Bioorganic & Medicinal Chemistry Letters 18 (2008) 4525–4528

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Improvement of the transactivation activity of phenylpropanoic acid-type peroxisome proliferator-activated receptor pan agonists: Effect of introduction of fluorine at the linker part

Jun-ichi Kasuga [a,*], Takuji Oyama [b], Yuko Hirakawa [b], Makoto Makishima [c], Kosuke Morikawa [b], Yuichi Hashimoto [a], Hiroyuki Miyachi [a,*]

[a] Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
[b] The Takara-Bio Endowed Division, Institute for Protein Research, Osaka University, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan
[c] Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan

ARTICLE INFO

Article history:
Received 15 May 2008
Revised 1 July 2008
Accepted 11 July 2008
Available online 15 July 2008

Keywords:
PPAR
Fluorine
PPAR pan agonist

ABSTRACT

We developed a potent peroxisome proliferator-activated receptor pan agonist (a candidate drug for treatment of altered metabolic homeostasis) by introducing fluorine atoms at appropriate position(s) of the known phenylpropionic acid-type pan agonist TIPP-703.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor (NR) superfamily, and three subtypes (PPARα, PPARδ, and PPARγ) have been identified to date. The PPARs are of great interest because they are considered to be the master regulators of lipid and glucose homeostasis.[1–5] PPARs are activated by fatty acids and metabolites, and regulate the expression of various genes downstream of the PPAR response element. Each PPAR subtype displays a distinct pattern of tissue distribution and a distinct pharmacological profile.

PPARα is expressed in tissues involved in lipid oxidation, such as liver, heart, muscle, and kidney. It regulates genes associated with fatty acid uptake and metabolism. Clinically, PPARα agonists, such as fenofibrate, are used for the treatment of dyslipidemia. PPARδ is expressed ubiquitously. Recently, the use of knock-out animals and PPARδ-selective agonists has revealed that PPARδ is associated with improved insulin sensitivity and elevated HDL levels. PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles, and it regulates genes related to adipogenesis and lipid storage. Clinically, PPARγ agonists, such as pioglitazone, are used for the treatment of type 2 diabetes mellitus.[1–4]

In recent years, many PPAR dual and pan agonists have been developed, as well as PPAR subtype-selective agonists.[5,6] Bezafibrate is a well-known PPAR pan agonist, which has been in clinical use for a long time. Bezafibrate increases HDL cholesterol and reduces triglycerides, improves insulin sensitivity, and reduces blood glucose level. Bezafibrate significantly lowers the incidence of cardiovascular events.[6]

We have been engaged in structural development studies of novel PPAR ligands based on the SAR results obtained from phenylpropanoic acid derivatives related to the PPARα-selective agonist KCL.[7] We have succeeded in the development of PPAR agonists with characteristic subtype selectivity, that is, PPARα/δ dual agonist TIPP-401,[8] PPARδ-selective agonist TIPP-204,[9] and PPAR pan agonist TIPP-703[10] (Fig. 1).

During the development of TIPP-401, we found that the introduction of fluorine at the ortho position of the amide carbonyl group greatly enhanced PPARδ activity. This was not unexpected, because the introduction of fluorine can improve metabolic stability by blocking metabolically labile sites, modulate physicochemical properties, such as lipophilicity or basicity, change molecular conformation, and so on.[11,12] Therefore, to evaluate the effect of fluorine substitution more comprehensively, we designed and synthesized a series of fluorinated phenylpropanoic acid PPAR agonists. In this letter we present the SAR of these fluorinated derivatives. We also report the creation of a potent PPAR pan agonist based on the SAR obtained.

