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Identification of novel PPARα ligands by the structural modification of a PPARγ ligand

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Abstract—To develop novel PPARα ligands, we designed and synthesized several 3-{3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}-propanoic acid derivatives. Compound **10**, the meta isomer of a PPARγ agonist **1**, has been identified as a PPARα ligand. The introduction of methyl and ethyl groups at the C-2 position of the propanoic acid of **10** further improved the PPARα-binding potency.

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily[1] and the PPAR subfamily consists of three members, PPARα, PPARγ, and PPARδ. These receptors act as ligand-activated transcription factors with other members of the nuclear receptor family,[2–4] and play a central role in the storage and catabolism of dietary fats by regulating the expression of a large number of genes involved in lipid metabolism and the energy balance.[5] PPARα is highly expressed in metabolically active tissues such as liver, heart, and muscle, and activation of PPARα decreases the serum triglyceride level and increases the HDL-c level.[6] Therefore, PPARα agonists such as clofibrate (Fig. 1) are being utilized as hypolipidemic agents. Meanwhile, PPARγ is expressed predominantly in adipose tissues and functions as a regulator of glucose and lipid homeostasis. The clinically useful thiazolidinedione (TZD) class of insulin sensitizers such as rosiglitazone[7] and pioglitazone[8] (Fig. 1) are potent PPARγ agonists used in the treatment of Type 2 diabetes. In addition, recent studies revealed that dual agonists of PPARα/γ decreased the free triglyceride plasma concentration and increased the plasma HDL concentration in an insulin resistant animal model.[9–11] Thus, many groups have ongoing research programs to find more potent and less toxic PPARα agonists and PPARα/γ dual agonists.

We previously reported compound **1**, which was designed based on the structure of rosiglitazone and 15d-PGJ_2,[12,13] as a potent PPARγ ligand.[14] To find novel PPARα agonists, we chose compound **1** as a lead structure, because recent reports on selective PPAR ligands indicated that minor structural modifications can affect selectivity.[15–17] In this letter, we report the design, synthesis, and binding affinity of PPAR ligands based on the structure of compound **1**.

In the course of our computational studies on compound **1** and its derivatives using Glide 3.5 software, we found that compound **10** (Fig. 2), the meta isomer of **1**, likely fits PPARα protein more tightly than **1**.[18] An inspection of the simulated PPARα/**1** complex showed that one of the two oxygen atoms of the carboxylate group forms hydrogen bonds with His 440 and Tyr 464, and that the nonyl group is located in the hydrophobic region formed by Ile 241, Leu 247, Ala 250, Leu 254, Ile 272, Val 332, and Ala 333 (Fig. 3, left). In the simulated PPARα/**10** complex, as well as the hydrogen bonds and the hydrophobic interaction found in the PPARα/**1** complex, the existence of added hydrogen bonds was expected between the other oxygen atom of the carboxylate and Ser 280, and between the tertiary nitrogen and Thr 279 (Fig. 3, right). These results prompted us to evaluate the affinity for PPAR of **10** and its derivatives **2**–**9** and **11**–**20** (Fig. 2).

