Bioorganic & Medicinal Chemistry Letters 18 (2008) 4959–4962

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

New PPARγ ligands based on barbituric acid: Virtual screening, synthesis and receptor binding studies

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ARTICLE INFO

Article history:
Received 15 May 2008
Revised 7 August 2008
Accepted 11 August 2008
Available online 14 August 2008

Keywords:
PPARγ
Virtual screening
Docking
Diabetes
Barbituric acid
Rosiglitazone

ABSTRACT

A new series of PPARγ ligands based on barbituric acid (BA) has been designed employing virtual screening and molecular docking approach. To validate the computational approach, designed molecules were synthesized and evaluated in in vitro radioligand binding studies. Out of the total 14 molecules, 6 were found to bind to the murine PPARγ with IC_{50} ranging from 0.1 to 2.5 μM as compared to reference standard, pioglitazone (IC_{50} = 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 identified and found to be important targets for the treatment of type 2 diabetes, dyslipidemia, atherosclerosis, etc.[2] The molecules belonging to fibrate class of drugs, such as clofibrate (**1**) and fenofibrate (**2**), are known to act as PPARα agonists (Scheme 1).[3] Thiazolidinediones (TZDs) class of insulin sensitizers, synthesized in early 1980s,[4] were later found to mediate hypoglycaemic effect through PPARγ.[5] Currently, rosiglitazone (**3**) and pioglitazone (**4**) are clinically available TZDs for the treatment of type 2 diabetes. Recently, PPARδ is also being studied as a target for the treatment of obesity.[6] Out of the three isoforms, PPARγ and its agonists have been studied extensively. In addition, the beneficial effects of PPARγ ligands have also been established for the treatment of inflammatory conditions and cancer.[7] However, many of the reported PPARγ ligands are associated with serious adverse effects such as fluid retention, weight gain, pro-carcinogenicity and hepatotoxicity.[8] Recently, use of rosiglitazone has been shown to be correlated with the increased incidences of heart attacks in patients.[8c] Thus, continued efforts are required to design and discover novel and safe ligands for these therapeutically valuable targets. The multiple activating ligands such as PPARα/γ, PPARγ/δ, PPARα/γ/δ and selective PPAR modulators (SPPARMs) are also being developed in this regard.[9] In continuation of our programme on design and synthesis of new PPAR agents,[10] we hereby report a virtual screening approach for this purpose, together with the experimental results.

A survey of various classes of PPARγ agonists, 3D-QSAR studies and crystal structure information reveals that the pharmacophoric features of these agents essentially consists of three parts: (i) an acidic head group, (ii) central aromatic region and (iii) a lipophilic side chain (Fig. 1).[11] The TZD ring of rosiglitazone is reported to make three important H-bonds with His323, Tyr473 and His449 that are important for the activation of the receptor.[11b] This observation was instrumental in the design of a variety of non-TZD PPAR agonists based on free carboxyl group, oxazolidinedione, tetrazoles, etc.[12] However, such reports are limited and most of the reported PPARγ agonists still belong to either 'glitazone' class (possessing TZD ring) or 'glitazar' class (possessing free carboxylic acid). Thus, we set our objective to explore newer acidic head groups for designing novel series of PPARγ ligands. It was realized that virtual screening, an important computational technique that can help in discovering newer scaffolds with desired features, can be employed for this purpose.[13] In fact, one of the recent reports suggests that this approach can be successfully applied for the discovery of novel PPARγ agonists.[14]

To start with, the bioactive (co-crystallized) conformation of rosiglitazone was extracted from the protein crystal structure (PDB code 2PRG) and a query was generated using the 'View Hypothesis' workbench of Catalyst program.[15] Based on the ligand-protein interactions, different features were assigned to

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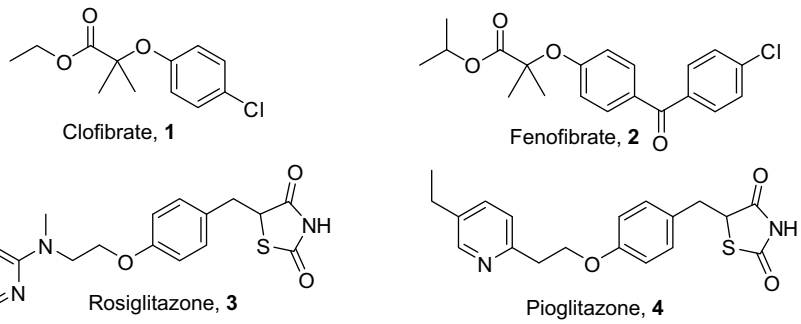
doi:10.1016/j.bmcl.2008.08.028



Scheme 1.