The synthetic route to racemic compounds is depicted in Scheme 1. Aldehydes **1a–e** were treated with Horner–Emmons re-

* Corresponding authors.
E-mail addresses: jk817@msn.com (J. Kasuga), miyachi@iam.u-tokyo.ac.jp (H. Miyachi).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.07.046

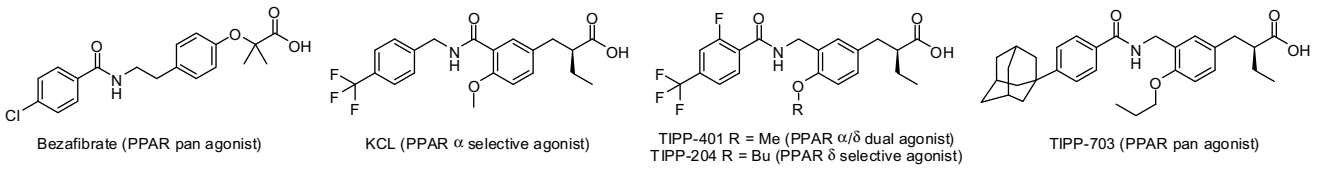
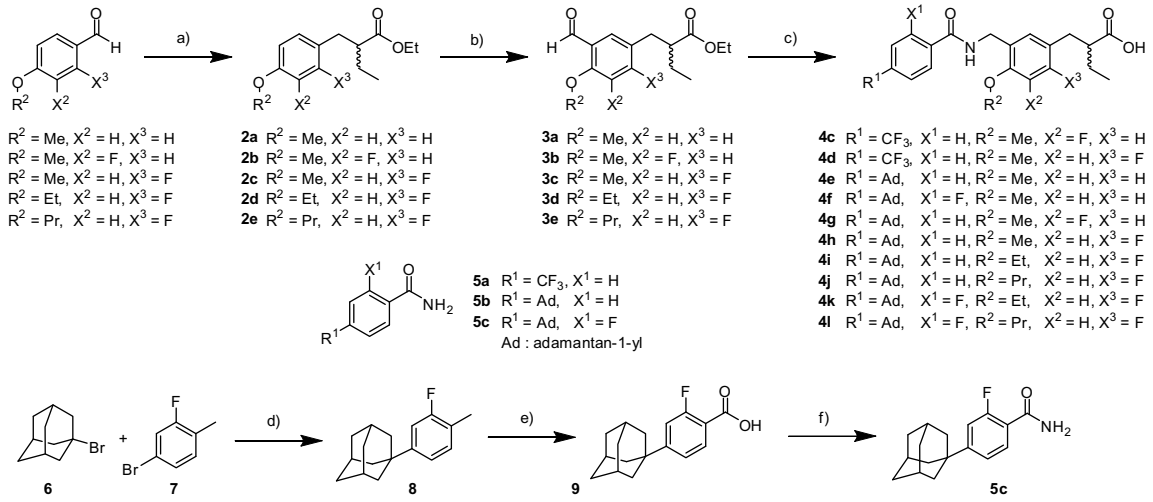


Fig. 1. Structures of PPAR agonists.

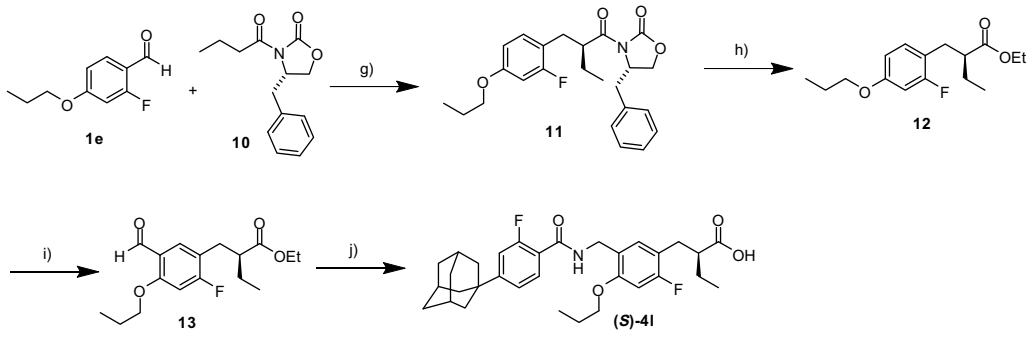


Scheme 1. Synthetic route to racemic compounds. Reagents and conditions: (a) 1—triethyl-2-phosphonobutyrate, *t*-BuOK, THF, 84–96%; 2—10% Pd–C, H_2, AcOEt, 95–100%; (b) $TiCl_4$, $CHCl_2OCH_3$, DCM, 77–89%; (c) 1—**5a–c**, Et_3SiH, TFA, toluene, 42–92%; 2—HCl, dioxane, 54–84%; (d) Mg, Et_2O, 49%; (e) $Co(OAc)_2$, $Mn(OAc)_2$, NaBr, O_2, aq AcOH, dioxane, 64%; (f) 1—$SOCl_2$; 2—acetone, aq NH_3, 90%.

