Bioorganic & Medicinal Chemistry Letters 21 (2011) 240–244

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of a novel human PPARδ selective agonist and its stimulatory effect on oligodendrocyte differentiation

Shogo Sakuma *, Tsuyoshi Endo, Takashi Kanda, Hideki Nakamura, Satomi Yamasaki, Tomio Yamakawa

Discovery Research Laboratories, Nippon Chemiphar Co Ltd, 1-22, Hikokawado, Misato, Saitama 341-0005, Japan

ARTICLE INFO

Article history:
Received 22 May 2010
Revised 29 October 2010
Accepted 4 November 2010
Available online 11 November 2010

Keywords:
Multiple sclerosis
PPARδ agonist
Compound **20**
Benzisoxazole ring
Oligodendrocyte

ABSTRACT

We successfully synthesized a novel peroxisome proliferator-activated receptor (PPAR)δ selective agonist, namely, compound **20**, with a characteristic benzisoxazole ring. Compound **20** exhibited potent human PPARδ transactivation activity and high δ selectivity. Further, it stimulated differentiation of primary oligodendrocyte precursor cells in vitro, indicating that it may be an effective drug in the treatment of demyelinating disorders such as multiple sclerosis.

Multiple sclerosis (MS) is an idiopathic disease suspected to be caused by an autoimmune disorder: the immune response is directed at the central nervous system (CNS; the brain and spinal cord), resulting in demyelination.[1] This disease usually develops in young adults, and it is more common in females.[2] Its prevalence ranges between 2 and 150 per 100,000 individuals.[3] However, there is no known cure for MS. Many patients do well without therapy, especially since many drugs have serious side effects and some pose significant risks. However, 3 forms of β-interferon have now been approved by the Food and Drug Administration (FDA) of USA for the treatment of relapsing-remitting MS. β-Interferon has been shown to reduce the number of exacerbations and thus slow the progression of physical disability. If and when attacks occur, they tend to be shorter and less severe. In clinical trials, monoclonal antibody therapy with natalizumab was shown to significantly reduce the frequency of attacks in people with relapsing forms of MS, and this drug was approved for marketing by the FDA in 2004. However, in 2005 the drug manufacturer voluntarily suspended marketing of the drug after several reports of significant adverse events. Although steroids do not affect the course of MS over time, they can reduce the duration and severity of attacks in some patients. Other drugs, including amantadine, pemoline, and the experimental drug aminopyridine, may reduce fatigue in some patients.

Although optic symptoms usually improve even without treatment, a short course of treatment with intravenous methylprednisolone followed by oral steroid treatment is sometimes administered.

As mentioned above, drugs for MS treatment that are currently in circulation present various problems in terms of effectiveness, administration routes, and even safety. Therefore, basic research on the possibility of developing peroxisome proliferator-activated receptor (PPAR)δ agonists as new candidates for MS treatment was conducted using data from recent studies.

PPARs are nuclear receptors that function after heterodimerization with other nuclear receptors, such as retinoid X receptors (RXRs). They regulate the transcription of the PPAR target gene cluster by identifying specific gene sequences and binding to them. Three PPAR subtypes, designated as PPARα, PPARδ (β), and PPARγ, have been identified. Recent advances in PPARα and PPARγ research have revealed important roles of these PPARs in lipid and glucose metabolism. However, the functions of PPARδ are not well understood. The availability of PPARδ transgenic mice and the discovery of PPARδ agonists, especially GW-501516[4] and L-165041[5] (Fig. 1), have stimulated PPARδ research, and many reports have shown that PPARδ activation leads to interesting effects such as antiobesity, improved lipid metabolism, and wound healing.[6–10]

PPAR is being closely monitored as the molecular target of a novel treatment agent for lifestyle-related diseases caused by glycolipidoses, and many molecular ligands have been studied. However, the use of PPARγ agonist poses many challenges such as occurrence of side effects in ischemic cardiac disease cases and carcinogenic effects. Therefore, many companies have stopped

Abbreviations: MS, multiple sclerosis; CNS, central nervous system; OPCs, oligodendrocyte precursor cells; PPAR, peroxisome proliferator-activated receptor.