Keywords: Peroxisome proliferator-activated receptor; PPARα ligand; PPARγ ligand; Nuclear receptor.
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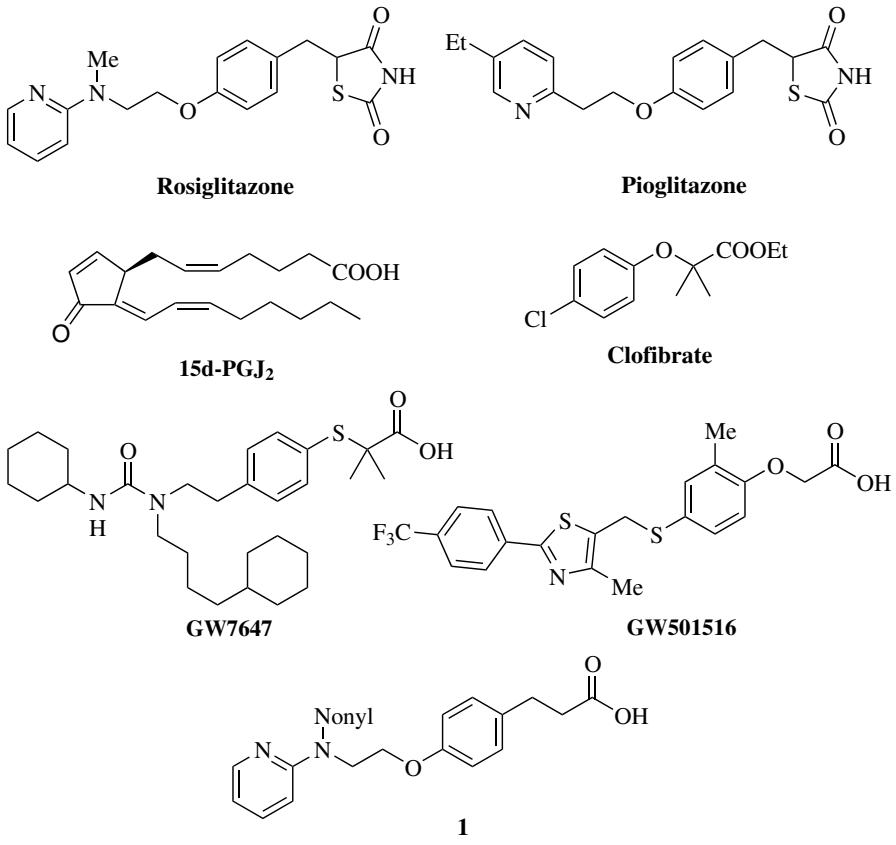


Figure 1. Structures of rosiglitazone, pioglitazone, 15d-PGJ$_2$, clofibrate, GW7647, GW501516, and compound **1**.

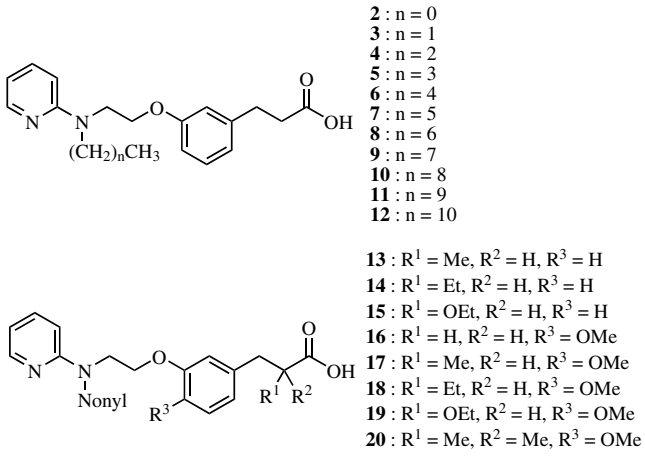


Figure 2. Structures of compounds **2**–**20**.

The compounds prepared for this study are shown in Figure 2, and the routes used for their synthesis are illustrated in Schemes 1 and 2. Scheme 1 shows the preparation of the 3-{3-[2-(alkylpyridin-2-ylamino)ethoxy]phenyl}propanoic acid derivatives **2**–**16**, **18**, and **19**. The yield of 2-alkylaminopyridine **22a**–**k** was 69–86%, using the method of Buchwald:[19] treatment of 2-bromopyridine **21** with *n*-nonylamine (4 equiv), $Pd_2(DBA)_3$, BINAP, and *t*-BuONa in toluene under reflux. *m*-Hydroxybenzaldehyde **23a** and isovaniline **23b** were allowed to react with 1,2-dibromoethane to give ethers **24a** and **24b**. The Horner–Wadsworth–Emmons reaction[20] was applied to the conversion of **24a**, **b** into acrylic acid derivatives **25a**–**g**. The double bond of **25a**–**g** was hydrogenated to yield compounds **26a**–**g**. Coupling between 2-alkylaminopyridine **22a**–**k** and propanoic acid ethyl esters **26a** afforded *N*-(2-pyridinyl)-*N*-alkylpropanoic acids **27a**–**k**. Propanoic acid ethyl esters **26b**–**g** were also allowed to react with 2-nonylaminopyridine **22i** to afford *N*-(2-pyridinyl)-*N*-nonylpropanoic acids **27l**–**q**. The subsequent hydrolysis of **27a**–**q** gave the desired carboxylic acids **2**–**16**, **18**, and **19**.

