docked into the PPARα receptor co-crystallized with AZ 242 (left panel), into the PPAR receptor co-crystallized with **5** (center panel) and into the PPARδ receptor co-crystallized with GW2433 (right panel), respectively.

produced a 51% BG and a 79% insulin lowering in the same experiment. After a further 2 days dosing, an oral glucose tolerance test (OGTT) was performed. The reduction of the blood glucose area under the curve (AUC) after the OGTT was considered to be a measure of improved insulin sensitivity. Compound **19** lowered the AUC by 52% compared to 55% after treatment with **3**. The pharmacokinetic profile of **19** in rat after oral administration (2.54 mg/kg) showed a preferred long half life of 5.4 h with a Cmax of 132 ng/mL. Further studies are, however, needed to fully evaluate the combined effect of activating all three PPAR receptors.

In conclusion, our results show that it is possible to design triple PPAR activators by keeping the acidic part of a dual PPAR activator unchanged and modifying only the lipophilic part of the dual activator. The data generated on compounds **18**–**20** suggests that these compounds could be tools for studies of the combined biological effects of PPARα/γ/δ activation.

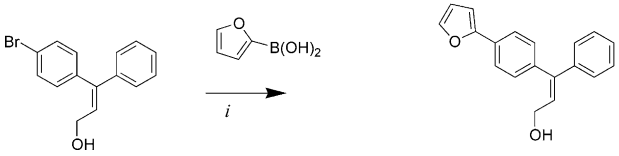
Chemistry

Compounds **6**–**13** and **16**–**19** were synthesized[12] as outlined in Scheme 1. Carrying out a Horner Emmons reaction on commercial available diphenyl ketones led to the ethyl 3,3-diphenyl-propenonates. The (*E*) and (*Z*) mixtures of **21** were then seperated by column chromatography and the conformations assigned using NMR. DIBAL-H reduction of the ester group of **21** allowed the preparation of the to the corresponding alcohol (**22**) which were subjected to a Mitsunobu reaction with (*S*) ethyl 3-(4-hydroxy-phenyl)-2-ethoxy-propionate to give the ethyl esters of the desired products **6**–**13** and **16**–**18**. Hydrolysis of the esters produced the target compounds **6**–**13** and **16**–**18**.



Scheme 1. (i) NaOEt, triethylphosphonoacetat, EtOH rt; (ii) DiBAL-H, THF, −70 °C, (iii) DEAD or ADDP, PPh_3 or P(*t*-Bu)$_3$ THF, 0 °C; (iv) 1 M NaOH, EtOH, rt −50 °C.

Compounds **14** and **15** were made using the (*E*) and (*Z*) 3-(4-bromo-phenyl)-3-phenyl-prop-2-en-1-ols as precursers for the introduction of the furanyl substituent via a Suzuki coupling with 2-furanyl boronic acid, (Scheme 2). The disubstituted analogues **19** and **20** were made the same way using ethyl 3,3-*bis*-(4-bromophenyl)-prop-2-en-1-ol as the precursor.



Scheme 2. (*i*) $Pd(PPh_3)_4$, DME, Na_2CO_3 aq 80 °C.

Modelling

FlexX version 1.10.0 was used.[7–10] Assignment of formal charges was made. FlexX was used with the Sybyl 6.8 interface.[13]

In vitro transactivation

The assay as done for PPARα and PPARγ has been described previously.[5] Briefly, the ligand binding domains of the two human PPAR receptor subtypes were fused to the DNA binding domain of the yeast transcription factor Gal4. HEK293 cells were transiently transfected with an expression vector for the respective PPAR chimera along with a reporter construct containing five copies of the Gal4 DNA binding site driving expression of a luciferase reporter gene. All compounds were dissolved in DMSO and diluted 1:1000 upon addition to the cells. Compounds were tested in five concentrations ranging from 0.01 to 30 μM. Cells were treated with compound for 24 h followed by luciferase assay. The PPARδ assay was done exactly as described above for PPARα and PPARγ using the human PPARδ ligand binding domain (LBD) spanning amino acids 128—end fused to the Gal4 DNA binding

domain in the vector pM1, yielding the plasmid pM1δLBD.

Acknowledgements

The technical assistance from Rikke Burgdorf, Hanne Nielsen, Lene Priskorn, Helle Bach, Anette Heerwagen, Anette Zeneca, Kirsten M. Klausen, Kent Pedersen, Brian Rosenberg, Otto Larsson, Sanne von Eyben, Bjørn Metzler, Per Klifforth, and Alice Ravn is highly appreciated.