* Corresponding author. Tel.: +81 48 952 4311; fax: +81 48 952 0743.
E-mail address: s-sakuma@chemiphar.co.jp (S. Sakuma).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.030

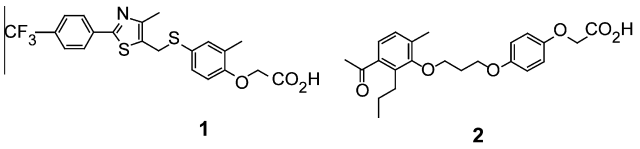
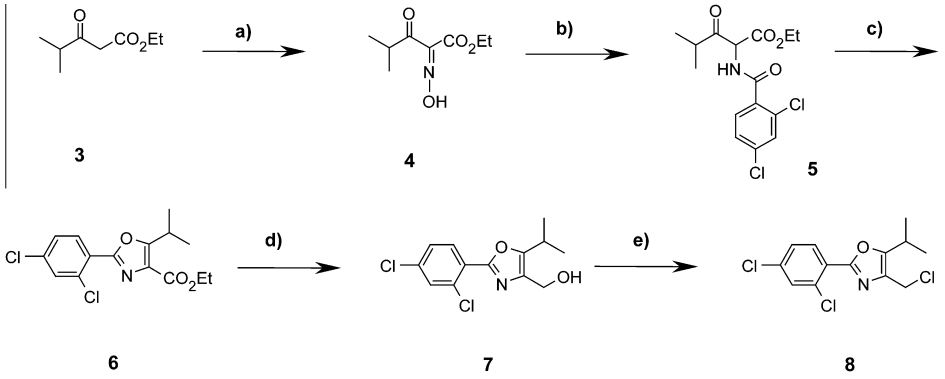


Figure 1. Structures of representative PPARδ agonists (1: GW-501516 and 2: L-165041).

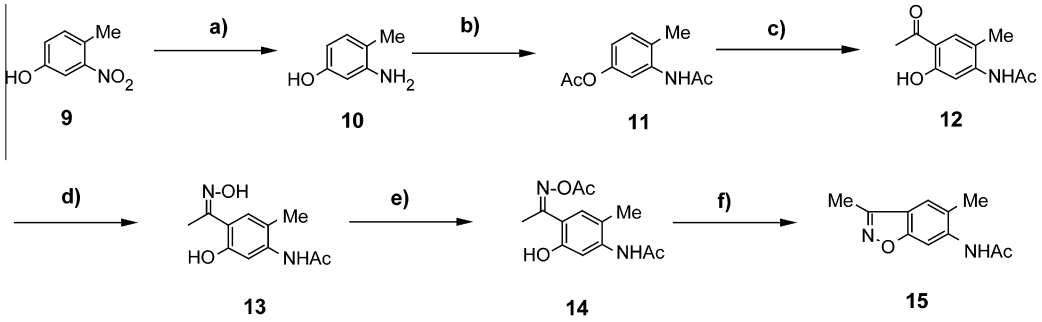
manufacturing PPAR agonist as a treatment agent for lifestyle-related diseases. Further, a high level of safety needs to be assured during long-term administration of a new PPAR agonist to patients. Taken together, the development of a PPAR agonist as a treatment agent for lifestyle-related diseases comes at a huge cost and risk. On the other hand, in recent years, research on the pleiotropic action of PPAR has shown that PPARδ has a wide tissue distribution, suggesting that PPARδ agonist may also be effective against conditions other than lifestyle-related diseases. A study was therefore carried out with an aim to determine conditions other than lifestyle-related diseases that could possibly be managed by the effective use of the PPARδ agonist. The results of this study showed that intractable multiple sclerosis could be a candidate disease. Currently, only few drugs have been found to be effective against multiple sclerosis. We therefore think that a revolutionary development of a new drug could be possible if a short-term clinical trial of the PPARδ agonist shows the effectiveness of this agent.

PPARδ agonists have been reported to have an oligodendrocyte differentiation stimulating effect.[11] Oligodendrocytes are members of the glial cell family and form the central myelin sheath. Any disease of the nervous system in which the myelin sheath of neurons is damaged is considered as a demyelinating disorder. The most common demyelinating disorder is MS, which is characterized by demyelination of the CNS. In the CNS tissue of MS patients, oligodendrocyte precursor cells (OPCs) are found to survive the demyelinating damage in MS, but they apparently fail to proliferate and differentiate.[12] Therefore, potent and selective PPARδ agonists, which can stimulate oligodendrocyte differentiation, are expected to be a possible remedy for dysmyelination and demyelinating diseases.