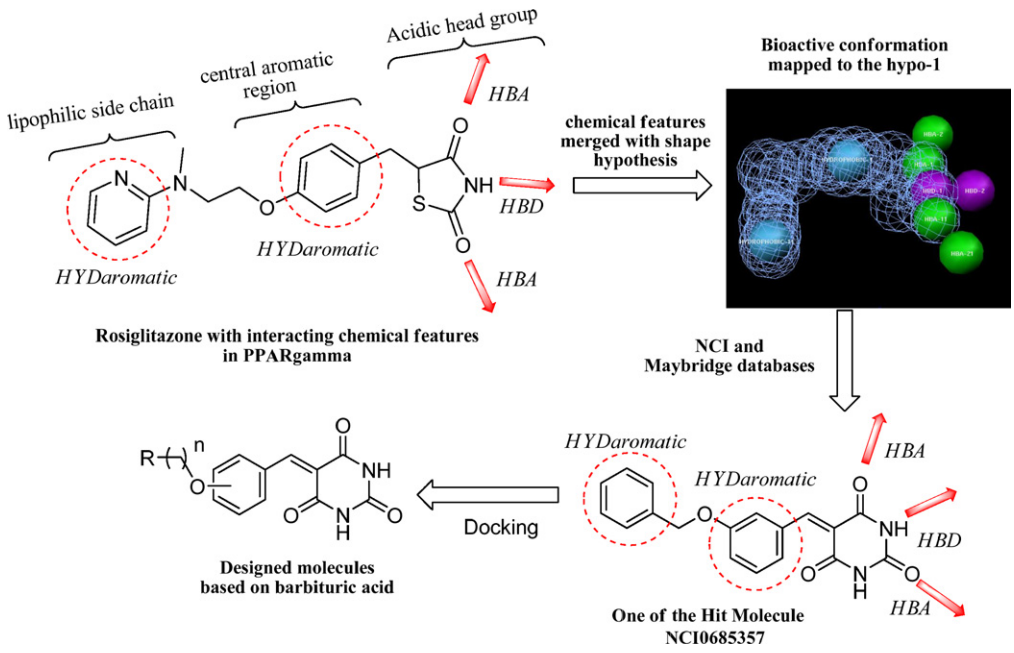


Figure 1. Design of barbituric acid derivatives as PPARγ ligands employing virtual screening approach.

rosiglitazone molecule that includes (i) two hydrogen bond acceptor (HBA) features corresponding to the two carbonyls of TZD ring and one hydrogen bond donor (HBD), related to the –NH of the TZD ring. (ii) hydrophobic aromatic (HYDaromatic) feature was assigned to the central aromatic region and (iii) HYDaromatic feature was also assigned to the pyridine ring in the lipophilic side chain (Fig. 1). These features were merged with the shape and hypothesis query, generated using the co-crystallized conformation of the rosiglitazone molecule leading to **hypo-1**. The later was used as a filter to screen NCI (total compounds = 238,819) and Maybridge (total compounds = 59,652) databases of small molecules for hits.

A total of 46 hits were obtained from NCI and 13 from the Maybridge. All hits were inspected visually individually to observe mappings of the molecules to various chemical features of the **hypo-1**. The analysis revealed that derivatives of barbituric acid (BA) such as NCI 0685357 can perfectly map to the **hypo-1** (Fig. 1). The BA ring mapped to the important HBA and HBD features, while other aromatic rings mapped to the two HYDaromatic features (Fig. 1). To the best of our knowledge, this ring has not been reported as a substitute for the TZD ring in PPARγ agonists. To further support the hypothesis, FlexX-based docking (implemented in SYBYL6.9)[16] was performed on a few designed derivatives of BA into the active site of the PPARγ (PDB code 2PRG). The docking results showed that the HBD and HBA features of BA ring overlaid the TZD ring and maintained the essential H-bonding interactions with His323, His449, Tyr473 and Gln286 as shown in Figure 2.

