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Diphenylmethane skeleton as a multi-template for nuclear receptor ligands: Preparation of FXR and PPAR ligands

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Abstract—Novel, potent farnesoid X receptor (FXR) and peroxisome proliferator-activated receptor α (PPARα) agonists were obtained by using a diphenylmethane skeleton as a substitute for a steroid skeleton.

Nuclear receptors (NRs) are a family of ligand-dependent transcription factors which modulate gene expression and thereby influence diverse biological processes, including cell growth, differentiation, and metabolism.[1,2] Analysis of the human genome sequence indicates that there are 48 NRs in humans.[1] So far, the ligands of only about half of them have been identified.[1,3] From the standpoint of chemical structure, the identified ligands for NRs most commonly contain a steroid skeleton, as typically found in the ligands for all of the classical steroid hormone receptors [estrogen receptors, progesterone receptor, androgen receptor (AR), glucocorticoid receptor, and mineral corticoid receptor]. In addition, oxysterols and cholic acid derivatives are considered to act as physiological ligands for liver X (oxysterol) receptors (LXRs) and farnesoid (bile acid) receptor (FXR). The ligand for vitamin D receptor (VDR) is 1α,25-dihydroxyvitamin D_3, which has a secosteroidal skeleton that is metabolically derived from cholesterol, although lithocholic acid, a steroid analog produced metabolically from cholesterol, is also reported to be a physiological ligand for VDR.[4] Because all members of NRs are thought to have evolved from a single gene,[5] it might be expected that a ligand superfamily also exists.[6,7]

There have been many structural development studies of non-steroidal/non-secosteroidal ligands for the above NRs from the standpoint of medicinal chemistry, including our studies on ligands for VDR and AR.[7,8] Recently, we developed diphenylmethane-type VDR ligands,[8] using LG190155 (**1**) (Fig. 1) reported by Boehm et al.[9] as a lead compound. In that work, we found that a nitrogen-containing diphenylpentane (DPP) derivative **2** (DPP-0113) (Fig. 1) acts as a dual ligand for both VDR and AR.[8] This result, as well as previous structural development studies on nuclear retinoic acid receptor (RAR) ligands,[7,10,11] implies that a diphenylmethane skeleton can substitute for a steroid skeleton as a multi-template for NR ligands.

To confirm the usefulness of the diphenylmethane skeleton as a multi-template for NR ligands, we designed novel ligands for FXR and peroxisome proliferator-activated receptor-α (PPARα). These NRs were chosen as targets because of the strict structural requirements for their ligands, and because steroidal ligands of FXR and PPARα, including chenodeoxycholic acid (CDCA: **3**)[12] and a diphenylmethane-related ligand, fenofibrate (**4**), respectively,[13] are already known (Fig. 1). In this paper, we describe the design, synthesis, and biological activity evaluation of novel diphenylmethane-based ligands for FXR and PPARα.

Keywords: Nuclear receptor ligands; FXR; PPAR; Drug design; Diphenylmethane.
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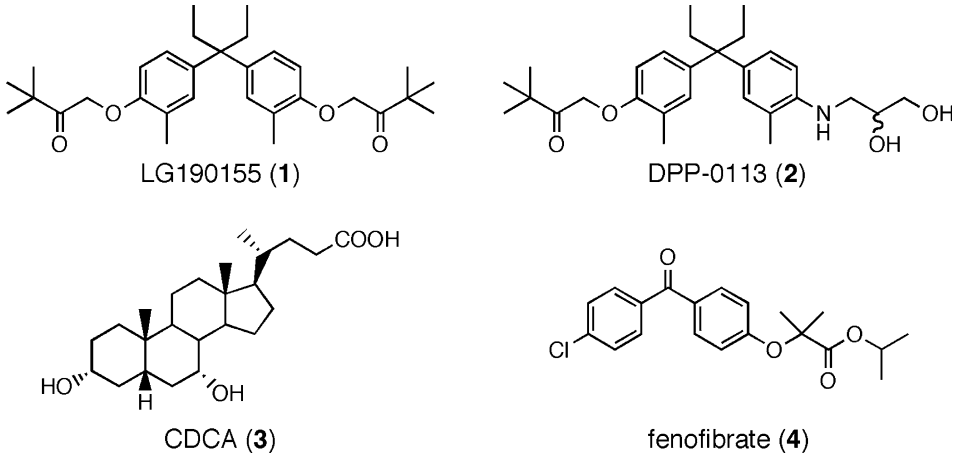


Figure 1. Structures of LG190155 (**1**), DPP-0113 (VDR/AR dual ligand: **2**), CDCA (FXR ligand: **3**) and fenofibrate (PPARα ligand: **4**).

