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Design and synthesis of 6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid derivatives as PPARγ activators

Rakesh Kumar,* Amit Mittal and Uma Ramachandran

Department of Pharmaceutical Technology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, Punjab 160 062, India

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Abstract—The design and synthesis of novel series of 6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid (pyrimidone) derivatives that are high affinity ligands for peroxisome proliferators activated receptor γ have been reported as a potential substitute of 2,4-thiazolidinedione head group. The FlexX docking and radioligand binding affinity of some promising compounds of this series is comparable to that of thiazolidinedione based antidiabetic drugs currently in clinical use.

Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetics.[1] It is predicted that world's diabetic population could rise to 220 million by the year 2010 partly due to a dramatic increase in the incidence of obesity and a sedentary lifestyle.[2] T2DM is a metabolic disorder which is associated with three basic pathophysiological abnormalities: impaired insulin secretion, excessive hepatic glucose production, and insulin resistance in skeletal muscle, liver, and adipose tissue.[3] It is now clear that aggressive control of hyperglycemia in patients with diabetes can prevent or delay the onset of complications such as retinopathy, nephropathy, and neuropathy.[4]

The peroxisome proliferators activated receptor γ (PPARγ) is a member of nuclear hormone receptor superfamily of ligand dependent transcription factors, which play a pivotal role in regulating adipogenesis, insulin sensitivity and glucose homeostasis.[5] Synthetic agonists of PPARγ including pioglitazone and rosiglitazone, had been proved clinically beneficial in decreasing the elevated plasma glucose levels in T2DM. However, edema and weight gain have been reported in patients after treatment with some of PPARγ agonists. It is unclear whether the side effects observed are PPAR receptor mediated or compound mediated.[6,7]

Therefore, there continues to be interest in new compounds for clinical development. This necessitated developing new antihyperglycemic agents that could be highly effective, safe, and devoid of side effects. This provides an opportunity to bring diverse class of ligands that could normalize both insulin and glucose levels (Chart 1).

The compounds of this class have few essential pharmacophoric elements. These comprise of an acidic group linked to a central flat ring and a large lipophilic substructure. Based on the crystal structure analysis and molecular modeling studies, a common U-shaped pharmacophore has been derived for PPARγ agonists. The thiazolidinedione (TZD) head group is anchored by four important H-bonds in the active site Ser289, His323, His449, and Tyr473.[8,9]

Current research trend has shifted toward non-thiazolidinedione insulin sensitizers. Bioisosteric replacement of thiazolidinedione by α-carbon substituted carboxylic acid, oxazolidine-2,4-dione, tetrazole, oxathiazole, carbonylated hydroxyurea, etc. had been reported in the literature.[10–15]

Earlier, we reported carbazole derivatives with the acyclic acidic head groups as PPARα/γ dual agonists with antioxidant property.[16,17] Sterically hindered open chain acid derivatives have been found efficient PPAR activators.[18] Recently compounds with pyran head instead of TZD were identified as novel PPARγ agonists.[19] The lipophilic fragment of promising compounds was

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* Corresponding author. Present address: 932, Sector 21, Gurgaon, Haryana, India. Tel.: +91 1722214682; e-mail: rakvats@gmail.com

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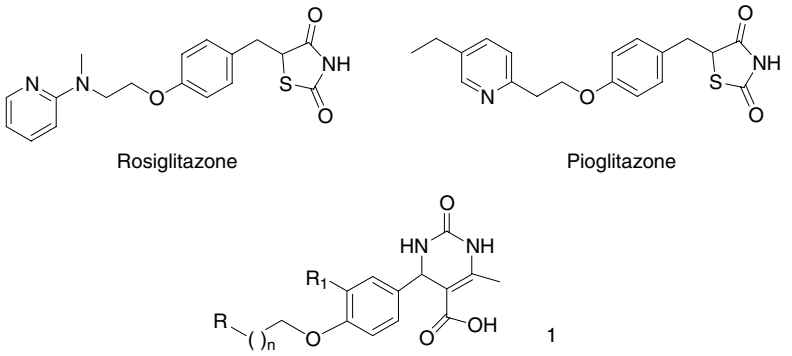


Chart 1. PPARγ agonists.