The preparation of 3-{4-methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic acids **17** and **20**, which have one or two methyl groups at the C-2 position of the propanoic acid, is outlined in Scheme 2. The aldehyde **24b** was reduced by $NaBH_4$ and allowed to react with acetic anhydride to give acetic acid 3-(2-bromoethoxy)benzyl ester **29**. Compound **29** was treated with 1-methoxy-1-trimethylsilyloxypropene or dimethylketene methyltrimethylsilyl acetal in the presence of magnesium perchlorate in anhydrous CH_2Cl_2 to give esters **30a**,**b**.[21] Coupling between 2-nonylaminopyridine **22i** and propanoic acid methyl esters **30a**,**b** afforded *N*-(2-pyridinyl)-*N*-nonyl compounds **31a**,**b** and subsequent hydrolysis gave carboxylic acids **17** and **20**.

The binding affinity of the compounds for PPARs was evaluated with a CoA-BAP System (Microsystems).[22] In this system, the alkaline phosphatase (AP) activity is directly proportional to the affinity of the ligands for PPARs.

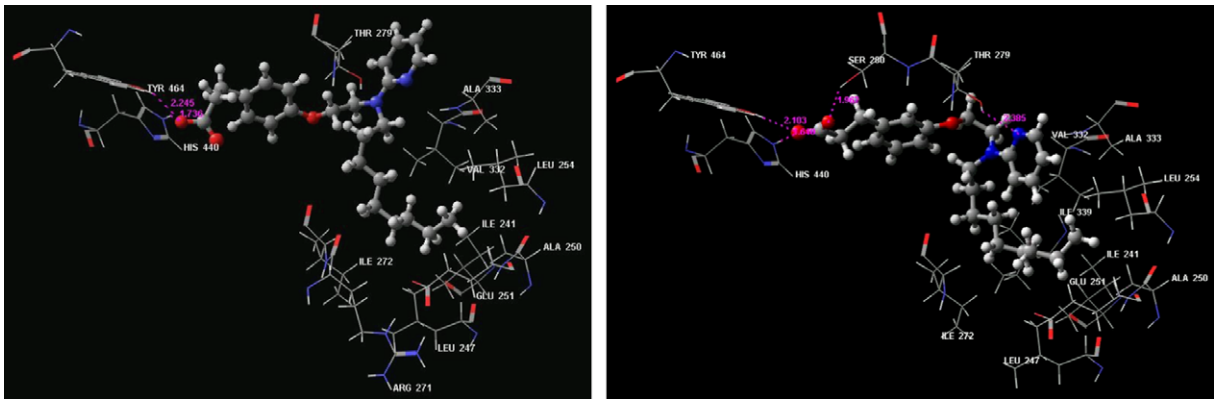
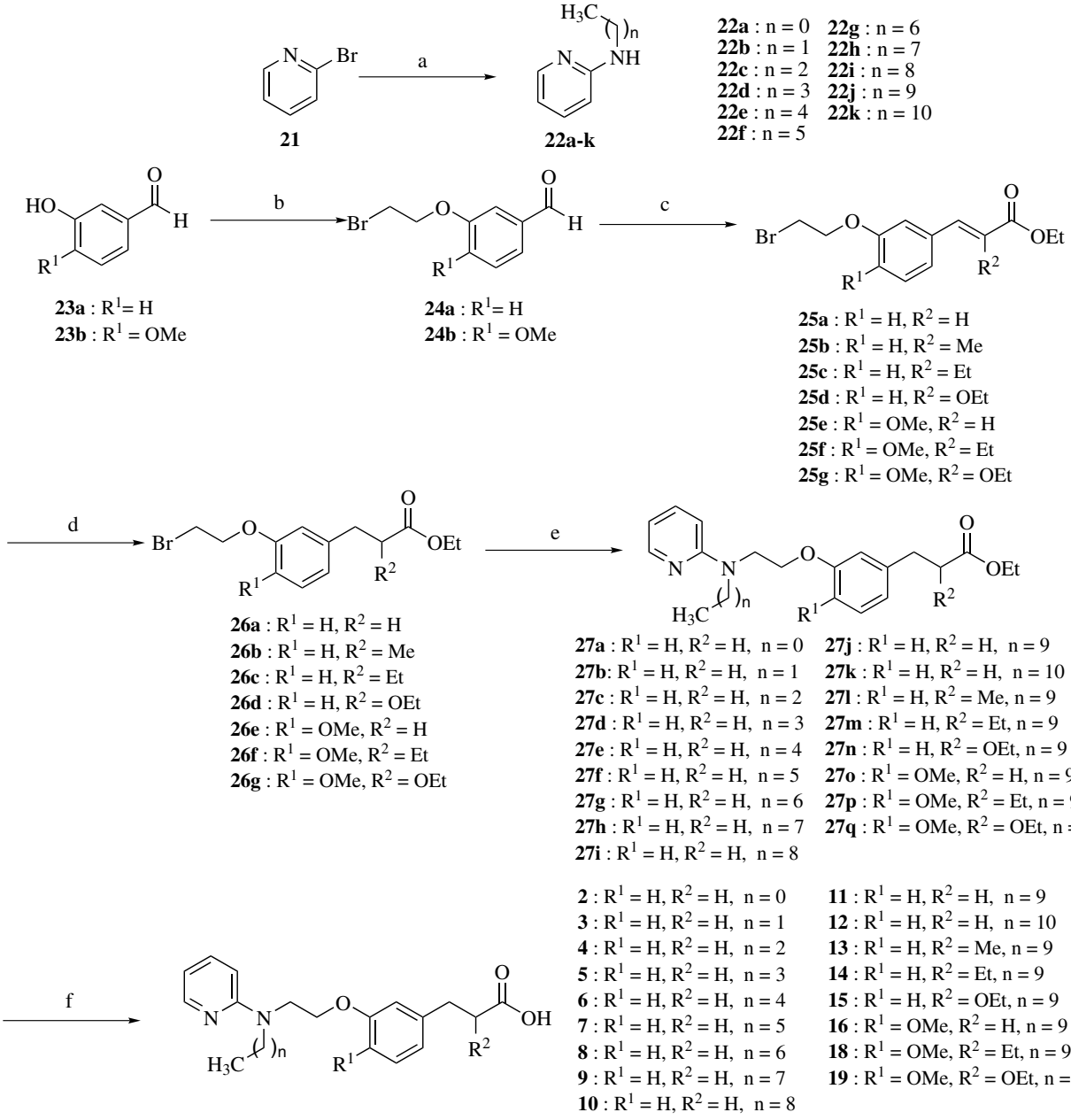
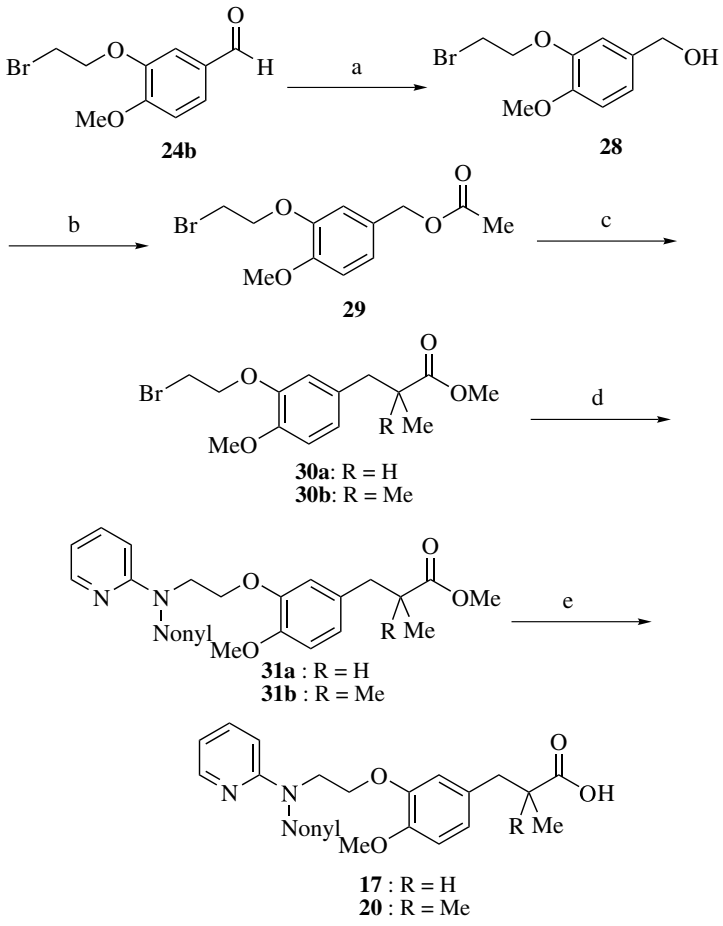


Figure 3. View of the lowest energy conformations of **1** (left) and **10** (right) docked in PPARα. Residues around compounds and hydrogen bonds are displayed as wires and dotted lines, respectively. Figures represent distances in angstrom.