agents, followed by hydrogenation to afford esters **2a–e**, which were formylated at the ortho position of the alkoxyl group to afford aldehydes **3a–e**. In step b, from **2c**, we had anticipated obtaining the 2-fluoro benzaldehyde derivative, as well as the 4-fluoro benzaldehyde derivative **3c**, but only **3c** was formed. Compounds **3a–e** were amidealkylated with **5a–c**, and then hydrolyzed to afford **4c–l**. Synthesis of **4a** and **4b** was previously reported.[8] Compound **5c**, which was used in step c, was prepared in three steps. Compounds **6** and **7** were condensed[13] to afford **8**, which was oxidized[14] and amidated to afford **5c**.

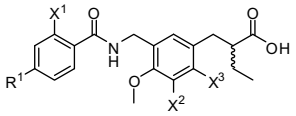
The synthetic route to the enantiomer of **41** is depicted in Scheme 2. The synthetic scheme used for the preparation of TIPP-703[10] was not applicable for the synthesis of optically active **4l**. According to the previous route, we had to prepare 4-fluoro-5-formyl-2-hydroxybenzoic acid as a starting material, but unfortunately we could not prepare it effectively. Therefore we adopted scheme 2. Evans' asymmetric aldol condensation[15] of **1e** with **10** followed by reductive dehydroxylation afforded **11**, which was treated with $Ti(OEt)_4$ to afford **12**. Then **12** was formylated, reductively amidated, and hydrolyzed to afford **(*S*)-4l**. The antipodal (*R*) enantiomer was prepared similarly from (*R*)-4-benzyl-3-butyryl-oxazolidin-2-one instead of **10** as the starting material.

First, we examined the effect of the introduction of fluorine at different positions, that is, ortho to the amide carbonyl group of the distal benzene ring (X^1), and ortho (X^2) and meta (X^3) to the methoxy group of the linker benzene ring (Table 1). In our previous study, the introduction of fluorine at the X^1 position of **4a** enhanced PPARδ activity, while PPARα activity was maintained (**4b**–**4a**). This SAR is also applicable to adamantyl-substituted compounds (**4f**–**4e**); PPARδ transactivation activity of **4f** (EC_{50}; δ 550 nM) is more potent than that of **4e** (EC_{50}; δ 940 nM). Introduction of fluorine at the linker benzene ring (X^2 and X^3) had the



Scheme 2. Synthetic route to chiral compound **(*S*)-4l**. Reagents and conditions: (g) 1—Bu_2BOTf, TEA, DCM, 98%; 2—Et_3SiH, TFA, DCM, 82%; (h) $Ti(OEt)_4$, EtOH, 53%; (i) $TiCl_4$, $CHCl_2OCH_3$, DCM, 84%; (j) 1—**5c**, Et_3SiH, TFA, toluene, 63%; 2—HCl, dioxane, 68%.

Table 1
PPARs transactivation activity of the present series of compounds



Compound	Structure				Activity (EC_{50} ± SE/nM)[a]		
	R1	X1	X2	X3	PPARα	PPARδ	PPARγ
4a	CF_3	H	H	H	10 ± 1	130 ± 20	2600 ± 100
4b	CF_3	F	H	H	7.9 ± 1.5	29 ± 5	2600 ± 200
4c	CF_3	H	F	H	54 ± 15	59 ± 6	>10,000
4d	CF_3	H	H	F	3.6 ± 0.8	20 ± 2	1100 ± 100
4e	Ad[b]	H	H	H	53 ± 5	940 ± 30	110 ± 10
4f	Ad[b]	F	H	H	60 ± 14	550 ± 70	140 ± 20
4g	Ad[b]	H	F	H	260 ± 30	2200 ± 300	190 ± 30
4h	Ad[b]	H	H	F	13 ± 4	280 ± 30	36 ± 6