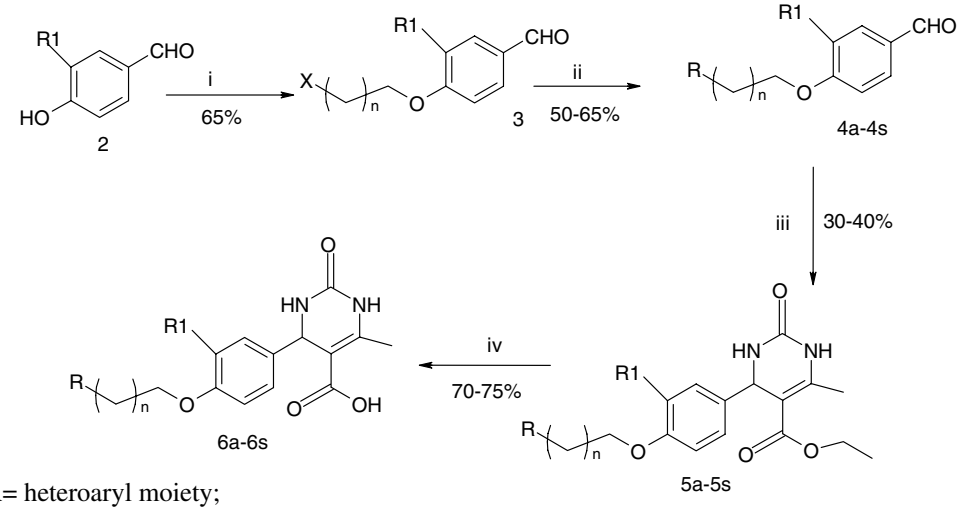
conjugated with a six-membered tetrahydropyrimidone group which is bioisosteric to thiazolidinedione with additional carbonyl functionality.

The prototype structures **1** are pyrimidone derivatives that are replacement for TZD group to develop new chemical entity that may retain the antihyperglycemic activity.

The molecular docking studies were performed on 1FM9 protein, viz. PPARγ employing the FlexX docking procedure using Sybyl 6.9 program installed on silicon graphics Octane2 workstation. FlexX is a fast automated program based on incremental construction procedure.[20] In this method flexibility of the ligands is considered including several conformations of ligands while maintaining a rigid structure for the biomolecule. Rosiglitazone was docked into the active site of the receptor to judge the discriminatory strength of the docking procedures and the choice of the crystal structure. The active sites were assigned at a radius of 8 Å around the reference ligand. FlexX run was submitted and the docking scores were obtained and analyzed. In the subsequent synthesis and biological evaluation of hit compounds, we found compounds **6a** to **6s** as high affinity ligands for PPARγ.

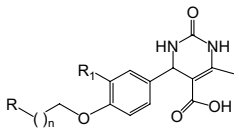
In this publication, we disclose the synthesis, in silico and in vitro evaluation of pyrimidone class of compounds for the first time. Further, we applied a receptor based approach to the validation of our hypothesis regarding substitution of pyrimidone moiety in place of TZD. This approach essentially searches for a ligand whose orientation and conformation achieve the highest degree of complementarity with respect to all details of the receptor's steric constraints and interaction geometrics.

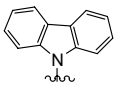
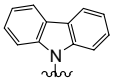
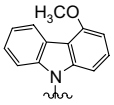
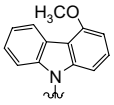
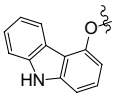
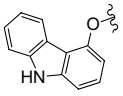
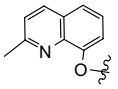
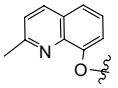
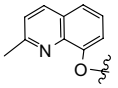
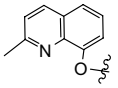
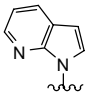
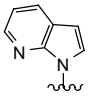
The synthesis of 6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid derivatives **6a** to **6s** is depicted in Scheme 1. Commercially available 1,2-dibromoethane ($n = 1$); 1-bromo-3-chloropropane ($n = 2$) were refluxed with 4-hydroxy benzaldehyde or 4-hydroxy-3-methoxybenzaldehyde (**2**) in acetone in presence of anhydrous K_2CO_3 as base to give compound **3** in good yield. The reaction mixture was concentrated and extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, and concentrated. Further



Scheme 1. Reagents and conditions: (i) 1,2-dibromoethane ($n = 1$); 1-bromo-3-chloropropane ($n = 2$), X = Cl, Br , anhydrous K_2CO_3, acetone, 70 °C, 12 h; (ii) heteroaryl moiety (R), NaH, THF, 0 °C to rt, 4 h or heteroaryl moiety (R), K_2CO_3, acetone, 70 °C, 15 h; (iii) ethyl acetoacetate, urea, zinc triflate, acetonitrile, 80 °C, 12 h; (iv) 20% NaOH, EtOAc, rt, 2 h.

