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New PPARγ ligands based on 2-hydroxy-1,4-naphthoquinone: Computer-aided design, synthesis, and receptor-binding studies

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Abstract—FlexX-based molecular docking study was employed to identify 2-hydroxy-1,4-naphthoquinone as a new 'acidic head group' for the design of a novel series of PPARγ ligands. To provide the proof of concept, designed molecules were synthesized and evaluated in a standard radioligand-binding assay. Out of eight molecules, four were found to bind to the murine PPARγ with IC_{50} ranging from 0.2 to 56.2 μM as compared to standard pioglitazone, with IC_{50} of 0.7 μM.

Peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear hormone receptor (NHR) superfamily.[1] Three subtypes, PPARα, PPARγ, and PPARδ for this receptor have been discovered and identified as important targets for the treatment of metabolic disorders such as type 2 diabetes, dyslipidemia, atherosclerosis, etc.[2] PPARγ first came into the picture when it was established as the target for the thiazolidinediones (TZD) class of insulin sensitizers,[3] synthesized in the early 1980s.[4] The molecules belonging to the fibrate class of drugs, such as clofibrate (**1**) and fenofibrate (**2**), are known to act as PPARα agonists. Recently, PPARδ is also being studied as the target for the treatment of obesity.[5] Out of the three isoforms, PPARγ and its agonists have been studied extensively. Recently, the role of PPARγ has been established in the treatment of inflammatory conditions and cancer, thus, providing more therapeutic value to this target.[6] Currently, rosiglitazone (**3**) and pioglitazone (**4**) are being used clinically (Fig. 1). However, in a recent study, rosiglitazone has been found to increase the incidences of heart attack among patients.[7] Thus, there is scope to discover novel and safer molecules as PPARγ agonists. In continuation of our previous work on PPAR ligands,[8] we hereby report the computer-aided design (CAD) and synthesis of a novel series of PPARγ ligands.

The crystal structure of PPARγ with co-crystallized rosiglitazone molecule showed that the TZD ring makes H-bonds with His323, Tyr473, Ser289 and His449 which are important for the activity.[9] This is in accordance with structure–activity relationship studies, which show that the acidic hydrogen of TZD is vital for the biological activity. Hence, a variety of 'acidic head groups' such as free carboxyl group, oxazolidinedione, tetrazoles, etc., were successfully used to replace the TZD ring to obtain a new class of such agents.[10] However, most of the PPARγ agonists described in the literature belong to either the 'glitazone' class (possessing a TZD ring) or to the 'glitazar' class (possessing free carboxylic acid). Many of these ligands are associated with adverse effects such as weight gain, edema, carcinogenicity, etc.[7,11] Thus, we recognized the importance of designing new series of PPAR ligands by exploring new 'acidic head groups'. We employed molecular docking as a computational tool for this purpose.

To start with, a few molecules were designed by replacing the 'acidic head group' of farglitazar (**5**), a PPARα/γ dual agonist, with a number of acidic moieties. However, the lipophilic group was kept same as that

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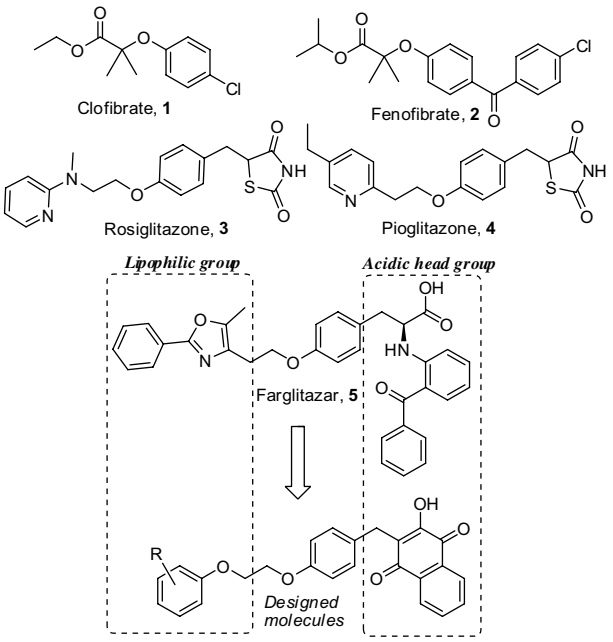


Figure 1. Structures of a few PPAR ligands and the design of new series of PPARγ ligands based on 2-hyroxy-1,4-naphthoquinone (HNQ).

of farglitazar in these molecules. These virtual molecules were then docked in the active site of PPARγ (PDB code 1FM9) using the FlexX algorithm implemented in Sybyl 6.9 and analyzed for binding interactions.[12] This study showed that a number of acidic moieties can maintain the important H-bonding interactions with the PPARγ active site as exhibited by the TZD ring and can act as 'acidic head group' for the design of novel series. One of such moieties was 2-hydroxy-1,4-naphthoquinone (HNQ) ring (Fig. 1), which was preferred for further studies over others on the basis of its commercial availability, low cost, and easy derivatization. Thus, different derivatives of HNQ ring with a variety of lipophilic groups were designed as potential PPARγ agonists. One of the designed molecules docked in the active site of PPARγ (1FM9) is shown in Figure 2 which was found to maintain the H-bonding interactions with His323, Tyr327, and Ser289 in the PPARγ active site, important for the receptor-binding and activation.