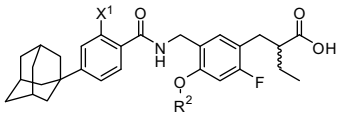
[a] Compounds were screened for agonist activity on PPAR–GAL4 chimeric receptors in transiently transfected HEK-293 cells. EC_{50} value is the concentration of the test compound that affords 50% of the maximal reporter activity.
[b] 'Ad' means adamantyl.

opposite effect. Introduction of fluorine at the X^2 position is not tolerated for PPARs activity (**4c** and **4g**). On the other hand, the introduction of fluorine at the X^3 position enhanced PPARs activity (**4d** and **4h**). **4d** (EC_{50}; α 3.6 nM, δ 20 nM, γ 1100 nM) is more potent than **4a** (EC_{50}; α 10 nM, δ 130 nM, γ 2600 nM) for PPARs of all subtypes. Likewise, adamantyl-substituted derivatives exhibited the same SAR (**4h**–**4e**).

Based on the same tendency of the effect of introduction of fluorine, we hypothesized that the SAR could be extended to other compounds. To test this hypothesis, we planned to design a more potent PPAR pan agonist than TIPP-703. Based on the SAR, we fixed X^3 as F, X^1 as H or F, and R^2 as ethyl or propyl for the compounds tested. The results are summarized in Table 2. All the compounds (**4i–l**) exhibited more potent activities than TIPP-703. In particular, compound **4l** exhibited the most potent and well-balanced PPAR activity. When the activity of optically active **4l** was tested, we found that **(*S*)-4l** is more potent than the antipodal *R*-enantiomer, as expected from previous studies.[8–10]

Although the reason(s) why fluorine at the X^1 and X^3 positions enhanced PPARδ activity is uncertain, recently the X-ray crystallographic structure of TIPP-401 complexed with human PPARδ is solved[16] (Figs. 2 and 3).

Table 2
PPARs transactivation activity of the present series of compounds



Compound	Structure			Activity (EC^{50}±SE/nM)[a]		
	X1	R2	Stereo	PPARα	PPARδ	PPARγ
4h	H	Me	*rac.*	13 ± 4	280 ± 30	36 ± 6
4i	H	Et	*rac.*	7.1 ± 1.1	94 ± 9	24 ± 2
4j	H	Pr	*rac.*	17 ± 2	60 ± 7	15 ± 2
4k	F	Et	*rac.*	7.5 ± 1.5	55 ± 3	22 ± 3
4l	F	Pr	*rac.*	13 ± 1	28 ± 2	35 ± 3
(*S*)-4l	F	Pr	S	12 ± 1	25 ± 2	38 ± 3
(*R*)-4l	F	Pr	R	200 ± 30	180 ± 10	160 ± 10
TIPP-703	—	—	—	58 ± 8	120 ± 10	43 ± 6

[a] Compounds were screened for agonist activity on PPAR–GAL4 chimeric receptors in transiently transfected HEK-293 cells. EC_{50} value is the concentration of the test compound that affords 50% of the maximal reporter activity.

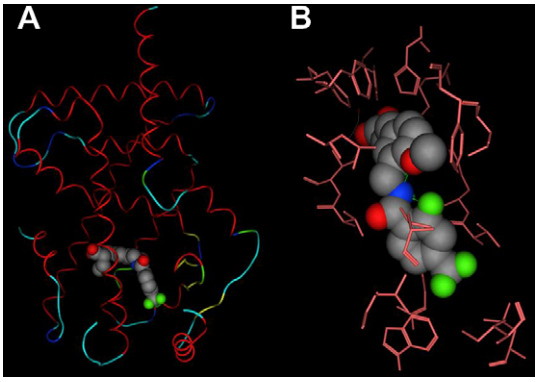


Fig. 2. Three-dimensional structure of TIPP-401 complexed with human PPARδ ligand binding domain (LBD). (A) Whole structure. (B) Zoomed view of TIPP-401. The interacting amino acids are colored pink.

