Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3507–3511
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and evaluation of a new class of noncyclic 1,3-dicarbonyl compounds as PPARα selective activators

Zhibin Li,[a,†] Chenzhong Liao,[a,b,†] Ben C. B. Ko,[c,d] Song Shan,[a] Edith H. Y. Tong,[c,d] Zihui Yin,[a] Desi Pan,[a] Vincent K. W. Wong,[c] Leming Shi,[a] Zhi-Qiang Ning,[a] Weiming Hu,[a] Jiaju Zhou,[b] Stephen S. M. Chung[e] and Xian-Ping Lu[a,*]

[a]*Chipscreen Biosciences, Ltd, Research Institute of Tsinghua University, Suite C301, PO Box 28, Shenzhen 518057, China*
[b]*Institute of Process Engineering, Chinese Academy of Sciences, PO Box 353, Beijing 100080, China*
[c]*Department of Chemistry, The University of Hong Kong, Hong Kong SAR, China*
[d]*Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Hong Kong SAR, China*
[e]*Insitute of Molecular Biology, The University of Hong Kong, Hong Kong SAR, China*

Received 7 March 2004; revised 19 April 2004; accepted 19 April 2004

Abstract—Lipid accumulation in nonadipose tissues is increasingly linked to the development of type 2 diabetes in obese individuals. We report here the design, synthesis, and evaluation of a series of novel PPARα selective activators containing 1,3-dicarbonyl moieties. Structure–activity relationship studies led to the identification of PPARα selective activators (compounds **10**, **14**, **17**, **18**, and **21**) with stronger potency and efficacy to activate PPARα over PPARγ and PPARδ. Experiments in vivo showed that compounds **10**, **14**, and **17** had blood glucose lowering effect in diabetic db/db mouse model after two weeks oral dosing. The data strongly support further testing of these lead compounds in other relevant disease animal models to evaluate their potential therapeutic benefits.

1. Introduction

Increasing evidence suggests that lipid accumulation in nonadipose tissues, such as pancreatic islet cells and skeletal muscle, is causally related to the development of type 2 diabetes in obese individuals.[1] Peroxisome proliferator activated receptor-α (PPARα), PPARγ and PPARδ are ligand-activated transcriptional factors that belong to the nuclear hormone receptor superfamily, which are essential in controlling lipid, glucose, and energy homeostasis.[2,3] At present, PPARγ agonists rosiglitazone[4] (**1**, Fig. 1) has been successfully prescribed for patients having type 2 diabetes. The fibrate agents, such as fenofibrate and Wy-14643[2] (**2**), are low affinity PPARα agonists; fenofibrate and beazfibrate have been widely used clinically as antihyperlipidemic drugs. These drugs increase HDL cholesterol levels and lower LDL and VLDL cholesterol levels with stronger triglyceride-lowering effect than statins, the HMG-CoA reductase inhibitors. Recently published data showed that PPARδ activators could also be used in the treatment of

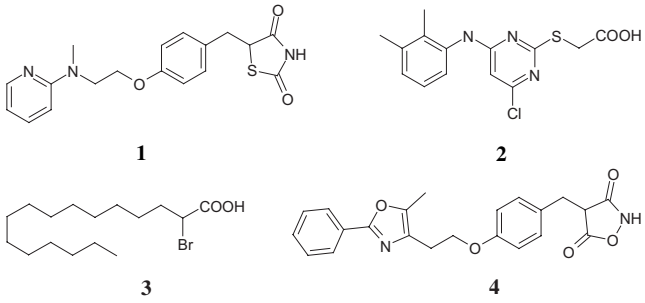


Figure 1. Reference compounds.