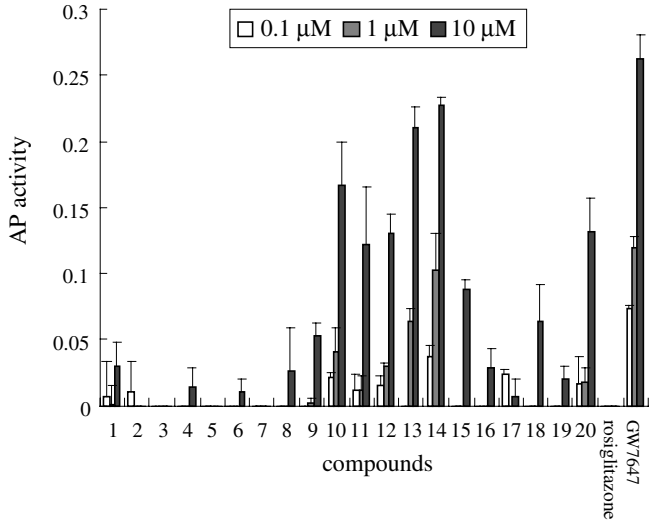
Scheme 1. Reagents and conditions: (a) $CH_3(CH_2)_nNH_2$, $Pd_2(DBA)_3$, BINAP, *t*-BuOH, toluene, 80 °C, 69–86%; (b) 1,2-dibromoethane, Cs_2CO_3, THF, 65 °C, 40–57%; (c) $(EtO)_2P(O)CH(R^2)CO_2Et$, NaH, anhydrous THF, 0 °C to rt, 47–95%; (d) H_2, Pd/C, EtOH, 79–97%; (e) **22a–k**, Et_3N, KI, 105 °C, 7–14%; (f) aq NaOH, EtOH–THF, rt, 89–95%.



Scheme 2. Reagents and conditions: (a) $NaBH_4$, EtOH, rt, 97%; (b) Ac_2O, DMAP, rt, 96%; (c) 1-methoxy-1-trimethylsilyloxypropene or dimethylketene methyltrimethyl silyl acetal, $Mg(ClO_4)_2$, rt, 94–95%; (d) **22i**, Et_3N, KI, 105 °C, 5–13%; (e) aq NaOH, EtOH, rt, 75–76%.

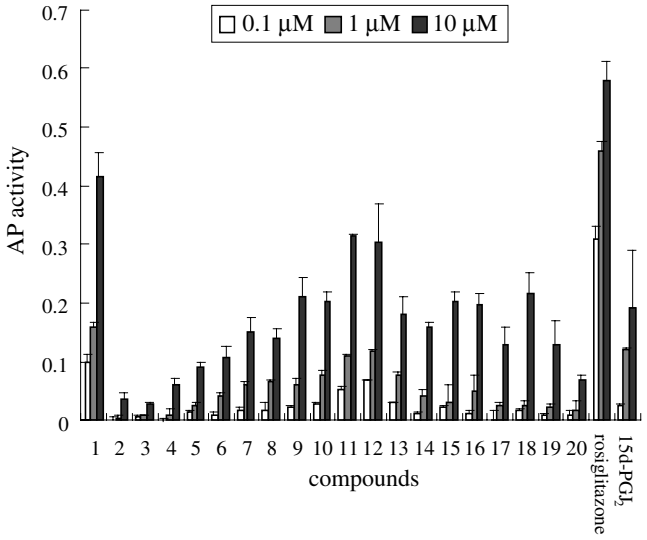
The ability of compounds **2**–**20** to bind PPARα, PPARγ, and PPARδ was evaluated and the results are shown in Tables 1–3, respectively. GW7647[23] (PPARα), rosiglitazone[7] (PPARγ), and GW501516[24] (PPARδ) were used as reference compounds (Fig. 1). The lead compound **1** showed high affinity for PPARγ and little affinity for PPARα and PPARδ (Tables 1–3, line 1). As we had expected from the computational study described above,