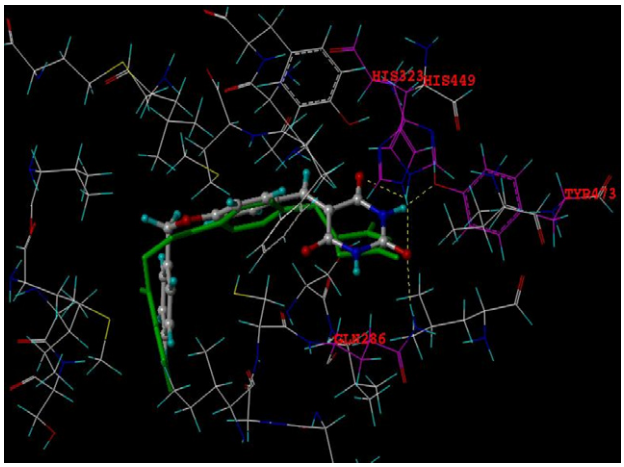
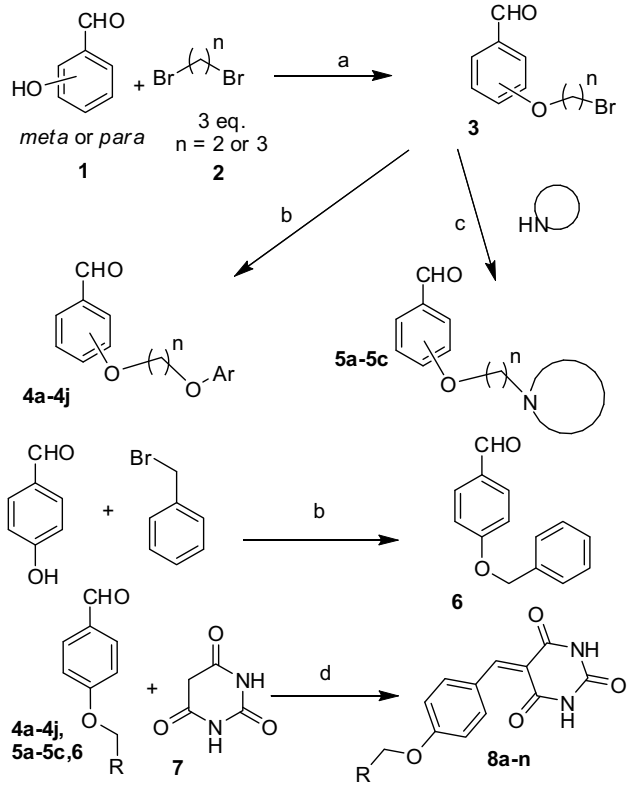


Figure 2. One of the designed barbituric acid derivative (**8n**) docked in the active site of PPARγ (2PRG). The co-crystallized ligand is shown in green colour for comparison while interacting amino acids (labeled in red) are shown in magenta.

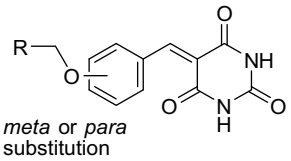
Hence, taking clues from this virtual screening exercise, we designed a variety of molecules with BA as acidic head group keeping a central aromatic ring and varying lipophilic side chain (Fig. 1). While developing synthetic strategy for this class of molecules, another advantage of using BA ring in place of TZD was observed. Both TZD and BA derivatives can be obtained by the Knovenagel condensation of an aromatic aldehyde with the corresponding acidic ring (TZD or BA) in the final step (Scheme 2). In case of TZD, the condensation results in the formation of mixture of '*E*' and '*Z*' isomers which may be difficult to separate.[10b] On the other hand, condensation of BA ring with aromatic aldehydes leads to a single product owing to the symmetry in the BA moiety. Thus, for the synthesis of different aromatic aldehydes, the first step adopted was the reaction between the *p*- or *m*-hydroxy benzaldehyde and 1,2-dibromoethane or 1,3-dibromopropane to give different monobromo products (**3**). The later were further reacted with different substituted phenols or 'NH' containing heterocyles under the basic conditions to yield the *O*- or *N*-alkylated aldehydes (**4a**–**4j** and **5a**–**5c**). The *para* analogue of the hit molecule NCI0685357 (Fig. 1) was also synthesized from the corresponding aldehyde (**6**) that was obtained by reacting *p*-hydroxy benzaldehyde and benzyl bromide. Finally, all aromatic aldehydes were condensed with BA (**7**) by a reported procedure[17] to yield final products (**8a**–**8n**). All the final products were characterized using Mass, ^{1}H NMR and ^{13}C NMR spectroscopy.[18]