It was decided to synthesize a few molecules in order to verify the docking results. The first step in the synthetic strategy was the O-alkylation of *p*-hydroxy benzaldehyde (**6**) with 1,2-dibromoethane (**7**) to yield the monobromo derivative (**8**) and also with benzyl bromide and 2-fluoro benzyl bromide to yield aldehydes **9a** and **9b** (Scheme 1). The monobromo aldehyde (**8**) was further used to alkylate different phenols to yield aldehydes **9c–h**. These were then reduced to benzyl alcohols, and further converted to the corresponding benzyl bromide derivatives using well-known procedures. In the final step, the benzyl bromide derivatives were used to alkylate the 2-hydroxy-1,4-naphthoquinone with a reported procedure[13] to yield the final products **11a–h**. In all cases, the isomeric O-alkylated product was also formed, which was separated from the desired C-alkylated product by column chromatography. All the final products were characterized using ^{1}H NMR, ^{13}C NMR, and mass spectrometry.[14]

Finally, molecules **11a–h** were evaluated for their ability to bind to the murine PPARγ using a standard receptor-

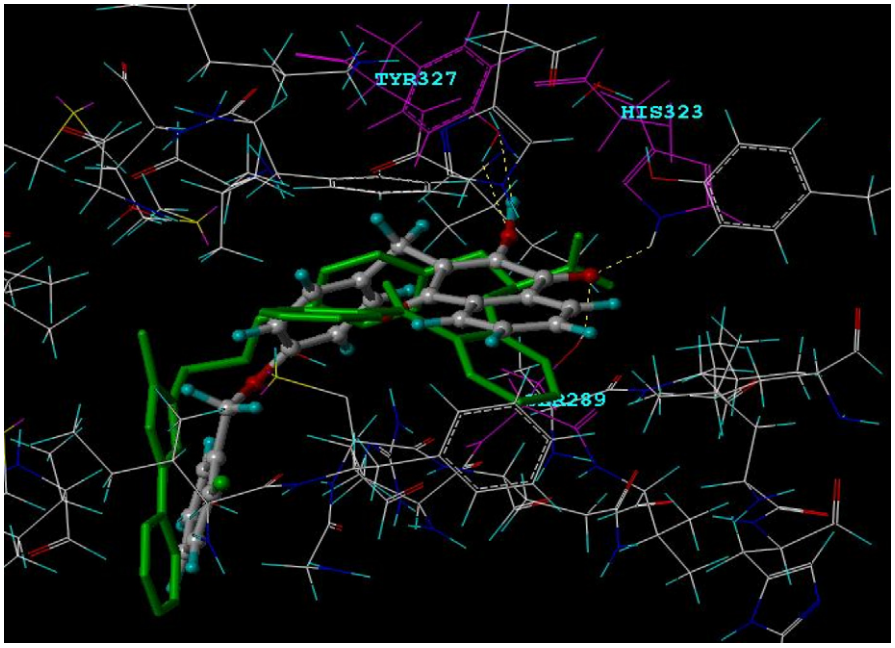
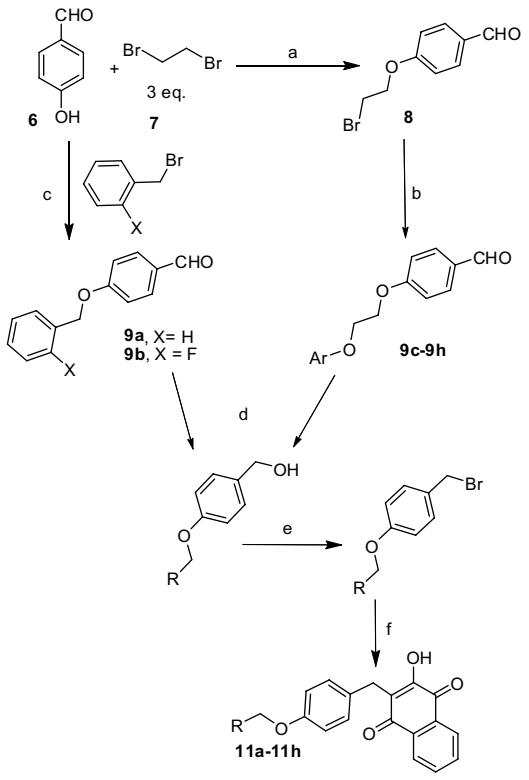


Figure 2. Predicted binding mode of the most potent compound (**11b**, ball and stick model) in the active site of PPARγ (PDB code 1FM9). The cocrystallized ligand (**5**, farglitazar) is shown in green color for comparison. The interacting amino acids (Tyr327, His323, and Ser289) are shown in magenta color and H-bonds are represented with yellow dashed lines.

