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Pimozide, a Novel Fatty Acid Binding Protein 4 Inhibitor, Promotes Adipogenesis of 3T3-L1 Cells by Activating PPARγ

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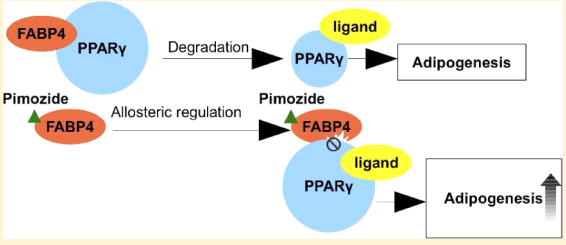
Supporting Information

ABSTRACT: Pimozide is a conventional antipsychotic of the diphenylbutylpiperidine class that has been clinically used for over 30 years. The obvious side effect of this drug is weight gain. However, the mechanism of pimozide-induced weight gain is still unknown. In the present study, we identified pimozide as a novel fatty acid binding protein 4 (FABP4) inhibitor using molecular docking simulation as well as biochemical characterizations. BMS309403, a well-known FABP4 inhibitor, elevated the basal protein levels of PPARγ, therefore stimulating adipogenesis in adipocytes. The present study showed that the inhibitory effect of pimozide on FABP4 promoted adipocyte differentiation with the potency proportional to their propensities for weight gain. These effects in adipogenesis by pimozide may help to explain the weight gain that is frequently observed in patients treated with pimozide.



KEYWORDS: *Pimozide, fatty acid binding protein 4 inhibitor, adipogenesis, PPARγ, molecular docking*

Pimozide is a conventional antipsychotic of the diphenylbutylpiperidine class. It acts as a selective blocker of dopamine D2 receptor and is widely used for schizophrenia and chronic psychosis treatment.[1,2] Antipsychotic drugs are associated with weight gain during the treatment periods, which has been documented in many clinical trials.[3] The systematic investigation of the mechanisms involved in antipsychotic-drug-induced weight gain is important in clinical practice. It might help optimize therapeutic treatments to reduce the serious metabolic side effects of these antipsychotic drugs.[4] Similarly to other typical antipsychotics, weight gain is a common side effect of pimozide administration. The induction of weight gain by pimozide has been demonstrated by previous clinical studies. After 6 weeks treatment of pimozide, the mean total weight gain in the 17 patients assessed was 4.2 kg.[5] Another clinical study showed that 4 weeks treatment of pimozide might result in a weight gain about 1.0 kg.[6] Weight gain, particularly fat deposits, predicts increased risks for cardiovascular diseases, metabolic syndromes, and type 2 diabetes.[7]

Up to date, the mechanistic studies of antipsychotic-drug-induced weight gain and metabolic abnormalities focus on the central nervous system. Antipsychotic drugs have been reported to inhibit histaminergic and serotoninergic pathways, therefore influencing on food intake or energy expenditure in patients.[8,9] Other studies showed that antipsychotic drugs directly induced white adipose tissue, and then disturbed the energy homeostasis in the body.[10] The induction of lipid accumulation within fully differentiated adipocytes increases adipocyte size, therefore resulting in increased adipose tissue mass associated with weight gain.[11] For example, weight gain is also the major adverse effect of olanzapine. Animal studies showed that olanzapine exhibited direct effects on mature rat adipocytes, increasing adipogenesis and decreasing adipolysis to induce lipid accumulation.[10,12] Further mechanistic study showed that the induction of adipogenesis by olanzapine in 3T3-L1 differentiation models was mediated through the regulation of sterol regulatory element binding protein-1 (SREBP-1).[13] However, the mechanistic details of pimozide-induced weight gain are still unknown. In the present study, we used 3T3-L1 cells as a model to investigate whether pimozide causes weight gain through the induction of adipogenesis.