Keywords: PPARα activators; Type 2 diabetes; Noncyclic 1,3-dicarbonyl compounds; Metabolic diseases.
* Corresponding author. Tel.: +86-755-26711889; fax: +86-755-2695-7291; e-mail: xplu@chipscreen.com
† Contributed equally to this work.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.053

dyslipidemia.[5] Fatty acids, for example, 2-bromo-hexadecanoic acid[6,7] (**3**) are known as weak activators of PPARδ. Because of the side effects of PPARγ agonists, such as weight gain and water retention,[8] and the potential beneficial effects of the PPARα and PPARδ,[9] balanced triple agonists for PPARα/γ/δ or selective agonists for PPARα or δ are of great interest for drug discovery efforts worldwide.[10]

Here, we describe the design, synthesis, and evaluation of a new class of PPARα selective activators containing 1,3-dicarbonyl moieties, leading to the identification of PPARα selective activators as candidate leads for the potential treatment of metabolic disorders such as diabetes, obesity, and hyperlipidemia.

2. Result and discussion

JTT-501[11] (**4**), a dual agonist of PPARα/γ, is an isoxazolidine-3,5-dione. Its activity is likely to be mediated through a malonic amide metabolite that is generated by hydrolysis of the heterocyclic ring.[12] We therefore took this moiety as template to develop structurally new human PPARα selective activators.

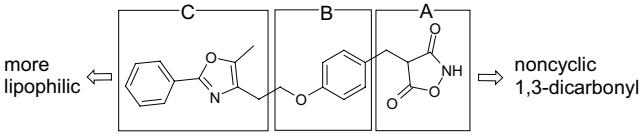
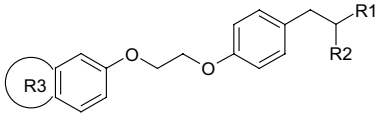


Figure 2. Chemical structure of the lead compound JTT-501.

The framework of JTT-501, like most of PPAR activators, can be divided into three key regions: (A) the acidic head part, (B) the linker part, and (C) the hydrophobic tail part (Fig. 2). Thus, chemical modifications of the acidic head part and the lipophilic part[13] might change their selectivity to PPARα, PPARγ, or PPARδ receptors.

We first modified the acidic head part into a series of 1,3-dicarbonyl compounds, and the lipophilic part into 2,2,5,5-tetramethyl-2,3,4,5-tetrahydronaphthalene. These two modifications generated compounds **5**–**9** that have no PPARα and PPARδ activities while remaining as partial PPARγ agonists (Table 1). Substitution of the four methyl groups with hydrogen atoms from this lipophilic tail formed compounds **10**–**13** that had activities to all three PPAR subtypes, suggesting that

Table 1. Cell-based in vitro transcription activation of test compounds on hPPARα, hPPARγ, and hPPARδ[a]



No.	R1	R2	R3	PPARα EC_{50} μM (%max[b])	PPARγ EC_{50} μM (%max[b])	PPARδ EC_{50} μM (%max[b])
5	$-COOCH_3$	$-COOCH_3$	2,2,5,5-Tetramethylcyclohexane	ia[c] (–)	0.15 (66)	ia (–)
6	$-COOCH_3$	–COOH	2,2,5,5-Tetramethylcyclohexane	ia (–)	0.09 (64)	ia (–)
7[d]	–COOH	–COOH	2,2,5,5-Tetramethylcyclohexane	ia (–)	2.05 (9)	ia (–)
8	$-COOCH_3$	$-CONH_2$	2,2,5,5-Tetramethylcyclohexane	ia (–)	0.83 (15)	ia (–)
9	–COOH	$-CONH_2$	2,2,5,5-Tetramethylcyclohexane	ia (–)	ia (–)	ia (–)
10	$-COOCH_3$	$-COOCH_3$	Cyclohexane	0.03 (210)	1.5 (55)	3.31 (134)
11	$-COOCH_3$	–COOH	Cyclohexane	ia (–)	0.69 (60)	2.45 (100)
12[d]	–COOH	–COOH	Cyclohexane	ia (–)	2.11 (39)	4.68 (80)
13	$-COOCH_3$	$-CONH_2$	Cyclohexane	ia (–)	3.43 (22)	ia (–)
14	$-COOCH_3$	$-COOCH_3$	Benzene	0.07 (264)	0.53 (78)	1.27 (144)
15[d]	–COOH	–COOH	Benzene	ia (–)	ia (–)	ia (–)
16	$-COOCH_3$	–COOH	Benzene	0.044 (264)	1.12 (59)	1.05 (125)
17	–COOH	$-CONH_2$	Benzene	0.25 (213)	3.02 (8)	2.63 (111)
18	$-COOCH_3$	$-COOCH_3$	Hexahydropyridine	0.17 (215)	3.16 (27)	3.16 (67)
19	$-COOCH_3$	–COOH	Hexahydropyridine	0.09 (207)	3.80 (14)	3.09 (56)
20[d]	–COOH	–COOH	Hexahydropyridine	ia (–)	ia (–)	ia (–)
21	–COOH	$-CONH_2$	Hexahydropyridine	0.14 (210)	2.88 (14)	3.62 (83)
1		Rosiglitazone		7.3 (16)	0.04 (100)	9.3 (42)
2		Wy-14643		15.5 (100)	ia (–)	1.46 (20)
3		2-Bro		nd[e]	nd	3.72 (100)
4		JTT-501[f]		1.9 (NA)	0.083 (NA)	ia