Ligands for FXR. FXR is a well-characterized member of the so-called 'metabolic' subfamily of NRs and is a transcriptional sensor for bile acids.[14] Its ligands, including CDCA (**3**) (Fig. 1), act as signaling molecules and participate in an intricate network of interactions that ultimately govern lipid, steroid, and cholesterol homeostasis, and are involved in processes such as glucose utilization, inflammation, and carcinogenesis.[14] Maloney et al. reported GW4064 (**5**) (Fig. 2) as a potent synthetic ligand for FXR.[15] Considering the structures of CDCA (**3**) and GW4064 (**5**), and the potential of diphenylpentane (DPP) as a steroid skeleton substitute, we designed DPP derivatives as FXR ligand candidates, that is, DPPF-01 (**6**) and DPPF-13 (**7**) (Fig. 2). The R group of DPPF-01 (**6**), containing a carboxylic acid group, was introduced to mimic the carboxylic acid group found in CDCA (**3**) and GW4064 (**5**). The R group of DPPF-13 (**7**), containing a diol moiety, was introduced to mimic side chains found in several oxycholesterols.

DPPF-01 (**6**) and DPPF-13 (**7**) were prepared by usual organic synthetic methods, as summarized in Scheme 1. Briefly, bisphenol (**8**) and its mono-anilino derivative (**9**) were prepared as already reported.[8] Coupling of **8** or **9** with 3-(2,6-dichlorophenyl)-4-bromomethyl-5-isopropyl-isozaxole (**10**) in DMF in the presence of NaH gave **11** or **12**, respectively. The phenolic hydroxyl group of **11** was alkylated with methyl 2-bromoethylate, followed by hydrolysis of the ester to give DPPF-01 (**6**).[16] Reaction of **12** with glycidol gave DPPF-13 (**7**).[17] CDCA (**3**) was purchased from Sigma Co. Ltd (Japan).

The FXR agonistic activity of DPPF-01 (**6**) and DPPF-13 (**7**) was evaluated by a reporter gene assay method

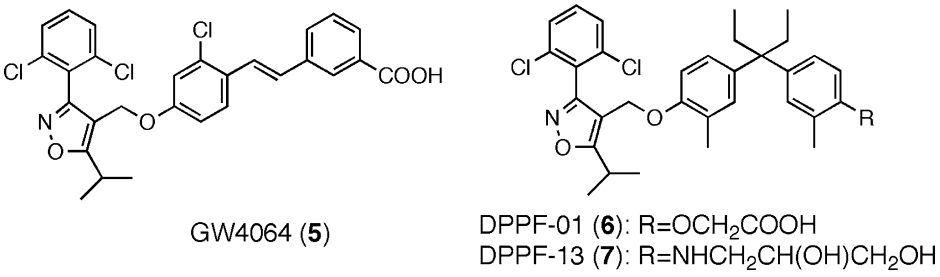
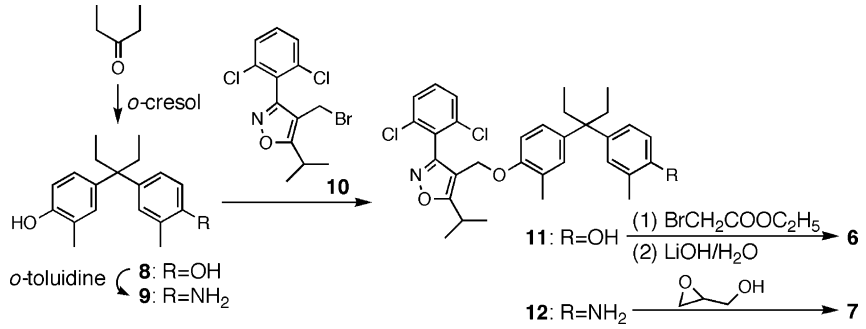


Figure 2. Structures of GW4064 (**5**) and compounds designed as diphenylmethane-type ligands for FXR (**6** and **7**).