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor that is encoded by the PPARG gene. PPARγ acts as a key regulator in fatty acid storage and glucose metabolism. The activation of PPARγ stimulates adipogenesis in fat cells. PPARG-deficient mice fail to generate adipose tissue when fed a high-fat diet.[14] Thiazolidinediones (TZD), a class of PPARγ agonists, is used in the treatment of type 2 diabetes.[15] Accumulating clinical studies showed that weight gain occurred with TZD therapy.[16]

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A

Critical residues of FABP4 interactions with ligands

PDB	Benchmark			Pimozide		
	Score	Hydrogen bond	π-π	Score	Hydrogen bond	π-π
1TOU	-8.2	Tyr19, Arg126, Tyr128	None	-10	Ser53, Ser55, Thr60	Phe16, Phe57
1TOW	-7.9	Ser53, Thr60, Arg126, Tyr128	Phe16, Phe57	-9.4	Ser53, Ser55, Lys58, Thr60	Phe16, Phe57
2NNQ	-11.1	Arg106, Arg126, Tyr128	Phe57	-10.9	Ser53, Ser55, Lys58, Thr60	Phe16, Phe57
3FR5	-10	Ser55, Arg126, Tyr128	Phe16	-10.5	Ser53, Lys58, Thr60	Phe16, Phe57

B

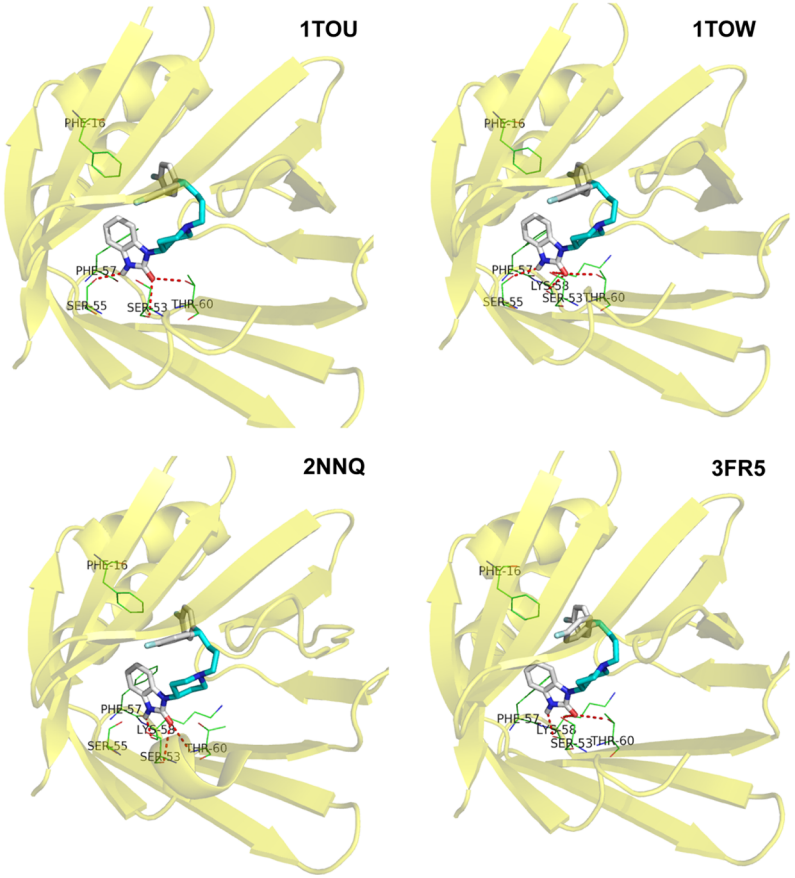

Figure 1. Pimozide is a FABP4 inhibitor identified by molecular docking. (A) Critical residues for inhibitor binding are demonstrated. The inhibitors in each crystal structure were used as benchmarks. The benchmark of 1TOU is 2-[4-hydroxy-6-(trifluoromethyl)pyrimidin-2-yl] sulfanyl-1-piperidin-1-yl-ethanone (B1V). The benchmark of 1TOW is 4-carbazol-9-ylbutanoic acid (CRZ). The benchmark of 2NNQ is 2-[3-[2-(5-ethyl-3,4-diphenyl-pyrazol-1-yl)phenyl]phenoxy]ethanoic acid (T4B). The benchmark of 3FR5 is 5-(3-carbamoylbenzyl)-5,6,7,8,9,10-hexahydrocyclohepta[*b*]indole-4-carboxylic acid (I4A). (B) Molecular docking analysis illustrates the favorable binding positions of pimozide with lowest binding free energy in the inhibitor-binding site of human FABP4 (PDB code 1TOU, 1TOW, 2NNQ, and 3FR5). The three-dimensional diagrams show the interactions of pimozide (gray stick) to human FABP4 (yellow cartoon) with labeled amino residues.