[a] Data represent the mean values from at least three independent experiments each in triplicate.
[b] %max responses were calculated as Wy-14643 at 5 μM as 100% for PPARα; Rosiglitazone at 1 μM as 100% for PPARγ; and 2-bromohexadecanoic acid at 5 μM as 100% for PPARδ.
[c] ia = inactive.
[d] Compounds **7**, **12**, **15**, and **20** are chemically instable and decomposed to CO_2 and monoacid at different rates when activity measured in vitro.
[e] nd = not determined.
[f] The EC_{50} originated from a same type of cell-based transcription activation assay reported for JTT-501 was adapted from reference 3; NA: not available.

the 2,2,5,5-tetramethyl-2,3,4,5-tetrahydronaphthalene group might be too big to interact with PPARα and δ favorably. Changing the lipophilic part to a planar group (naphthalene), such as compounds **14**–**17**, led to stabilization of the interaction between the compounds and PPARα as evidenced by strong transcription activation of PPARα, although the substitutions were made in the acidic head part of the dimethyl malonate group. The substitution of the dimethyl malonate group in compound **14** with 2-methoxycarbonyl propionic acid (compound **16**) resulted in weakening of PPARγ agonist activity while marginally enhancing PPARα and δ activities; the substitution in compound **14** with 2-carbamoyl propionic acid (compound **17**) caused significant decrease in PPARγ activity, as indicated in Table 1, where the maximal response of the PPARγ activity to the compound was only 8% of that of rosiglitazone, but the activation of PPARα by the compound remained strong. Replacement of a carbon atom with a nitrogen atom in the 2,3,4,5-tetrahydronaphthalene ring in compounds **18**–**21** further decreased their ability to activate PPARγ and δ, while relatively strong PPARα activation by these compounds still remained. The activity profiles in Table 1 indicate that the compounds **10** and **19** are potent activators with good selectivity for PPARα while the compounds **17**, **18**, and **21** also exhibit good selectivity for PPARα since the efficacies for PPARγ were significantly decreased; the compounds **14** and **16** show only modest selectivity for PPARα.

AutoDock[14–17] was used to dock compound **17** into PPARα co-crystallized with AZ 242,[18] PPARγ co-crystallized with rosiglitazone,[19] and PPARδ co-crystallized with GW2433,[20] respectively. Figure 3 shows that compound **17** docks into the active sites with the carboxylic acid in the same position as the co-crystallized ligands in PPARα and PPARδ, but not in PPARγ. The calculated pK_i by AutoDock were 8.14, 5.89 and 7.06 to PPARα, PPARγ, and PPARδ. This data is in good agreement with the in vitro activities measured.

Compounds **10**, **14**, and **17** were tested in preliminary experiments using diabetic db/db mouse model. As shown in Table 2, all three compounds were able to lower the increased blood glucose levels as compared to the vehicle treatment after 12-days oral dosing, but they were less effective than rosiglitazone. The blood glucose-lowering effect of compound **17** is interesting since it is almost inactive for PPARγ stimulation, suggesting that the glucose-lowering effect may indirectly result from its lipid modulation. It should be noted that the compounds **10** and **14** are pro-drug forms that are converted to the acid form quickly in vivo. It is speculated that these two compounds may have short half-lives that could generate a favorable side-effect profile. Efforts are currently underway to test the efficacy of these compounds in other animal models of type 2 diabetes and dyslipidemia.