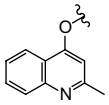
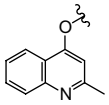
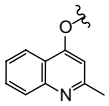
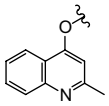
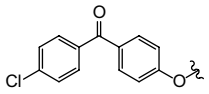
Table 1. Compounds with their FlexX Docking scores in 1FM9 (PPARγ) and Radioligand binding affinities of pyrimidone derivatives



S. No.	Compound	R	*n*	R^1	FlexX score	Radioligand binding affinity[a] IC_{50} (μM)
1	**6a**		1	H	−18.1	0.9
2	**6b**		1	OCH_3	−17.7	0.82
3	**6c**		1	H	−16.1	0.8
4	**6d**		1	OCH_3	−14.8	ND
5	**6e**		1	H	−23.7	1.0
6	**6f**		2	H	−20.8	0.9
7	**6g**		2	H	−16.4	ND
8	**6h**		2	OCH_3	−14.5	ND
9	**6i**		1	H	−19.5	0.6
10	**6j**		1	OCH_3	−18.8	0.8
11	**6k**		2	H	−19.4	0.75
12	**6l**		2	OCH_3	−16.1	ND
13	**6m**		1	H	−21.4	1.0
14	**6n**		1	OCH_3	−17.6	0.72

(*continued on next page*)

Table 1 (*continued*)

S. No.	Compound	R	*n*	R^1	FlexX score	Radioligand binding affinity[a] IC_{50} (μM)
15	**6o**		1	H	−18.1	0.76
16	**6p**		1	OCH_3	−21.2	0.94
17	**6q**		2	H	−19.8	0.75
18	**6r**		2	OCH_3	−15.8	ND
19	**6s**		1	H	−21.7	0.93

Note. The 3D coordinates of the active sites were taken from the X-ray crystal structures of PPARγ proteins reported as complexes with their corresponding agonists, Farglitazar deposited in Brookhaven Protein Data Bank with PDB code 1FM9.

[a] IC_{50} is the concentration of test compound required to inhibit 50% of the specific binding of the radioligand [^{3}H]-rosiglitazone. IC_{50} of the reference Pioglitazone is 0.8 μM.

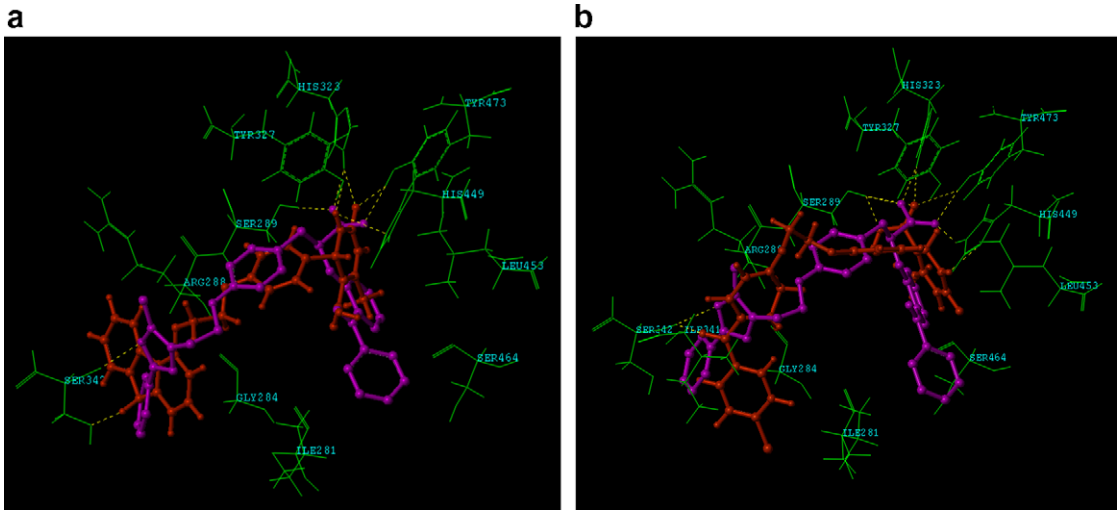


Figure 1. (a) Compound **6e** with PPARγ (1FM9). (b) Compound **6s** with PPARγ (1FM9). The molecules in pink are the reference ligands found as complexes with the crystal structures (farglitazar in 1FM9).