Fatty acid-binding proteins (FABPs) is a cluster of ∼15 kDa cytoplasmic proteins that serve as carriers of endogenous fatty acids. Fatty acids are transported by FABPs in cytoplasm for metabolic process or storage. The adipocyte FABP, FABP4 (aP2), is highly expressed in adipocytes.[17] The gene of FABP4 contains peroxisome proliferator response elements (PPREs) and therefore can be regulated by PPARγ.[18] Recently, FABP4 was reported to directly interact with PPARγ;[19] this kind of interaction might trigger the ubiquitination and the subsequent proteasomal degradation of PPARγ.[20] BMS309403, the well-known FABP4 inhibitor, elevated the basal protein levels of PPARγ, therefore stimulating adipogenesis in adipocytes.[20]

We first identified pimozide as an inhibitor of FABP4 using molecular docking simulation in our previous work.[21] In the present study, biochemical characterization showed that pimozide inhibited FABP4 activity in cell-free and cell-based assay. Like BMS309403, pimozide elevated the protein levels of PPARγ during differentiation and subsequently promoted adipogenesis in 3T3-L1 adipocytes. It is strongly suggested that pimozide acts as a FABP4 inhibitor and promotes adipogenesis in adipose tissues that attributes to weight gain after long-term usage of this drug.

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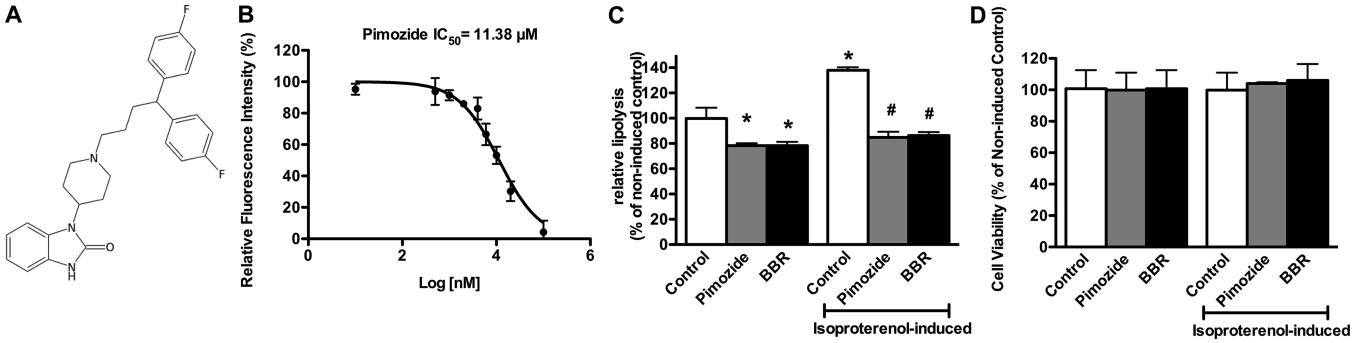

Figure 2. Pimozide inhibits FABP4 activity in both cell-free and cell-based assay. (A) Dose−response curves of pimozide in FABP4 activity inhibition assay. The IC_{50} values were calculated using the inhibitor dose−response function in Prism 5. (B) Pimozide inhibited both basal and isoproterenol-stimulated adipolysis in 3T3-L1 cells. Benzbromarone (BBR) (10 μM) was used as a positive control. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test. $^{*}P < 0.05$, compared with the control group. $^{\#}P < 0.05$, compared with the isoproterenol-treated group. There were no effects on the cell proliferation at 10 μM pimozide in the presence or absence of isoproterenol. 3T3-L1 cells were treated with pimozide for 24 h followed by incubation with isoproterenol (100 nM) for another 24 h. Afterward, the viability was detected by MTT. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test.

RESULTS AND DISCUSSION

Four crystal structures of human FABP4 (PDB code 1TOU,[22] 1TOW,[23] 2NNQ,[24] and 3FR5[25]) were selected for molecular docking analyses. Pimozide exhibited good binding affinities with all of protein models (Figure 1A). As shown in Figure 1B, pimozide exhibited similar conformation in the four protein models. Generally, a carbonyl group of pimozide formed hydrogen bonds with Ser53, Ser55, Lys58, and Thr60 of human FABP4. In addition, two fluorobenzenes of pimozide interacted with Phe16 of FABP4 through $\pi-\pi$ interactions. The benzimidazole of pimozide also formed $\pi-\pi$ interaction with Phe57 of FABP4. A previous study has shown that the key residues in FABP4, including Ser53, Ser55, and Thr60, can form hydrogen bonds with FABP4 inhibitors.[23] Furthermore, both Phe16 and Phe57 play key roles in the FABP4 inhibitor binding by $\pi-\pi$ interactions.[23] Pimozide was found to interact with the key residues in FABP4 inhibitor binding pocket, similar to the known FABP4 inhibitors.