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12. Target compounds were synthesized according to the following general procedure, using the synthesis of compound **19** as an example: Step 1: To a solution of NaH (3.53 g, 88.2 mmol) in dry toluene (300 mL) was added dropwise at 0 °C a solution of trietylphosphonoacetate (13.2 g, 58.8 mmol) in toluene (100 mL). The reaction mixture was stirred for 30 min after which a solution of 4,4-dibromobenzophenone (10.0 g, 29.4 mmol) in THF (100 mL) was added. The reaction mixture was stirred for 48 h. EtOH (10 mL) and water (300 mL) were added and the mixture was extracted with EtOAc–MeOH (2%, 2×150 mL). The combined organic phases were washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (eluent: ether) to give ethyl 3,3-*bis*-(4-bromophenyl)-acrylate as a gum. Crystallization from hexanes gave white crystals in 8.77 g (73%) yield. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.20 (3H, t), 4.05 (2H, q), 6.35 (1H, s), 7.0–7.1 (4H, m), 7.40–7.52 (4H, m). Step 2: Ethyl 3,3-bis-(4-bromophenyl)-acrylate (8.75 g, 21.3 mmol) was dissolved in dry THF (35 mL). DIBAL-H (1.5 M in toluene, 43 mL, 64.0 mmol) was added at −15 °C and the reaction mixture was stirred for 30 min. A solution of NH_4Cl in water was added and the mixture was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried with $MgSO_4$, filtered and evaporated to give 3,3-bis-(4-bromophenyl)-pro-2-en-1-ol in 6.0 g (76%) yield. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.15 (1H, br s), 4.16–4.20 (2H, dd), 6.25 (1H, t), 7.0–7.1 (4H, m), 7.40-7.52 (4H, m). Step 3: To a solution of 3,3-*bis*-(4-bromophenyl)-pro-2-en-1-ol (1.00 g, 2.72 mmol) and $Pd(PPh_3)_4$ (0.11 g, 0.1 mmol) in DME (40 mL) was added phenylboronic acid (1.33 g, 10.88 mmol) and the reaction was stirred for 10 min at rt under N_2. Na_2CO_3 (2 M aq, 16.3 mL, 32.6 mmol) was added and the reaction was stirred at 80 °C overnight followed by 3 days at rt. Water was added to the reaction mixture and the product extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified on column chromatography [eluent/EtOAc/hexanes (3:1)] to give 3,3-*bis*-(biphenyl-4-yl)-pro-2-en-1-ol in 630 mg (64%) yield. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.4 (1H, t), 4.32 (2H, dd), 6.45 (1H, t), 7.2–7.7 (18H, m). Step 4: 3,3-*bis*-(biphenyl-4-yl)-2-pro-2-en-1-ol (363 mg, 1.0 mmol) and (*S*)-ethyl-2-ethoxy-3-(4-hydroxyphenyl)-propionate (262 mg, 1.1 mmol) and Bu_3P (303 mg, 1.5 mmol) were dissolved in dry benzene (20 mL) and cooled to 0 °C. 1,1′-(Azodicarbonyl)dipiperidine (ADDP) (378 mg, 1.5 mmol) was added and the reaction mixture was stirred at room temp. for 48 h. Water (50 mL) was added and the mixture was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography [eluent/EtOAc/hexanes (3:1)] to give (*S*)-ethyl-3-(4-(3,3-*bis*-(biphenyl-4-yl)-allyloxy)-phenyl)-2-ethoxy-propionate in 445 mg (76%) yield. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.15 (3H, t), 1.25 (3H, t), 2.92 (1H, d), 3.28-3.41 (1H, m), 3.52-3.66 (1H, m), 4.0 (1H, dd), 4.22 (2H, q), 4.65 (2H, d), 6.40 (1H, t), 6.79 (2H, d), 7.12 (2H, d), 7.30-7.70 (18H, m). Step 5: A suspension of (*S*)-ethyl-3-(4-(3,3-*bis*-(biphenyl-4-yl)-allyloxy)-phenyl)-2-ethoxy-propionate (370 mg, 0.64 mmol) in EtOH (4 mL) and 1 M NaOH (1.27 mL, 1.27 mmol) was heated to give a clear solution that was stirred for 5 days at rt. The reaction mixture was washed with EtOAc and 1 N HCl was added to pH < 2. The water phase was extracted with EtOAc (3×50 mL) and the combined organic phases were washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (eluent: 5% MeOH in CH_2Cl_2) to give (*S*)-3-(4-(3,3-*bis*-(biphenyl-4-yl)-allyloxy)-phenyl)-2-ethoxy-propionic acid in 275 mg (78%) yield. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.13 (3H, t), 2.93 (1H, dd), 3.05 (1H, dd), 3.35-3.50 (1H, m), 3.50-3.64 (1H, m), 4.02 (1H, dd), 4.62 (2H, d), 6.39 (1H, t), 6.80 (2H, dm), 7.02 (2H, dm), 7.27–7.72 (18H, m).
13. SYBYL® 6.8; Tripos Inc.: 1699 South Hanley Rd., St. Louis, MO, 63144, USA.