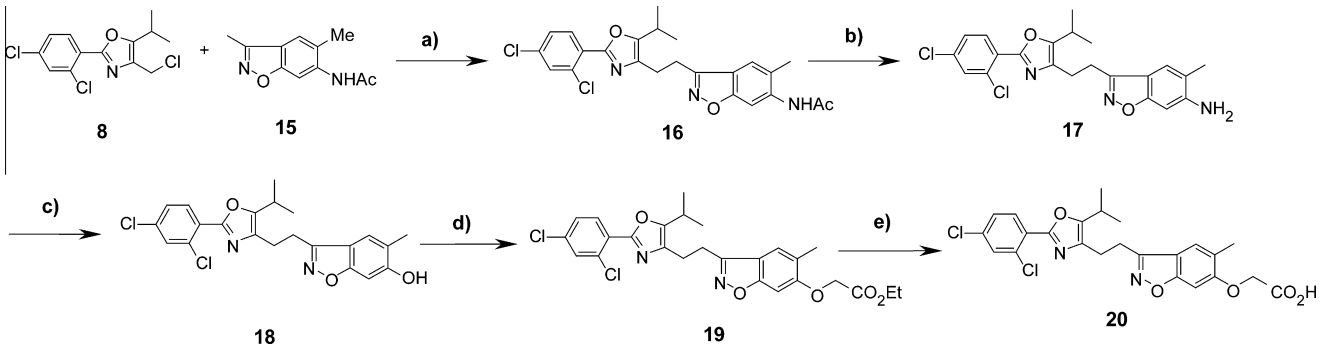
The synthesis of compound **20** and its key intermediates **8** and **15** is described below. The key intermediate **8** was synthesized as shown in Scheme 1. Compound **4**, which was obtained by reacting ethyl isobutyrylacetate (**3**) with hydroxylamine, was reduced by zinc to produce the amine form and then treated with 2,4-dichlorobenzoyl chloride by the Schotten–Baumann method to obtain compound **5**. Compound **5** was treated with phosphorus oxychloride to obtain compound **6**, an oxazole derivative. This three-step procedure to synthesize compound **6** was achieved with a 43% yield. Compound **6** was treated with LAH to obtain the alcohol compound **7**, which was then treated with thionyl chloride to produce 4-chloromethyl-2-(2,4-dichlorophenyl)-5-isopropyloxazole (**8**).[13] The key intermediate **15** was synthesized as shown in Scheme 2. Aniline **10**, obtained by catalytic hydrogenation of 4-methyl-3-nitrophenol (**9**), was acetylated to produce **11**. In the next step, compound **11** was treated with $AlCl_3$ at a high temperature to produce compound **12** with a 79% yield. Compound **12** was treated with hydroxylamine to obtain compound **13**, which was treated with acetic acid anhydride to obtain compound **14** and then treated with pyridine to obtain the key intermediate 6-acetoamido-3,5-dimethyl benzisoxazole (**15**).[14] Compound **20** was synthesized as shown in Scheme 3. Compounds **8** and **15** were condensed with LDA to obtain compound **16**, which was then treated with hydrochloric acid to produce compound **17**. This compound was then treated with sodium nitrite and then hydrolyzed with



Scheme 1. Synthesis of compound **8**. Reagents and conditions: (a) $NaNO_2$, AcOH–H_2O, 0 °C, 100%; (b) (1) Zn, 30% H_2SO_4, 0 °C and (2) 2,4-dichlorobenzoylchloride, 0 °C to rt; (c) $POCl_3$, Ph–H, reflux, three-steps 43%; (d) LAH, THF, 0 °C, 96%; (e) $SOCl_2$, Ph–H, reflux, 100%.