The replacement assay showed that pimozide directly inhibited FABP4 activity with the IC_{50} value of 11.38 μM (Figure 2B). The inhibitory activity of pimozide is similar to that of arachidonic acid (an endogenous ligand of FABP4, IC_{50} value of 7.39 μM). The inhibition of FABP4 was reported to decrease adipolysis in 3T3-L1 cells. Benzbromarone is a newly identified FABP4 inhibitor, which inhibits adipolysis at 10 μM.[26] Thus, we assessed whether pimozide could modulate the levels of adipolysis. The results showed that pimozide (10 μM) inhibited both basal and isoproterenol-stimulated adipolysis in 3T3-L1 cells (Figure 2C). This result is consistent with pimozide as an inhibitor of FABP4 in adipocytes. In addition, pimozide has no obvious effect on 3T3-L1 cell proliferation (Figure 2D). Together, we confirmed the inhibitory effects of pimozide on FABP4 activity in both cell-free assay and cell-based assay.

Recently FABP4 has been reported to inhibit adipogenesis in 3T3-L1. The FABP4-null preadipocytes exhibited a remarkably enhanced adipogenesis compared with the wild-type cells.[20] Here we tested whether pimozide as a FABP4 inhibitor could promote adipogenesis in 3T3-L1 cells. First, we confirmed that the pimozide induced lipid accumulation during adipocyte differentiation. Pimozide was added at the beginning of differentiation induction and kept in the medium throughout the differentiation period (days 0−6). As shown in Figure 3A, pimozide significantly induced lipid accumulation. Quantification of the extracted oil red dye revealed that lipid accumulation levels increased 1.3-fold with the addition of pimozide at 10 μM (Figure 3A, right panel).

Given that FABP4 attenuates adipogenesis through the blockade of PPARγ,[20] we hypothesized that pimozide-induced adipogenesis requires PPARγ activation. The conversion of preadipocytes into adipocytes in response to the PPARγ requires the exposure of 3T3-L1 cells to an exogenous ligand, such as insulin. To investigate the role of PPARγ in pimozide-induced adipogenesis, we tested whether pimozide could promote 3T3-L1 adipogenesis without the differentiation cocktail. As shown in Figure 3A, the induction of lipid accumulation by pimozide was not observed without the addition of differentiation cocktail.

Recent study demonstrated that BMS309403, a well-known FABP4 inhibitor, binding to FABP4 might lead to an allosteric regulation of FABP4, and therefore resulting in the elevation of PPARγ protein expression. However, the blockade of FABP4 by BMS309403 did not affect the mRNA levels of PPARγ.[20] Here, we demonstrated that pimozide has no effect on mRNA levels of PPARγ (Figure 3B). Like BMS309403, pimozide upregulated PPARγ expression at protein levels at the end of the differentiation processes (Figure 3C). The elevated PPARγ protein level consequently enhanced the expression of lipogenic genes, including FABP4, CCAAT-enhancer-binding protein α (C/EBPα), and adiponectin. Unlike C/EBPα, the gene of C/EBPβ cannot be regulated by PPARγ.[14] Our work also demonstrated that the upregulation of PPARγ induced by pimozide did not alter mRNA expression level of C/EBPβ (Figure 3D).

To further explore the role of PPARγ in pimozide-induced adipogenesis, we applied PPARγ antagonist GW 9662 to establish the involvement of PPARγ in 3T3-L1 differentiations. As shown in Figure 4A, we found that pimozide-induced lipid accumulation was significantly attenuated by GW 9662. Furthermore, the cotreatment with pimozide and GW 9662 did not change mRNA expression of PPARγ or protein level of PPARγ in 3T3-L1 adipocytes (Figure 4B and C). GW 9662 subsequently decreased the upregulation of PPARγ target genes induced by pimozide (Figure 4D). Taken together, these results