the product was crystallized with dichloromethane and hexane. The different heteroaryl moieties were then condensed with **3** using NaH in THF at 0 °C to rt for 4 h or K_2CO_3 in acetone at 70 °C for 15 h to furnish corresponding aldehydes **4a** to **4s**. These latter aldehydes were refluxed under Biginelli pyrimidone reaction condition utilizing ethyl acetoacetate and urea with zinc triflate as catalyst in acetonitrile solvent to give 6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester derivatives **5a** to **5s**. The compounds **5a** to **5s** were hydrolyzed in presence of 20% aqueous NaOH and ethyl acetate as solvent to give corresponding acid derivatives **6a** to **6s**. The structures of all the synthesized compounds were confirmed by spectral analysis techniques.

The newly synthesized compounds **6a** to **6s** were evaluated in the PPAR Scintillation Proximation Assay binding assay to ascertain γ binding profiles. Radioligand binding assay: The newly synthesized compounds for PPAR receptor are not available in a radioactive form (unlabeled). The affinity of the unlabeled ligand can be determined indirectly by measuring its ability to compete with the radiolabeled ligand ([^{3}H]-rosiglitazone) for the receptor (PPARγ). In the competition experiment, various concentrations of unlabeled ligands were

allowed to compete with a fixed concentration of a radiolabeled ligand, [^{3}H]-rosiglitazone (40 nM), for the PPARγ binding.[21] As the concentration of unlabeled ligand increased, the amount of [^{3}H]-rosiglitazone bound to the receptor decreased. A sigmoidal curve was plotted as percent inhibition of [^{3}H]-rosiglitazone vs log concentrations of compounds and IC_{50} (concn that inhibits 50% of [^{3}H]-rosiglitazone binding) value for each compound was found by nonlinear regression curve fitting using Graph Pad Prism software.

From the radioligand binding assay, it was found that compounds **6a** to **6s** could inhibit the [^{3}H]-rosiglitazone binding to PPARγ in a dose dependent manner similar to the commercially available PPARγ agonist, pioglitazone (reference standard). The expansion of SAR was done by investigating effects of replacing thiazolidinedione group with pyrimidone, so that they may modulate the binding with the receptor. Molecular docking studies have been performed on all the molecules in order to study their binding mode in the active sites of PPARγ. The docking results have been analyzed in terms of FlexX docking scores obtained by the molecules (using the Bohm scoring function, employed within FlexX). The scores of compounds **6a** to **6s** are better in active sites of the receptor and also showing good binding affinity.

Molecular docking studies were carried out on the synthesized compounds to get insight about their binding preferences at the active site of the receptor. Rosiglitazone when docked in the active site of PPARγ receptor attained a score of −15.5 kcal/mol. It showed the important H-bonding interactions with the residues at the active site of PPARγ. These results are on the expected lines as rosiglitazone is a PPARγ agonist. The FlexX docking scores of the hit compounds are listed in Table 1.

From observed results, the following conclusions can be drawn: The most active compound **6e** fitted the best in the active site of PPARγ and attained the best score of −23.7 kcal/mol amongst all the molecules. It showed all the prime interactions to anchor well in the active sites of the receptor (Fig. 1a). The 4-methoxy carbazole derivative **6c**, the oxygen of methoxy group shows the hydrogen bonding with Ser342 and water molecule which is in close proximity. The pyrimidone carboxylic acid group of **6c** shows double hydrogen bonding with Tyr327.

In substituted 4-hydroxycarbazole compound **6e**, there is additional hydrogen bonding with NH of carbazole ring with Ser342 and water molecule. In compound **6f** carbon chain linker is varied from 2 to 3 between the hydrophobic region and the central aromatic unit. It also shows additional hydrogen bonding with NH of carbazole and Ser342 and water molecule. Compound **6s**, where 4-chlorobenzophenone moiety is hydrophobic head, shows almost same interaction of middle carbonyl group oxygen with Ser342 and water (Fig. 1b). The extra double hydrogen bonding is seen in **6s**, between pyrimidone carboxylic acid group and Leu453. Similar interaction in the other analogues has been observed.

All the compounds with methoxy substituted central ring have lower score than corresponding unsubstituted compounds except for **6p**. The reason for the better activity of 4-hydroxy substituted carbazole derivatives over compounds with N-substitution can be attributed to the fact that the 4-hydroxy substituent orients its oxygen such that there is an additional H-bonding interaction that is observed with this oxygen atom and a water molecule lying in close vicinity. Hence the strategic position of this group is important for its activity. Thus with these studies we could get some insights into the nature of binding of the ligands and these studies can be applied for designing better molecules. Some of representative compounds having better docking score **6a** to **6s** were synthesized and radioligand binding assay was performed. It has been observed that all the synthesized compounds have inhibited [^{3}H]-rosiglitazone binding to PPARγ in dose dependent manner.