Scheme 1. Synthesis of compounds **6** and **7**.

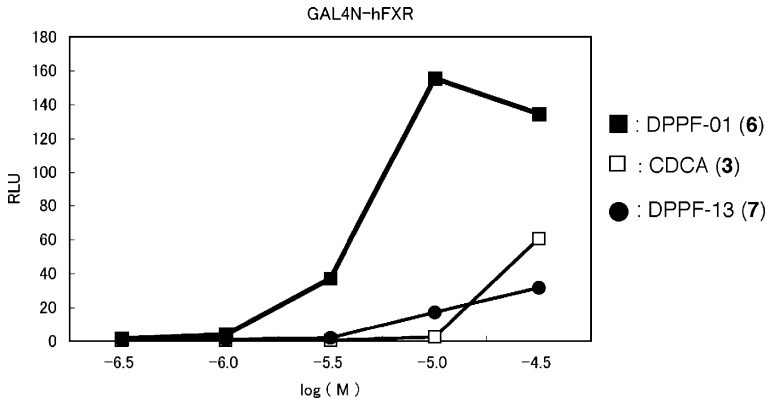


Figure 3. Transcriptional activation of FXR by DPPF-01 (**6**), DPPF-13 (**7**) and CDCA (**3**). Horizontal scale: Concentration of added test compounds. Vertical scale: Relative luminescence intensity arising from luciferase reporter gene expression.

using CMX-GAL4N-hFXR as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene, and the CMX β-galactosidase gene for normalization, as previously reported.[4,12,18] Briefly, human embryonic kidney 293 cells were cultured in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections were performed by the calcium phosphate coprecipitation method. Test compounds were added 8 h after the transfection, and luciferase and β-galactosidase activities were assayed using a luminometer and microplate reader. The experiment was repeated three times, and the normalized average values are presented in Figure 3. Transcriptional activation activities (and antagonistic activities) of the test compounds toward other NRs (Figs. 4 and 7) were similarly evaluated.[4,12,18]

As shown in Figure 3, the agonistic activity of DPPF-01 (**6**) for transcriptional activation of FXR was far more potent than that of the physiological ligand, CDCA (**3**). The EC_{50} values are 3.4 μM for DPPF-01 (**6**) and 11.7 μM for CDCA (**3**) under the experimental conditions employed. DPPF-13 (**7**) also showed agonistic activity toward FXR, though it was less potent than CDCA (**3**). No apparent antagonistic activity of DPPF-13 (**7**) was observed.

The transcriptional activation activity of DPPF-01 (**6**) seems to be specific for FXR, because DPPF-01 did not activate VDR, PPARα, PPARγ, PPARδ, LXRα, RARα or retinoid X receptor α (RXRα) at 10 μM under these experimental conditions (Fig. 4). This specificity of DPPF-01 (**6**) toward FXR would be attributed to its side chain's structure which had been optimized by Maloney et al. for GW4064 (**5**).[15] The results suggest that the strategy adopted in this study of utilizing the diphenylmethane skeleton as a steroid substitute is useful to create superior/specific ligands. The computer-assisted overlaying studies of DPPF-01 (**6**) and CDCA (**3**) are under way.