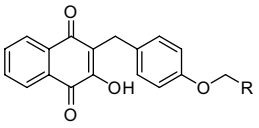


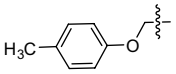
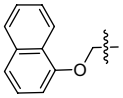
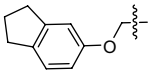
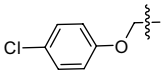
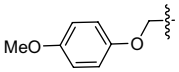
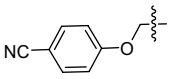
Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C, 5 h, 50%; (b) ArOH, K_2CO_3, DMF, reflux, 2–4 h, 84–93%; (c) K_2CO_3, DMF, reflux, 2–4h, 64–85%; (d) $NaBH_4$, MeOH–THF, 0 °C–RT, 0.5–1 h, 90–95%; (e) $SOBr_2$, Toluene, 0 °C–RT; (f) 2-hydroxy-1,4-naphthoquinone (HNQ), Li_2CO_3, DMF, 80 °C, 15–25%.

binding assay, with pioglitazone as a reference standard.[15] Interestingly, out of the eight molecules, four were found to be active in this assay and one molecule (**11b**) was found to possess IC_{50} value of 0.2 μM (Table 1), comparable to the reference molecule. However, only one molecule (**11a**) inhibited nearly 100% binding of the radioligand [^{3}H]-rosiglitazone, while others were found to be partial inhibitors (Fig. 3). Addition of a 'F' atom in the lipophilic group of **11a** improved the binding strength tremendously in **11b**. Molecules with bulky lipophilic group (**11d** and **11e**) were found to be inactive, possibly due to unfavorable steric clashes with the amino acid residues in the active site. Nonetheless, these experimental results verify the molecular docking studies and demonstrate that the HNQ ring can actually be employed as the 'acidic head group' for the design of novel class of PPAR ligands.

In summary, using docking studies we have identified a novel series of PPAR ligands based on 2-hydroxy-1,4-naphthoquinone, as a 'acidic head group'. The designed molecules were predicted to retain the H-bonding interactions important for the receptor activation. Preliminary synthetic and receptor binding studies were undertaken to provide the experimental evidence. The synthesized molecules were indeed found to bind to the receptor with IC_{50} comparable to the reference standard, pioglitazone. Further structure–activity relationship studies for this series are in progress.

Table 1. Structures of final molecules with corresponding IC_{50} values



Compound	R	IC_{50} (μM)
11a[a]		3.6
11b		0.2
11c		i.a.
11d		i.a.
11e		i.a.
11f		i.a.
11g		56.2
11h		1.9

Pioglitazone (reference standard) IC_{50} = 0.7 μM. i.a., inactive (less than 20% inhibition at 10^{-7} M).

[a] Inhibited nearly 100% binding of [^{3}H]-rosiglitazone.

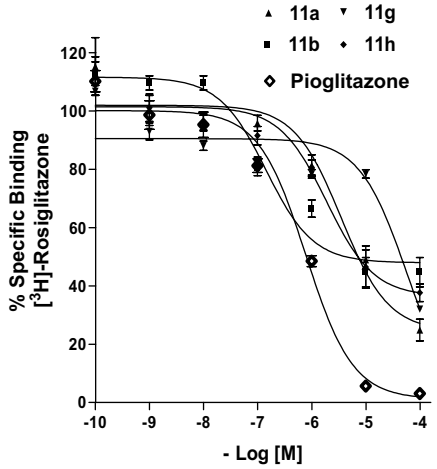


Figure 3. Plot of specific binding versus concentration of the active PPARγ ligands.

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14. *Spectral data of a representative molecule* (**11b**)*:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13–8.05 (m, 2H), 7.74–7.66 (m, 2H), 7.50–7.46 (m, 2H), 7.36 (d, J = 8.71 Hz, 2H), 7.16–7.01 (m, 2H), 6.90 (d, J = 8.61 Hz, 2H), 5.09 (s, 2H), 3.90 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.4, 181.7, 162.0, 158.8, 157.1, 152.9, 134.9, 132.9, 131.3, 130.2, 129.7, 129.4, 126.9, 126.1, 124.4, 123.5, 115.4, 115.1, 63.7, 28.2; IR (KBr) 3334, 2955, 2925, 2853, 1729, 1660, 1509, 1369, 1179; MS (MALDI) *m*/*z* 389.1 $[M+H]^+$.
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