As a part of our ongoing efforts to find a potential PPAR agonist, 6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid derivatives have been studied for the first time and are found to be high affinity ligands for PPARγ. Further advanced pharmacological studies on these compounds are underway.

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Supplementary data

Experimental details of synthesis and biological evaluation are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.081.

References and notes

1. Joe, M. C.; Arshag, D. M. *Drugs* **2000**, *60*, 95.
2. Rotella, D. P. *J. Med. Chem.* **2004**, *47*, 4111.
3. Ramarao, P.; Kaul, C. L. *Drugs Today* **1999**, *35*, 895.
4. Skyler, J. S. *J. Med. Chem.* **2004**, *47*, 4113.
5. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
6. Ramachandran, U.; Kumar, R.; Mittal, A. *Mini-rev. Med. Chem.* **2006**, *6*, 563.
7. Miller, A. R.; Etgen, G. J. *Expt. Opin Investig. Drugs* **2003**, *12*, 1489.
8. Khanna, S.; Sobhia, M. E.; Bharatam, P. V. *J. Med. Chem* **2005**, *48*, 3015.
9. Ramachandran, U.; Mital, A.; Bharatum, P. V.; Khanna, S.; Rao, P. R.; Srinivasan, K.; Kumar, R.; Chawla, H. P. S.; Kaul, C. L.; Raichur, S.; Chakrabarti, R. *Bioorg. Med. Chem.* **2004**, *12*, 655.
10. Kumar, R.; Ramachandran, U.; Khanna, S.; Bharatam, P. V.; Raichur, S.; Chakrabarti, R. *Bioorg. Med. Chem.* **2007**, *15*, 1547.

11. Haigh, D.; Birell, H. C.; Cantello, B. C. C.; Hindley, R. M.; Ramaswamy, A.; Rami, H. K.; Stevens, N. C. *Tet. Asymm.* **1999**, *10*, 1335.
12. Momose, Y.; Maekawa, T.; Yamano, T.; Kawada, M.; Odaka, H.; Ikeda, H.; Sohda, T. *J. Med. Chem.* **2002**, *45*, 1518.
13. Kim, B. Y.; Ahn, H. W.; Kang, S. K.; Lee, J. H.; Shin, J. S.; Ahn, S. K.; Hong, C.; Yoon, S. S. *Eur. J. Med. Chem.* **2004**, *39*, 433.
14. Adams, A. D.; Yuen, W.; Hu, Z.; Santini, C.; Jones, A. B.; MacNaul, K. L.; Berger, J. P.; Doebber, T. W.; Moller, D. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 931.
15. (a) Lu, Y.; Guo, Z.; Guo, Y.; Feng, J.; Chu, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 915; (b) Neogi, P. Novel compounds to treat diabetes and associated conditions. WO **2001,** 01/34094.
16. Kumar, R.; Ramachandran, U.; Srinivasan, K.; Rao, P. R.; Raichur, S.; Chakrabarti, R. *Bioorg. Med. Chem.* **2005**, *13*, 4279.
17. Ramachandran, U.; Kumar, R.; Mital, A.; Ramarao, P.; Srinivasan, K.; Dey, C.S.; Ishrath, A.; Chawla, H.P.S.; Kaul, C.L.; WTO Patent **2003**, DEL/1268.
18. Cvetovich, R. J.; Chung, J. Y. L.; Kress, M. H.; Amato, J. S.; Matty, L.; Weingarten, M. D.; Tsay, F. R.; Li, Z.; Zhou, G. *J. Org. Chem.* **2005**, *70*, 8560.
19. Sharon, A.; Pratap, R.; Vatsyayan, R.; Maulik, P. R.; Roy, U.; Goel, A.; Ram, V. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3356.
20. (a) Nolte, R. T.; Wisley, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. *Nature* **1998**, *395*, 137; (b) Cronet, P.; Petersen, J. F. W.; Folmer, R.; Blomberg, N.; Sjoblom, K.; Karlsson, U.; Lindstedt, E.-L.; Bamberg, K. *Structure* **2001**, *9*, 699.
21. Arun, K. H. S.; Kaul, C. L.; Ramarao, P. *Cardiovas. Res.* **2005**, *65*, 374.