Scheme 2. Synthesis of compound **15**. Reagents and conditions: (a) H_2, Pd–C, EtOH, rt, quantitative yield; (b) Ac_2O, pyridine, rt, 71%; (c) $AlCl_3$, 130 °C, 79%; (d) NH_2OH·HCl, EtOH, rt, 91%; (e) Ac_2O, 130 °C, 94%; (f) pyridine, reflux, 86%.



Scheme 3. Synthesis of compound **20**. Reagents and conditions: (a) LDA, THF, −78 °C, 45%; (b) 3 M HCl, AcOH, reflux, 83%; (c) (1) $NaNO_2$, 25% H_2SO_4, 0 °C and (2) 75% H_2SO_4, 120 °C, 43%; (d) ethyl bromoacetate, K_2CO_3, acetone, rt, 96%; (e) 1 M NaOH, EtOH, rt, 76%.

Table 1
PPARs transactivation activity of compound **20** and representative PPARs agonists as assessed by a cell-based transactivation assay, using GAL4 DBD-hPPAR LBD

Compound	Transactivation activity EC_{50} (μM)		
	PPARα	PPARγ	PPARδ
20	>10	>10	0.025
GW-590735	0.010	>10	2.6
Rosiglitazone	>10	0.10	>10
GW-501516	0.99	4.1	0.0017

Table 2
PPARs transactivation activity of compound **20** as assessed by cell-based transactivation assay, using the full-length receptor (full-length hPPAR + hRXRα)

Compound	Transactivation activity EC_{50} (μM)		
	PPARα	PPARγ	PPARδ
20	>10	>10	0.0045

75% sulfuric acid to produce compound **18**. Compound **18** was condensed with ethyl bromoacetate in the presence of potassium carbonate in acetone to obtain compound **19**, which was then treated with sodium hydroxide in ethanol to obtain compound **20**.[15]

The human PPAR transactivation activity of compound **20** was evaluated by the following method. The mammalian expression vectors used were pSG5-GAL4-hPPARα, pSG5-GAL4-hPPARγ, and pSG5-GAL4-hPPARδ, which express the ligand binding domains of human PPARα, PPARγ, and PPARδ; each of these vectors were fused to the yeast transcription factor GAL4 DNA binding domain.[16] Each receptor expression vector and the UASx4-TK-LUC[17] reporter plasmid were co-transferred into CV-1 cells (kidney fibroblasts isolated from an African green monkey). After the addition of compound **20** (0.001–100 μM), CV-1 cells were incubated for 40 h, and luciferase activity was measured. PPARα, PPARγ, and PPARδ transactivation activity of the test compounds was calculated relative to the luciferase activity induced by 1 μM GW-590735[18] (PPARα selective agonist), 10 μM rosiglitazone (PPARγ selective agonist), and 0.1 μM GW-501516 (PPARδ selective agonist), respectively.

The calculated 50% effective concentration (EC_{50}) of compound **20** confirmed that this compound had potent hPPARδ transactivation activity (EC_{50} = 0.025 μM) and a high δ selectivity (Table 1) (Fig. 2). In the cell-based assay using the full-length receptor and peroxisome proliferator response element, compound **20** acted as a complete human PPARδ agonist with an EC_{50} of 4.5 nM. With the full-length human receptor, compound **20** showed about 2000-fold selectivity for PPARα and PPARγ (Table 2) (Fig. 3).

This research was conducted on the basis of the forecast that if the PPARδ agonist stimulated the differentiation of Adult Oligodendrocyte Precursor Cells (Adult OPCs) inside the brain of patients with MC, it could regenerate myelin. With these Adult OPCs as the target cells, the OPCs of the brain of a rat fetus were used as the model. As the OPCs differentiate into mature oligodendrocyte in the cell lineage, they specifically develop proteins. Among the proteins, O1 and MBP were selected as the differentiation markers. Within the cell lineage, these proteins exist in the relatively late stage; in other words, they reach the mature oligodendrocyte stage just before myelin is formed in the immature