Ligands for PPARα. PPARα is a NR whose physiological ligands are considered to be endogenous fatty acids, and it is well known as the target molecule of fenofibrate (**4**) (Fig. 1),[13] a drug used to treat dyslipidemia and type 2 diabetes, and whose active form is considered to be its hydrolyzed analog, fenofibric acid (FA: **13**) (Fig. 5). Various synthetic PPARα ligands have been reported, including our phenylpropionic acid derivatives derived from KCL (**14**) (Fig. 5).[18–20] PPARα has a large Y-shaped ligand-binding pocket of approximately 1300–1400 $Å^3$.[21] Based on the structures of FA (**13**) and KCL (**14**), as well as the shape of the ligand-binding pocket of PPARα and the potential usefulness of the

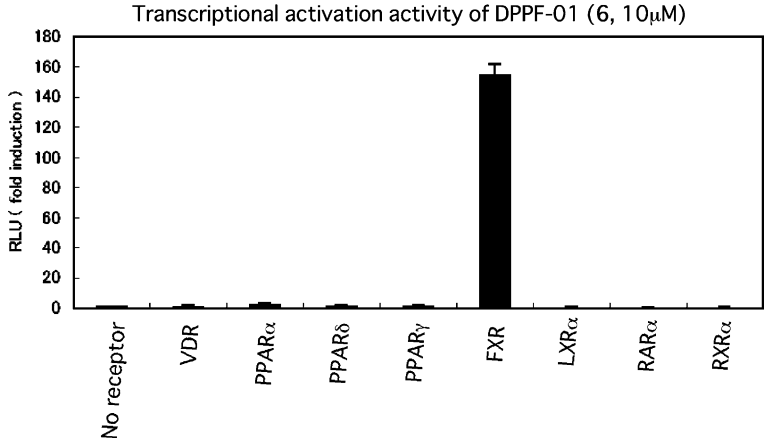


Figure 4. Transcriptional activation of various NRs by DPPF-01 (**6**). Vertical scale: Relative luminescence intensity arising from luciferase reporter gene expression.

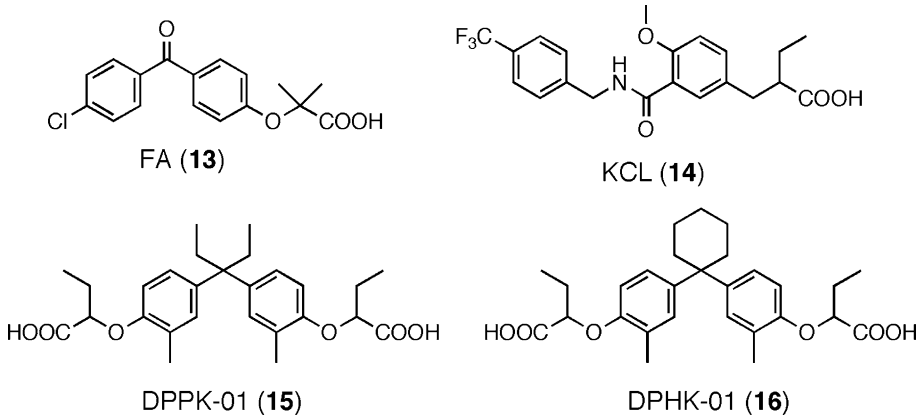


Figure 5. Structures of FA (**13**), KCL (**14**), DPPK-01 (**15**) and DPHK-01 (**16**).

diphenylmethane skeleton as a scaffold for PPARα ligands, we designed a diphenylpentane derivative DPPK-01 (**15**) and a diphenylcyclohexane derivative DPHK-01 (**16**) (Fig. 5), both of which possess a butyric acid moiety. The butyric acid moiety was introduced to mimic the carboxylic acid side chain of KCL (**14**), and a cyclohexyl moiety was adopted to provide a rigid Y-shape of the molecule, as the carbonyl group of FA (**13**) does.

DPPK-01 (**15**)[22] was prepared by reaction of a bisphenol derivative **8** with ethyl 2-bromobutylate in DMF in the presence of NaH. DPHK-01 (**16**)[23] was similarly prepared, but the starting bisphenol derivative was synthesized by condensation of cyclohexanone with *o*-cresol. FA (**13**) was generously supplied by ASKA Pharm. Co. Ltd (Japan).

The PPARα agonistic activity of DPPK-01 (**15**) and DPHK-01 (**16**) was evaluated with a reporter gene assay method similar to that adopted for evaluation of FXR agonistic activity (vide supra), except that CMX-GAL4N-hPPARα was used as the recombinant receptor gene in place of CMX-GAL4N-hFXR. The results are shown in Figures 6 and 7.