Table 2. In vivo anti-diabetes activity of tested compounds in db/db mice

Compound	Dosage (mg/kg)	Reduction in BG (%) after two weeks of oral dosing[a]
Vehicle	0	0
10	30	38.15
14	10	35.08
14	30	46.76
17	20	42.22
Rosiglitazone	5	62.96

[a] Data represent the mean of the percent reduction in blood glucose (BG) level measured using blood samples from mice ($N = 4$ to 6 mice).

In conclusion, the in vitro and in vivo results show that by modifying the acidic head part and the lipophilic part of the template compound JTT-501, we obtained PPARα selective activators that are potentially useful for the development of drugs against type 2 diabetic or other metabolic diseases.

3. Experimental Section

3.1. Chemistry

Compounds **5**–**21** were synthesized as outlined in Scheme 1. The commercially available phenols upon reaction with *p*-bromoethoxy benzaldehyde gave benzaldehyde derivatives **a**. Knoevenagel condensation between the aldehydes **a** and dimethyl malonate gave the benzylidene **b**. Catalytic hydrogenation of **b** with 5% palladium on carbon gave the dimethyl malonate **c**. Partial hydrolysis of **c** with 1 equiv of sodium hydroxide gave the half-ester **d**. The Schotten–Baumann reaction

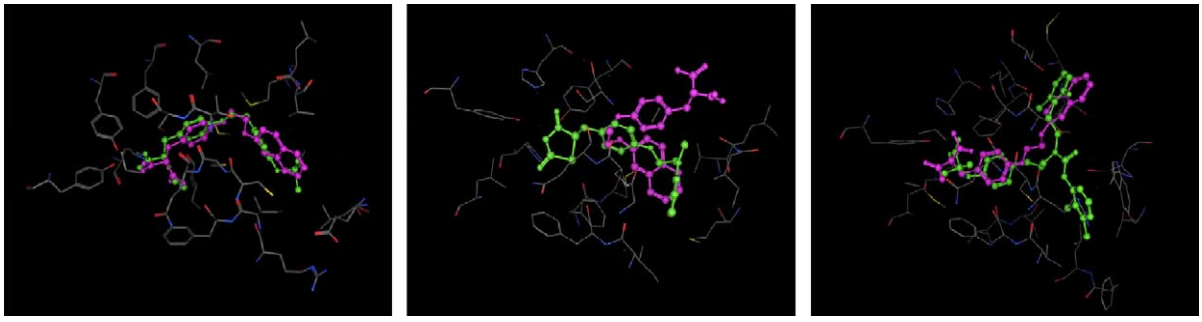
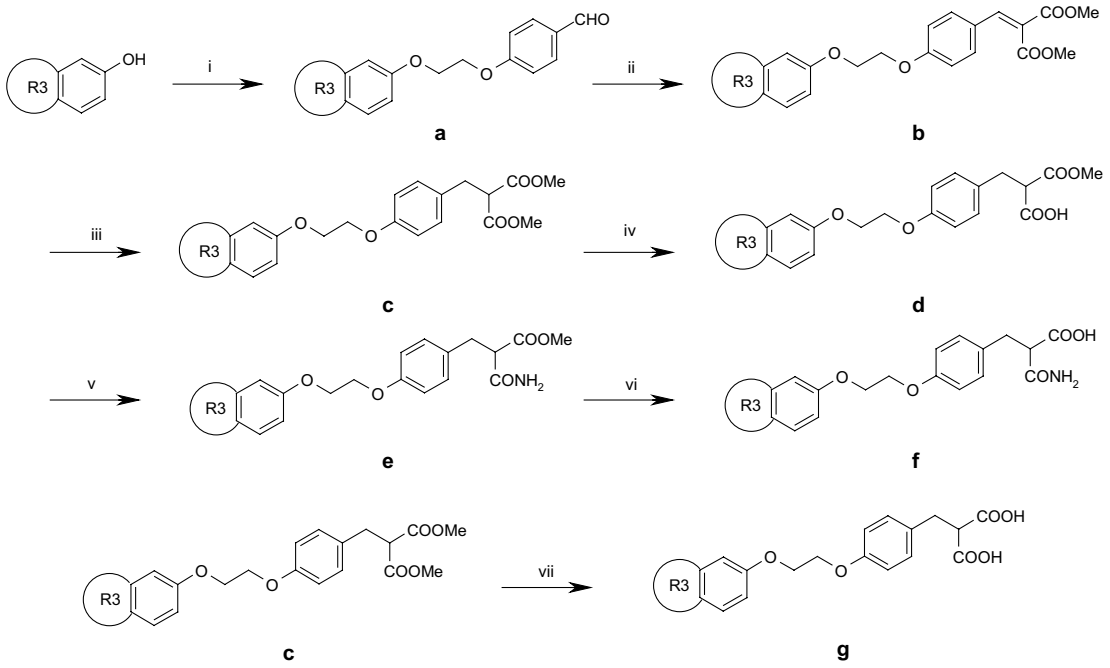