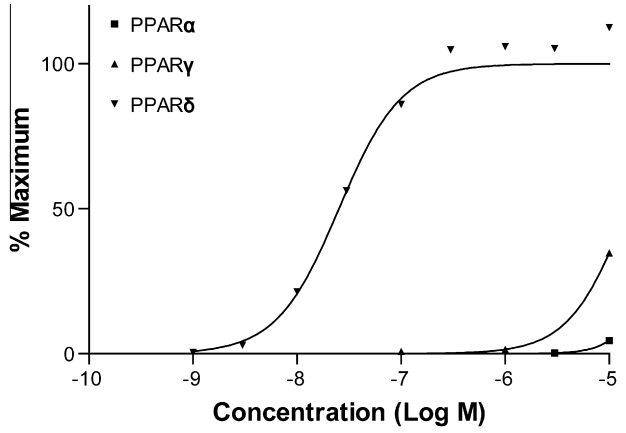


Figure 2. PPAR transactivation activities of compound **20** in the cell-based transactivation assay, using GAL4 DBD-hPPAR LBD.

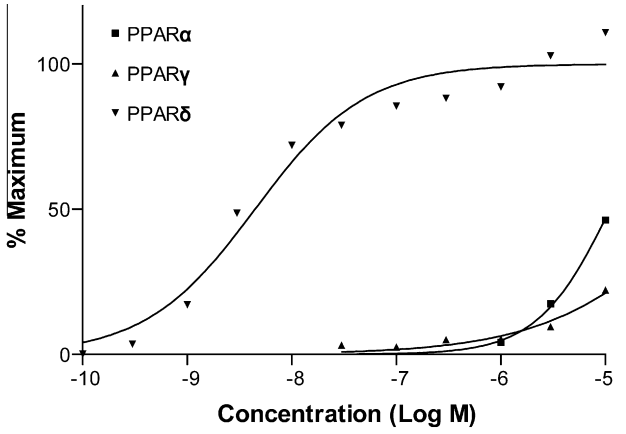


Figure 3. PPAR transactivation activities of compound **20** in the cell-based transactivation assay, using full-length hPPAR + hRXRα.

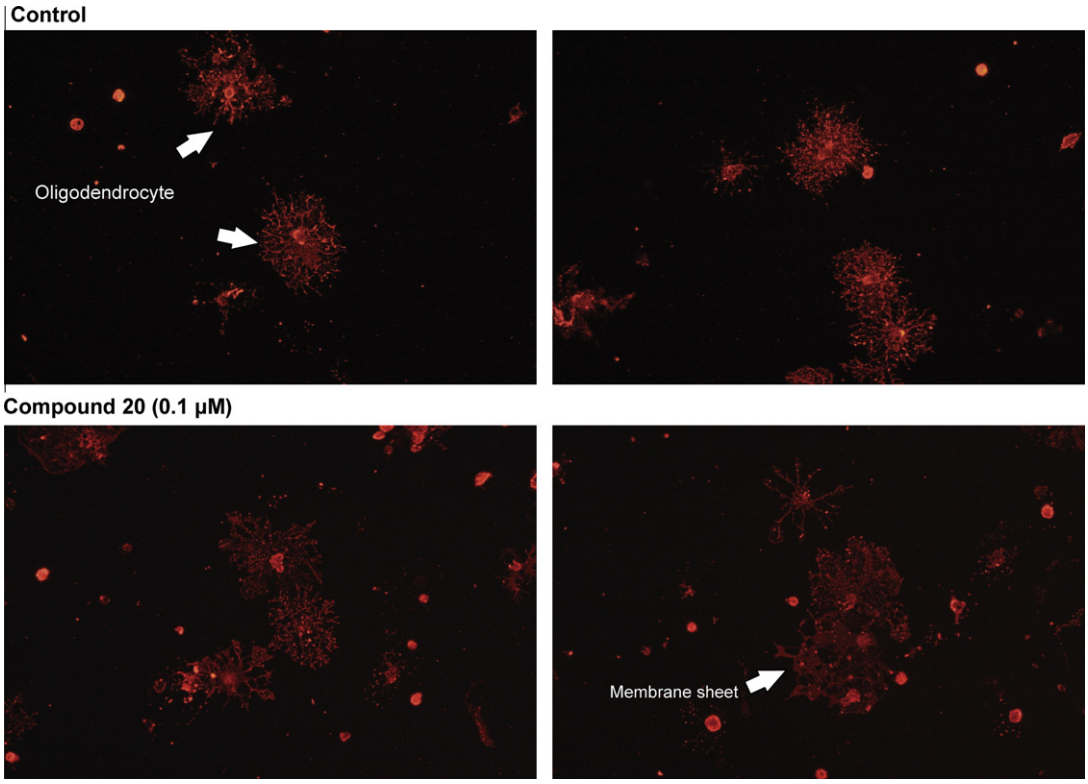


Figure 4. Oligodendrocyte differentiation stimulating effect of compound **20**. Seven-day-old primary oligodendrocyte cultures were immunostained with anti-O1 antibody. Compound **20** was added to the cultures 3 days prior to fixation. In the cultures treated with compound **20**, an increase in the number of stained cells, membrane sheet formation, and increased cell diameter were observed.