As shown in Figure 6, DPHK-01 (**16**) showed more potent agonistic activity for transcriptional activation of PPARα than did FA (**13**), the active form of fenofibrate (**4**). The EC_{50} values were 3.5 μM for DPHK-01 (**16**) and 9.2 μM for FA (**13**) under the experimental conditions used. The less rigid derivative, DPPK-01 (**15**), showed only very weak agonistic activity toward PPARα (no apparent antagonistic activity was observed). In these preliminary studies, we used diastereomeric mixtures of DPPK-01 (**15**) and DPHK-01 (**16**). Among PPARα-activating phenylpropionic acid derivatives, the isomers with *S*-configuration at the α-carbon of the propionic acid moiety have been established to be the eutomers (active isomers), while the *R*-isomers are inactive. Therefore, existence of a eutomer of DPHK-01 (**16**) that would be far more potent than FA (**13**) can be expected. Evaluation of the biological activity of the optical isomers of our compounds is in progress. The transcriptional activation activity of DPHK-01 (**16**) seems to be specific for PPARα, because DPHK-01 did not activate VDR, FXR, PPARγ, PPARδ, LXRα, RARα or RXRα at 10 μM under these experimental conditions (Fig. 7). These results again suggest the usefulness of the diphenylmethane skeleton as a multi-template for NR ligands.

In conclusion, we have prepared novel ligands for FXR, DPPF-01 (**6**), and for PPARα, DPHK-01 (**16**), based on a diphenylmethane skeleton. DPPF-01 (**6**) is more potent than the physiological ligand CDCA (**3**), and the potency of DPHK-01 (**16**) is

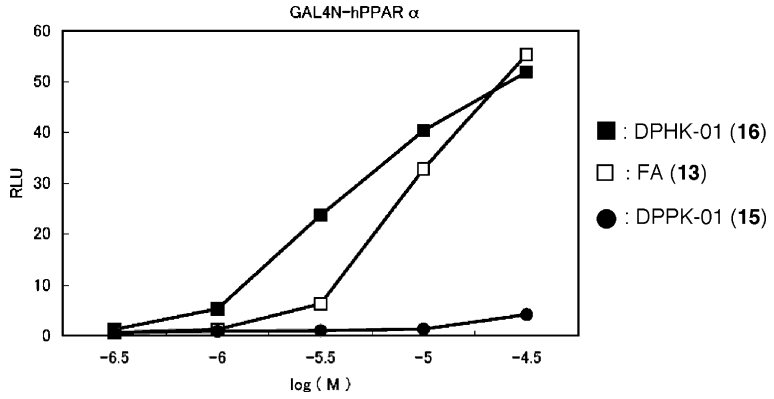


Figure 6. Transcriptional activation of PPARα by DPPK-01 (**15**), DPHK-01 (**16**) and FA (**13**). Horizontal scale: Concentration of added test compounds. Vertical scale: Relative luminescence intensity arising from luciferase reporter gene expression.

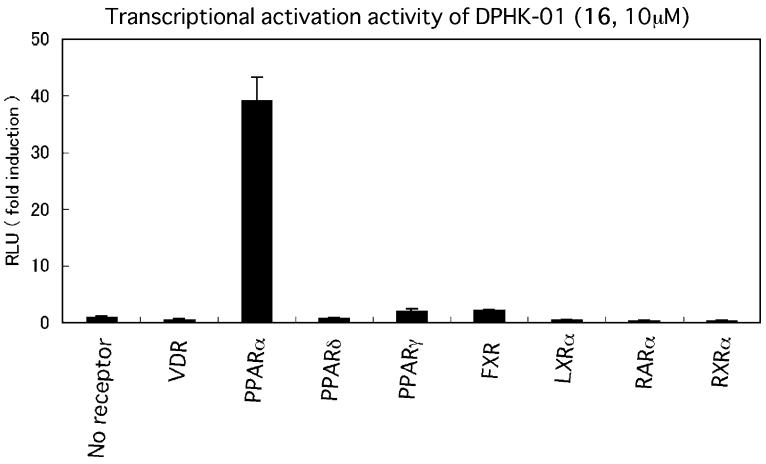


Figure 7. Transcriptional activation by DPHK-01 (**16**) toward various NRs. Vertical scale: Relative luminescence intensity arising from luciferase reporter gene expression.