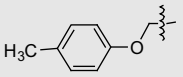
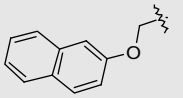
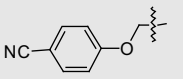
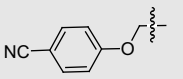
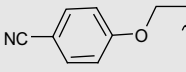
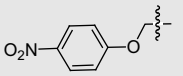
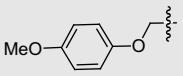
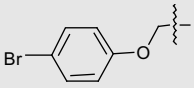
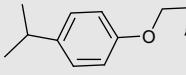
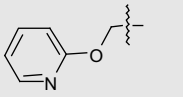
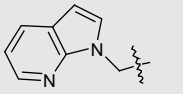
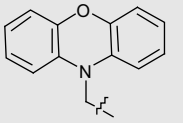
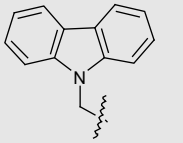
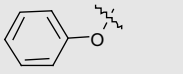
Final molecules (**8a**–**8n**) were evaluated in vitro for their ability to bind to murine PPARγ in a standard radioligand binding assay (Table 1).[19] Out of the total 14 molecules, 6 were found to be active in this assay. However, these molecules were found to act as partial inhibitors in this assay except for **8d** and **8f** which inhibited nearly 100% binding of [^{3}H]-rosiglitazone (see Supplementary information). The molecule **8n** was found to be most potent with IC_{50} of



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C, 3–5 h, 40–72%; (b) ArOH, K_2CO_3, DMF, reflux, 2–4 h, 52–93%; (c) NaH, DMF, 0 °C-RT, 4–5 h, 63–85%; (d) MeOH, RT, 2–6 h, 76–92%.

Table 1
Structure and IC_{50} values of the final molecules



Compound	R	IC_{50} (μM)	FlexX score
8a		i.a.	−28.0
8b		i.a.	−25.5
8c		0.2	−27.6
8d[*,#]		0.8	−27.5
8e		i.a.	−27.1
8f[*]		2.5	−28.1
8g		0.2	−25.5
8h		i.a.	−26.8
8i		i.a.	−27.6
8j		i.a.	−27.3
8k		i.a.	−30.8
8l		0.5	−34.0
8m		i.a.	−32.6
8n		0.1	−20.7

Pioglitazone (reference standard) IC_{50} = 0.7 μM.
i.a., inactive (less than 20% inhibition at 10 μM).
[*] Inhibited nearly 100% binding of [^{3}H]-rosiglitazone.
[#] Meta substitution (all other compounds have para substitution).

0.1 μM. However, experimentally observed IC_{50} values of these molecules did not correlate with the docking scores. This may be due to the inability of some of the compounds to penetrate the adipocyte cells under the given bioassay conditions for their inappropriate physicochemical properties. In addition, the problem may arise due to the limitation of docking programme that considers only the flexibility of the ligand and not the protein. Nonetheless, experimental findings demonstrate the proof of concept for the adopted virtual screening approach and show that BA ring can be employed as the acidic group for the design of novel class of PPAR ligands.

In conclusion, computer-aided design of a novel series of PPAR ligands based on barbituric acid has been reported. Preliminary synthetic and receptor binding studies were taken up to validate the adopted computer-aided design strategy. The synthesized molecules were indeed found to bind to the receptor with IC_{50} values comparable to the reference standard, pioglitazone.

Acknowledgment

Authors thank Department of Science and Technology (DST), New Delhi, for providing financial assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.028.