oliogodendrocyte. For this evaluation, O1 was mainly used. The evaluation methods and an outline of the results are described below.

In vitro tests were conducted to evaluate the stimulatory effects of compound **20** on the differentiation of OPCs. These cells were prepared from the primary mixed cell cultures of the fetal brain cortex from Wistar rats,[19] and an OPC-rich fraction was prepared using the Percoll gradient separation technique. After 4 days of culture, OPCs were treated with the test compounds. The differentiation stimulatory effect was evaluated by immunostaining. Compound **20** was dissolved in dimethylsulfoxide (DMSO), and the final concentration of DMSO in the medium was 0.01%. Each dissolved compound was added to the culture medium such that the final concentration was 0.1 μM. For the control groups, 0.01% DMSO was added to the culture medium. The cells on a cover glass were incubated for 3 days at 37 °C under a 5% CO_2 atmosphere. Thereafter, the cells were immunostained using an anti-oligodendrocyte marker O1 antibody and placed on a glass slide. Random slide images of the specimens were selected and processed, and the areas of stained cells were measured. The mean stained area of the control specimen was considered as 100%, and the mean stained area of the treated specimens was calculated relative to this control value. The degree of differentiation was determined on the basis of the increase in the number of stained cells, increase in the diameter of stained cells, and membrane sheet formation. The images of the stained cells in the control and test-compound-treated specimens are shown in Figure 4. As compared to the control cells, the cells treated with 0.1 μM of compound **20** showed a greater area of staining.

In conclusion, the results of this study show that the novel PPARδ agonist compound **20**, which has a characteristic benzisoxazole ring, has strong hPPARδ transactivation activity and high δ selectivity. Compound **20** was examined in vitro using OPCs from rat fetal brains by an immunostaining method and confirmed to have a strong stimulatory effect on oligodendrocyte differentiation. The lack of mutagenicity of compound **20** has previously been confirmed with Ame's test; moreover, it passed the hERG test. We believe that compound **20** may be an effective drug for the treatment of demyelinating diseases such as MS.

Acknowledgements

The authors would like to thank Dr. Masui for extending support during the length of our research period. We are also deeply grateful to Mr. Kobayashi and Mr. Tashiro for their contribution to the synthesis and analysis of compound **20** and also to Dr. Asou from the Tokyo Metropolitan Institute of Gerontology for the guidance related to the establishment of a system to assess oligodendrocytes.

References and notes

1. Compston, A.; Coles, A. *Lancet* **2008**, *372*, 1502.
2. Debouverie, M.; Pittion-Vouyovitch, S.; Louis, S.; Guillemin, F. *Eur. J. Neurol.* **2008**, *9*, 916.
3. Rosati, G. *Neurol. Sci.* **2001**, *2*, 117.
4. Oliver, W. R.; Sheck, J. L.; Sanith, M. R.; Russell, D. A.; Sznaidman, M. L.; Xu, H. E.; Sternbatch, D. D.; Kliewer, S. A.; Hansen, B. C.; Wilson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5306.
5. Berger, J.; Leibowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M. S.; Berger, G. D.; Mosley, R.; Marquis, R.; Santini, C.; Sahoo, S. P.; Tolman, R. L.; Smith, R. G.; Moller, D. E. *J. Biol. Chem.* **1999**, *274*, 6718.
6. Tanaka, T.; Yamamoto, J.; Iwasaki, S.; Asaba, H.; Hamura, H.; Ikeda, Y.; Watanabe, M.; Magoori, K.; Ioka, R. X.; Tachibana, K.; Watanabe, Y.; Uchimura, Y.; Sumi, K.; Iguchi, H.; Ito, S.; Doi, T.; Hamakubo, T.; Auwerx, J.; Yanagisawa, M.; Kodama, T.; Sakai, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15924.
7. Wang, Y. X.; Lee, G. H.; Tiep, S.; Yu, R. T.; Ham, J.; Kang, H.; Evans, R. M. *Cell* **2003**, *113*, 159.
8. Dressel, U.; Allen, T. L.; Pippal, J. B.; Rohde, P. R.; Lau, P.; Muscat, G. E. *Mol. Endocrinol.* **2003**, *17*, 2477.