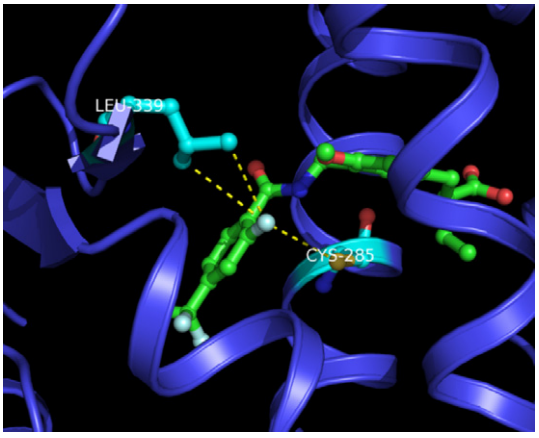


Fig. 3. Zoomed view of the fluorine at X^1 position of TIPP-401 and amino acids Cys285 and Leu339. Lengths of the yellow lines are shorter than 4 Å.

The structure reveals that the two amino acids (Cys285 and Leu339) are positioned within 4 Å around fluorine atom at the X^1 position of TIPP-401(Fig. 3). This indicates the possibility of some interaction(s) between fluorine and these amino acids, and the possible interaction(s) might be one reason why fluorine at the X^1 position enhanced PPARδ activity. Further structural analysis is in progress.

In summary, introduction of fluorine at appropriate position(s) enhances the activity of the present phenylpropionic acid-type PPAR agonists. Based on this SAR, we developed a potent PPAR pan agonist, which is a candidate drug for the treatment of altered metabolic homeostasis.

Acknowledgments

The work described in this letter was partially supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and grant from Keimeikai Foundation.

References

1. Evans, R. M.; Barish, G. D.; Wang, Y. X. *Nat. Med.* **2004**, *10*, 355.
2. Shulman, A. I.; Mangelsdorf, D. J. *N. Engl. J. Med.* **2005**, *353*, 604.
3. Xu, H. E.; Lambert, M. H.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisely, G. B.; Willson, T. M.; Kliewer, S. A.; Milburn, M. V. *Mol. Cell* **1999**, *3*, 397.
4. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.

5. Gross, B.; Staels, B. *Best Pract. Res. Clin. Endocrinol. Metab.* **2007**, *21*, 687.
6. Tenenbaum, A.; Motro, M.; Fisman, E. Z. *Cardiovasc. Diabetol.* **2005**, *4*, 14.
7. Nomura, M.; Tanase, T.; Ide, T.; Tsunoda, M.; Suzuki, M.; Uchiki, H.; Murakami, K.; Miyachi, H. *J. Med. Chem.* **2003**, *46*, 3581.
8. Kasuga, J.; Yamasaki, D.; Araya, Y.; Nakagawa, A.; Makishima, M.; Doi, T.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2006**, *14*, 8405.
9. Kasuga, J.; Nakagome, I.; Aoyama, A.; Sako, K.; Ishizawa, M.; Ogura, M.; Makishima, M.; Hirono, S.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2007**, *15*, 5177.
10. Kasuga, J.; Yamasaki, D.; Ogura, K.; Shimizu, M.; Sato, M.; Makishima, M.; Doi, T.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2008**, *18*, 1110.
11. Böhm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. *Chembiochem* **2004**, *5*, 637.
12. Müller, K.; Faeh, C.; Diederich, F. *Science* **2007**, *317*, 1881.
13. Olah, G. A.; Farooq, O.; Farnia, S. M. F.; Wu, A. H. *J. Org. Chem.* **1990**, *55*, 1516.
14. Krasnikov, S. V.; Obuchova, T. A.; Yasinskii, O. A.; Balakin, K. V. *Tetrahedron Lett.* **2004**, *45*, 711.
15. Evans, D. A.; Starr, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 13531.
16. PDB code = 2ZNQ. Detailed X-ray results will be published in the near future.