comparable to that of FA (**13**), the active form of the antihyperlipidemic drug fenofibrate (**4**). These results indicate that a diphenylmethane skeleton is useful as a steroid skeleton substitute and a multi-template for NR ligands. It was also demonstrated that DPPF-01 (**6**) and DPHK-01 (**16**) are highly specific for FXR and PPARα, respectively. It is well documented that ligands with a steroidal skeleton often show cross reactivity with several NRs. For example, lithocholic acid acts as a ligand for both VDR and FXR.[4,12] Application of a diphenylmethane skeleton as a scaffold of NR ligands might overcome this problem. In addition, NR ligands with a diphenylmethane skeleton might elicit selectivity on cell-type/co-factor(s) which is different from that of the physiological ligands. In this preliminary study, we examined only the diphenylmethane skeleton. However, it is very likely that hetero-atom analogs of a diphenylmethane skeleton, such as diphenylamine (as found in some RAR/RXR ligands)[7,24] and diphenylether (as found in thyroxine hormones),[25] would also available as substitutes for a steroid skeleton. Further structural development studies of the present ligands and synthesis of candidate NR ligands based on the above 'diphenyl X' skeletons are under way.

Acknowledgments

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16. DPPF-01 (**6**): MS (FAB) *m/z* 610 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3/\delta$): 7.38 (d, $J = 8.0$ Hz, 2H), 7.33–7.31 (m, 1H), 6.93 (s, 2H), 6.88–6.83 (m, 2H), 6.63–6.59 (m, 2H), 4.68 (s, 2H), 4.64 (s, 2H), 3.40–3.30 (m, 1H), 2.22 (s, 3H), 2.01–1.97 (m, 7H), 1.41 (d, $J = 6.7$ Hz, 6H), 0.57 (t, $J = 7.3$ Hz, 6H). HRMS (FAB, $M+H^+$) calcd. for $C_{34}H_{38}Cl_2NO_5$, 610.2127, found 610.2119.
17. DPPF-13 (**7**): MS (FAB) *m/z* 625 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3/\delta$) (racemate): 7.38 (d, $J = 8.0$ Hz, 2H), 7.33–7.29 (m, 1H), 6.91–6.82 (m, 4H), 6.59 (d, $J = 7.9$ Hz, 1H), 6.53 (d, $J = 7.9$ Hz, 1H), 4.68 (s, 2H), 4.00 (br, 1H), 3.83–3.80 (m, 1H), 3.69–3.66 (m, 1H), 3.34–3.30 (m, 3H), 3.24–3.20 (m, 1H), 2.10 (s, 3H), 2.00–1.96 (m, 7H), 1.41 (d, $J = 6.7$ Hz, 6H), 0.57 (t, $J = 7.3$ Hz, 6H). HRMS (FAB, $M+H^+$) calcd. for $C_{35}H_{43}Cl_2N_2O_4$, 625.2600, found 625.2597.

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22. DPPK-01 (**15**): MS *m*/*z* 456 $(M)^+$. ^1H NMR (500 MHz, $CDCl_3$/δ) (diastereomeric mixture) 6.93 (m, 2H), 6.87 (m, 2H), 6.54 (m, 2H), 4.58 (m, 2H), 2.23 (m, 6H), 2.0 (m, 8H), 1.11 (m, 6H), 0.58 (m, 6H). HRMS (FAB, M^+) calcd. for $C_{27}H_{36}O_6$, 456.2512, found 456.2509.
23. DPHK-01 (**16**): MS *m*/*z* 468 $(M)^+$. ^1H NMR (500 MHz, $CDCl_3$/δ) (diastereomeric mixture): 7.06 (m, 2H), 6.95 (m, 2H), 6.58 (m, 2H), 4.58 (m, 2H), 2.24 (m, 6H), 2.17 (m, 4H), 2.02 (m, 4H), 1.47 (m, 6H), 1.09 (m, 6H). HRMS (FAB, M^+) calcd. for $C_{28}H_{36}O_6$, 468.2512, found 468.2541.
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