9. Luquet, S.; Lopez-Soriano, J.; Holst, D.; Fredenrich, A.; Melki, J.; Rassoulzadegan, M.; Grimaldi, P. A. *FASEB J.* **2003**, *17*, 22994.
10. Chong, H. C.; Tan, M. J.; Philippe, V.; Tan, S. H.; Tan, C. K.; Ku, C. W.; Goh, Y. Y.; Wahli, W.; Michalik, L.; Tan, N. S. *J. Cell Biol.* **2009**, *184*, 817.
11. Saluja, I.; Granneman, J. G.; Skoff, R. P. *Glia* **2001**, *33*, 191.
12. Wolswijk, G. *J. Neurosci.* **1998**, *18*, 601.
13. 4-Chloromethyl-2-(2,4-dichlorophenyl)-5-isopropyloxazole (**8**) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (d, 6H, J = 7 Hz), 3.0–3.2 (m, 1H), 4.63 (s, 2H), 7.33 (dd, 1H, J = 2 Hz, 9 Hz), 7.51 (d, 1H, J = 2 Hz), 7.92 (d, 1H, J = 9 Hz).
14. 6-Acetoamido-3,5-dimethylbenzisoxazole (**15**) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.26 (s, 3H), 2.38 (s, 3H), 2.53 (s, 3H), 7.14 (s, 1H), 7.39 (s, 1H), 8.34 (s, 1H).
15. [3-[2-[2-(2,4-Dichloro) phenyl-4-isopropyl-4-oxazolyl]ethyl]-5-methyl-1,2-benzisoxazol-6-yloxy]acetic acid (**20**) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.12 (d, 6H, J = 7 Hz), 2.24 (s, 3H), 2.9–3.0 (m, 1H), 3.04 (dd, 2H, J = 7 Hz, 8 Hz), 3.27 (dd, 2H, J = 7 Hz, 8 Hz), 4.74 (s, 2H), 6.82 (s, 1H), 7.20 (s, 1H), 7.33 (dd, 1H, J = 2 Hz, 9 Hz), 7.52 (d, 1H, J = 2 Hz), 7.88 (d, 1H, J = 9 Hz).
Mp (dec) 182–184 °C. HRMS calcd for $C_{24}H_{22}Cl_2N_2O_5$: 488.0906 $[M]^+$, found 488.0885.
16. Lehmann, J. M.; Lenhard, J. M.; Oliver, B. B.; Ringold, G. M.; Kliever, S. A. *J. Biol. Chem.* **1997**, *272*, 3406.
17. Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. *Cell* **1995**, *81*, 541.
18. Sierra, M. L.; Beneton, V.; Boullay, A. B.; Boyer, T.; Brewster, A. G.; Donche, F.; Forest, M. C.; Fouchet, M. H.; Gellibert, F. J.; Grillot, D. A.; Lambert, M. H.; Laroze, A.; Le Grumelec, C.; Linget, J. M.; Montana, V. G.; Nguyen, V. L.; Nicodème, E.; Patel, V.; Penfornis, A.; Pineau, O.; Pohin, D.; Potvain, F.; Poulain, G.; Ruault, C. B.; Saunders, M.; Toum, J.; Xu, H. E.; Xu, R. X.; Pianetti, P. M. *J. Med. Chem.* **2007**, *50*, 685.
19. Yoshimura, K.; Sakurai, Y.; Nishimura, D.; Tsuruo, Y.; Nomura, M.; Kawato, S.; Seiwa, C.; Iguchi, T.; Itoh, K.; Asou, H. J. *Neurosci. Res.* **1998**, *54*, 79.