Figure 3. From left panel to right panel: compound **17** (purple) docked into the PPARα receptor co-crystallized with AZ 242 (green), the PPARγ receptor co-crystallized with rosiglitazone (green) and the PPARδ receptor co-crystallized with GW 2433 (green).



Scheme 1. (i) *p*-Bromoethoxy benzaldehyde, KOH, EtOH, refluxing; (ii) dimethyl malonate, toluene, piperidinium acetate, refluxing; (iii) 5% Pd/C, H_2, rt; (iv) 1 equiv of NaOH, rt; (v) $SOCl_2$, 28% ammonia; (vi) NaOH, CH_3 OH, rt; (vii) 2 equiv of NaOH, rt.

between an acid chloride of **d** and ammonia gave the amide ester **e**. Hydrolysis of **e** with sodium hydroxide gave the amide acid **f**. Hydrolysis of **c** with more than 2 equiv of sodium hydroxide gave the malonic acid **g**.

3.2. Modeling

The docking program used in this study was AutoDock 3.0.[14–17] Polar hydrogens were added and Kollman partial atomic charges were assigned to the macromolecule. Gasteiger charges were assigned to the ligands. All molecular modeling works except docking were done in Sybyl 6.81 package.[21]

3.3. In vitro transactivation

cDNAs for Human RXR, PPAR were obtained by RT-PCR from the human liver or adipose tissues. Amplified cDNAs were cloned into pcDNA3.1 expression vector and the inserts were confirmed by sequencing. U2OS cells were cultured in McCoy's 5A with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. Cells were seeded in 96-well plates the day before transfection to give a confluence of 50–80% at transfection. A total of 60 ng of DNA containing 10 ng of hRXR, 10 ng of pCMV Gal, 10 ng of nuclear receptor expression vectors and 30 ng of the corresponding reporters were cotransfected per well using FuGene6 transfection reagent according to the manufacturer's instructions. Following 24 h after transfection, cells were incubated with 10% charcoal-stripped FBS DMEM and were treated with the individual compound dissolved in DMSO. The final concentration of DMSO in culture medium was 0.1%.

Cells were treated with compound for 24 h, and then collected with Cell Culture Lysis buffer. Luciferase activity was monitored using the luciferase assay kit according to the manufacturers instructions. Light emission was read in a Labsystems Ascent Fluoroskan reader. To measure galactosidase activity to normalize the luciferase data, 50 μL of supernatant from each transfection lysate was transferred to a new microplate. Galactosidase assays were performed in the microwell plates using a kit from Promega and read in a microplate reader.

3.4. In vivo animal study

Six-week old male db/db mice purchased from Jackson Lab (USA) were given a standard diet and kept at 12 h light/darkness cycle, a temperature of 21 °C, and a relative humidity of 50% throughout the accommodation (two weeks) and dosing periods. All tested compounds were suspended in water containing 0.2% of methylcellulose and 0.1% Tween-80 (w/v). Mice were given tested compounds by gavage once daily for 12 days. Blood was collected from tail vein after 3 h fasting in the morning for blood glucose measurement using Roche's Accu-Chek advantage II Glucose strip together with Accu-Chek Advantage glucose meter.