Table 1. Binding affinity for PPARα of compounds **1**–**20** at 0.1, 1.0, and 10 μM



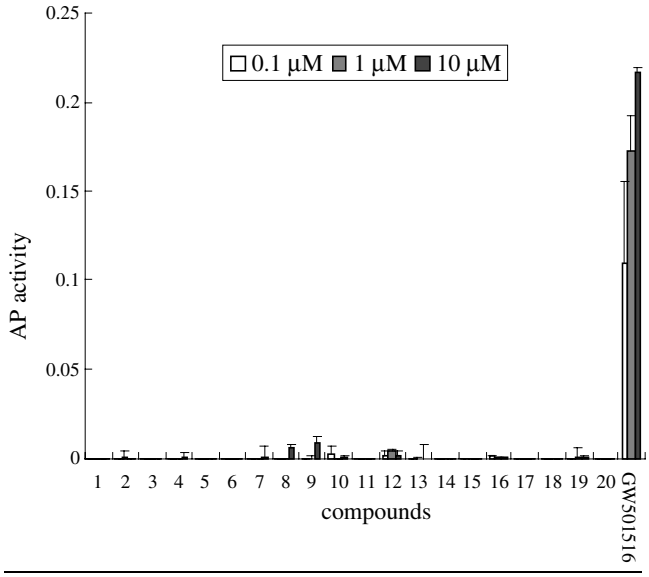
Values are means of at least three experiments.

Table 2. Binding affinity for PPARγ of compounds **1**–**20** at 0.1, 1.0, and 10 μM



Values are means of at least three experiments.

Table 3. Binding affinity for PPARδ of compounds **1**–**20** at 0.1, 1.0, and 10 μM



Values are means of at least three experiments.

compound **10**, the meta isomer of compound **1**, displayed much higher affinity for PPARα than did **1** (Table 1, line 1 vs 10). Furthermore, the affinity for PPARγ of **10** is lower than that of **1** (Table 2, line 1 vs 10), and compound **10** exhibited no affinity for PPARδ (Table 3, line 10).

To study the structure–activity relationship of 3-{3-[2-(alkylpyridin-2-ylamino)ethoxy]phenyl}propanoic acid derivatives and to find more potent PPARα ligands, we initially evaluated the PPAR-binding affinity of compounds **2**–**12** which have alkyl chains of various lengths on their nitrogen atom. It was found that the affinity of these compounds was closely related to chain length. Among compounds **2**–**12**, nonyl **10** showed the greatest affinity for PPARα, while decyl **11** and undecyl **12** were most active toward PPARγ, and heptyl **8** and octyl **9** showed little affinity for PPARδ (Tables 1–3, lines 2–12).

We next examined the effect of substituents at the C-2 position of the propanoic acid of **10**, because it has been reported that the introduction of an alkyl or alkoxy group at this position increases activity for PPARα.[15,25–27] Methyl **13**, ethyl **14**, and ethoxy **15** were tested, and much to our satisfaction, **13** and **14** showed strong affinity for PPARα and slightly weak affinity for PPARγ as compared with the parent compound **10**. In addition, compounds **13**–**15** had no affinity for PPARδ (Tables 1–3, lines 13–15).

To examine the effect of the introduction of a methoxy group at the C-4 position of the benzene ring, compounds **16**–**20** were investigated. However, these compounds did not show a pronounced affinity for PPARα compared to compounds **10**, **13**, and **14** (Tables 1, lines 16–20).

In summary, to find novel PPARα ligands, we prepared several 3-{3-(2-nonylaminoethoxy)phenyl}propanoic acid derivatives which were designed based on the structure of the PPARγ agonist **1**. Compound **10**, the meta isomer of **1**, was found to be a PPARα ligand. The introduction of methyl (**13**) and ethyl (**14**) groups at the C-2 position of the propanoic acid of **10** further improved the PPARα-binding potency. The findings of this study will help provide an effective agent for hyperlipidemia. Currently, further detailed studies pertaining to compounds **13** and **14** are under way.

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