References and notes

1. (a) Desvergne, B.; Wahli, W. *Endocr. Rev.* **1999**, *20*, 649; (b) Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
2. (a) Guo, L.; Tabrizchi, R. *Pharmacol. Ther.* **2005**, *111*, 145; (b) Kersten, S. *Eur. J. Pharmacol.* **2002**, *440*, 223; (c) Duval, C.; Chinetti, G.; Trottein, F.; Fruchart, J.-C.; Staels, B. *Trends Mol. Med.* **2002**, *8*, 422.
3. Schoonjans, K.; Staels, B.; Auwerx, J. *J. Lipid Res.* **1996**, *37*, 907.
4. Sohda, T.; Mizuno, K.; Imamiya, E.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3580.
5. Lehmann, J. M.; Moore, L. M.; Smith-Oliver, T. A.; Wilkinson, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.* **1995**, *270*, 12953.
6. Oliver, W. R., Jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5306.
7. (a) Moraes, L. A.; Piqueras, L.; Bishop-Bailey, D. *Pharmacol. Ther.* **2006**, *110*, 371; (b) Koeffler, H. P. *Clin. Cancer Res.* **2003**, *9*, 1; (c) Alarcon de la Lastra, C.; Sanchez-Fidalgo, S.; Villegas, I.; Motilva, V. *Curr. Pharm. Des.* **2004**, *10*, 3505.
8. (a) Peraza, M. A.; Burdick, A. D.; Marin, H. E.; Gonzalez, F. J.; Peters, J. M. *Toxicol. Sci.* **2006**, *90*, 269; (b) Rubenstrunk, A.; Hanf, R.; Hum, D. W.; Fruchart, J.-C.; Staels, B. *Biochim. Biophys. Acta* **2007**, *1771*, 1065; (c) Nissen, S. E.; Wolski, K. N. *N. Engl. J. Med.* **2007**, *356*, 2457.
9. Pourcet, B.; Fruchart, J. C.; Staels, B.; Glineur, C. *Exp. Opin. Emerging Drugs* **2006**, *11*, 379.
10. (a) Kumar, R.; Ramachandran, U.; Khanna, S.; Bharatam, P. V.; Raichur, S.; Chakrabarti, R. *Bioorg. Med. Chem.* **2007**, *15*, 1547; (b) Ramachandran, U.; Mittal, A.; Bharatam, P. V.; Khanna, S.; Ramarao, P.; Srinivasan, K.; Kumar, R.; Chawla, H. P. S.; Kaul, C. L.; Raichur, S.; Chakrabarti, R. *Bioorg. Med. Chem.* **2004**, *12*, 655; (c) Khanna, S.; Sobhia, M. E.; Bharatam, P. V. *J. Med. Chem.* **2005**, *48*, 3015; (d) Sundriyal, S.; Viswanad, B.; Bharathy, E.; Ramarao, P.; Chakraborti, A. K.; Bharatam, P. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3192–3195.
11. (a) Kulkarni, S. S.; Gediya, L. K.; Kulkarni, V. M. *Bioorg. Med. Chem.* **1999**, *7*, 1475; (b) Nolte, R. T.; Wisely, 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; (c) Khanna, S.; Bahal, R.; Bharatam, P. V.. In *Topics in Heterocyclic Chemistry*; Gupta, S. P., Ed.; Springer: Berlin, Heidelberg, 2006; Vol. 3, pp 149–180.
12. (a) Momose, Y.; Maekawa, T.; Yamano, T.; Kawada, M.; Odaka, H.; Ikeda, H.; Sohda, T. *J. Med. Chem.* **2002**, *45*, 1518; (b) Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L.; Harrington, W. W., Jr.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A., Jr.; Noble, S. A.; Oliver, W., Jr.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. J. *Med. Chem.* **1998**, *41*, 5020; (c) Momose, Y.; Maekawa, T.; Odaka, H.; Ikeda, H.; Yamano, T.; Sohda, T. *Chem. Pharm. Bull.* **2002**, *50*, 100.
13. (a) Klebe, G. *Drug Discov. Today* **2006**, *11*, 580; (b) Muegge, I.; Oloff, S. *Drug Discov. Today: Technologies* **2006**, *3*, 405.
14. Lu, I.-L.; Huang, C.-F.; Peng, Y.-H.; Lin, Y.-T.; Hsieh, H.-P.; Chen, C.-T.; Lien, T.-W.; Lee, H.-J.; Mahindroo, N.; Prakash, E.; Yueh, A.; Chen, H.-Y.; Goparaju, C. M. V.; Chen, X.; Liao, C.-C.; Chao, Y.-S.; Hsu, J. T.-A.; Wu, S. Y. *J. Med. Chem.* **2006**, *49*, 2703.
15. Catalyst Program, Version 4.10. Accelrys Inc., San Diego, CA, USA.
16. (a) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470; (b) SYBYL6.9, Tripos Inc., 1699 South Hanley Road, St. Louis, MO 631444, USA. www.tripos.com.
17. Jursic, B. S. *J. Heterocyclic Chem.* **2001**, *38*, 655.
18. Spectral data for a representative molecule (**8n**) ^{1}H NMR (DMSO- d_6; 300 MHz) δ 8.32 (2H, d, J = 9 Hz), 8.20 (1H, s), 7.44–7.28 (5H, m), 7.10 (2H, d, J = 9 Hz), 5.20 (2H, s); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 164.2, 162.9, 162.5, 155.2, 150.5, 137.8, 136.7, 128.9, 128.4, 128.2, 125.7, 116.0, 115.0; Mass (MALDI-TOF/TOF) m/z 323.6 (M^++1).
19. (a) Kumar, R.; Mittal, A.; Ramachandran, U. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4613; (b) Arun, K. H. S.; Kaul, C. L.; Ramarao, P. *Cardiovasc. Res.* **2005**, *65*, 374.