Acknowledgements

The authors gratefully acknowledge financial supports from the national '863' project of People's Republic of China and project of 'Most Important Biopharmaceutical Projects' by Guangdong provincial and Shenzhen

municipal governments in China, and University Grants Committee of the Hong Kong Special Administrative Region Areas of Excellence Scheme Grant AoE/P-10/01. The authors also acknowledge the excellent technical assistances from Kun Zhang, Fei Yu, Yin Zhang, Baoshan Ma, Zhongwen Wu, and Jingzhong Zhu.

References and notes

1. Kelly, D. E.; Goodpaster, B. H. *Diabetes Care* **2001**, *24*, 933.
2. Issemann, I.; Green, S. *Nature* **1990**, *347*, 645.
3. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
4. Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.* **1995**, *270*, 12953.
5. Oliver, W. R.; 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, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *PNAS* **2001**, *98*, 5306.
6. Bastie, C.; Luquet, S.; Holst, D.; Jehl-Pietri, C.; Grimaldi, P. A. *J. Biol. Chem.* **2000**, *275*, 38768.
7. Son, C.; Hosoda, K.; Matsuda, J.; Fujikura, J.; Yonemitsu, S.; Iwakura, H.; Masuzaki, H.; Ogawa, Y.; Hayashi, T.; Itoh, H.; Nishimura, H.; Inoue, G.; Yoshimasa, Y.; Yamori, Y.; Nakao, K. *Endocrinology* **2001**, *142*, 4189.
8. Larsen, T. M.; Toubro, S.; Astrup, A. *Int. J. Obesity* **2003**, *27*, 147.
9. Wang, Y. X.; Lee, C. H.; Tiep, S.; Yu, R. T.; Ham, J.; Kang, H.; Evans, R. M. *Cell* **2003**, *113*, 159.
10. Etgen, G. J.; Mantlo, N. *Curr. Topics Med. Chem.* **2003**, *3*, 1649.
11. Shibata, T.; Matsui, K.; Nagao, K.; Shinkai, H.; Yonemori, F.; Wakitani, K. *Eur. J. Pharmacol.* **1999**, *364*, 211.
12. Shinkai, H.; Onogi, S.; Tanaka, M.; Shibata, T.; Wao, M.; Wakitani, K.; Uchida, I. *J. Med. Chem.* **1998**, *41*, 1927.
13. Brown, P. J.; Winegar, D. A.; Plunket, K. D.; Moore, L. B.; Lewis, M. C.; Wilson, J. G.; Sundseth, S. S.; Koble, C. S.; Wu, Z.; Chapman, J. M.; Lehmann, J. M.; Kliever, S. A.; Willson, T. M. *J. Med. Chem.* **1999**, *42*, 3785.
14. Goodsell, D. S.; Olson, A. J. *Proteins* **1990**, *8*, 195.
15. Goodsell, D. S.; Morris, G. M.; Olson, A. J. *J. Mol. Recognition* **1996**, *9*, 1.
16. Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. *J. Comput. Aided Mol. Des.* **1996**, *10*, 293.
17. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
18. Cronet, P.; Petersen, J. F. W.; Folmer, R.; Blomberg, N.; Sjoblom, K.; Karlsson, U.; Lindstedt, E.-L. *Structure* **2001**, *9*, 699.
19. Gampe, R. T., Jr.; Montana, V. G.; Lambert, M. H.; Miller, A. B.; Bledsoe, R. K.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M.; Xu, H. E. *Mol. Cell* **2000**, *5*, 545.
20. Xu, H. E.; Lambert, M.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisely, G. B.; Willson, T. M.; Kliewer, S. A.; Milburn, M. V. *Mol. Cell* **1999**, *3*, 397.
21. SYBYL® 6.81. Tripos Inc., 1699 Hanley Road, St. Louis, MO 63144, USA.