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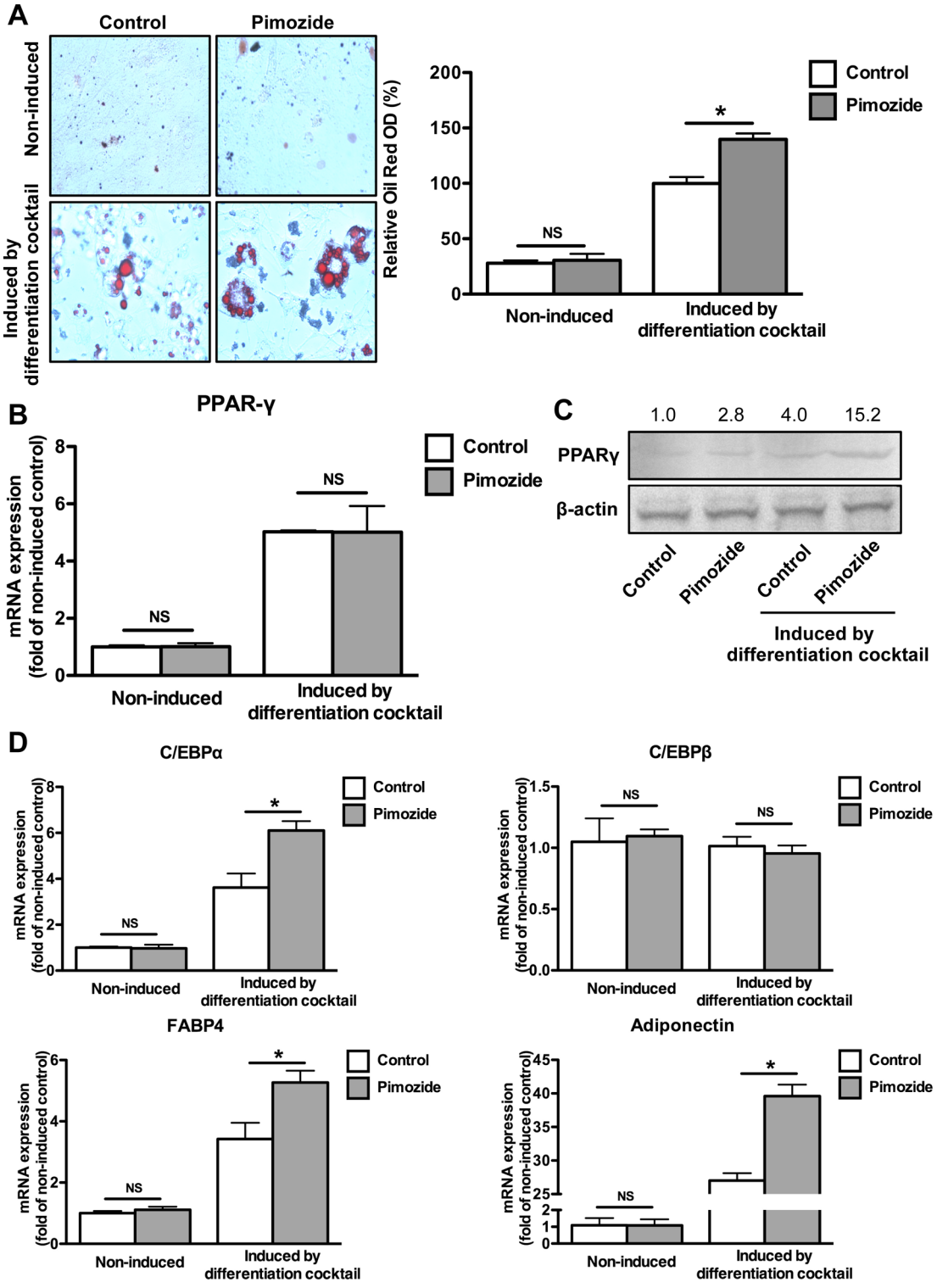

Figure 3. Pimozide induces adipogenesis and upregulates PPARγ expression. Confluent preadipocytes (day 0) were treated with pimozide and cultured for 6 days in the absence or in the presence differentiation cocktail. (A) At day 6, 3T3-L1 cells were stained with red oil and photographed macroscopically (A, left panel); then the stained red oil was washed by isopropanol and quantitative analysis of lipid droplet was determined by absorbance values of red oil (A, right panel). Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test. *$P < 0.05$, compared with the control group. (B) At day 6, the cells were harvested, the total mRNA was collected for reverse transcription, and quantitative PCR measured. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test. (C) At day 6, the cells were harvested and the total protein was collected for Western blot to measure PPARγ protein level. Numbers above bands correspond to the fold change compared with the control. (D) At day 6, the cells were harvested and the total mRNA was collected for reverse transcription and quantitative PCR to measure mRNA levels of PPARγ target genes. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test.

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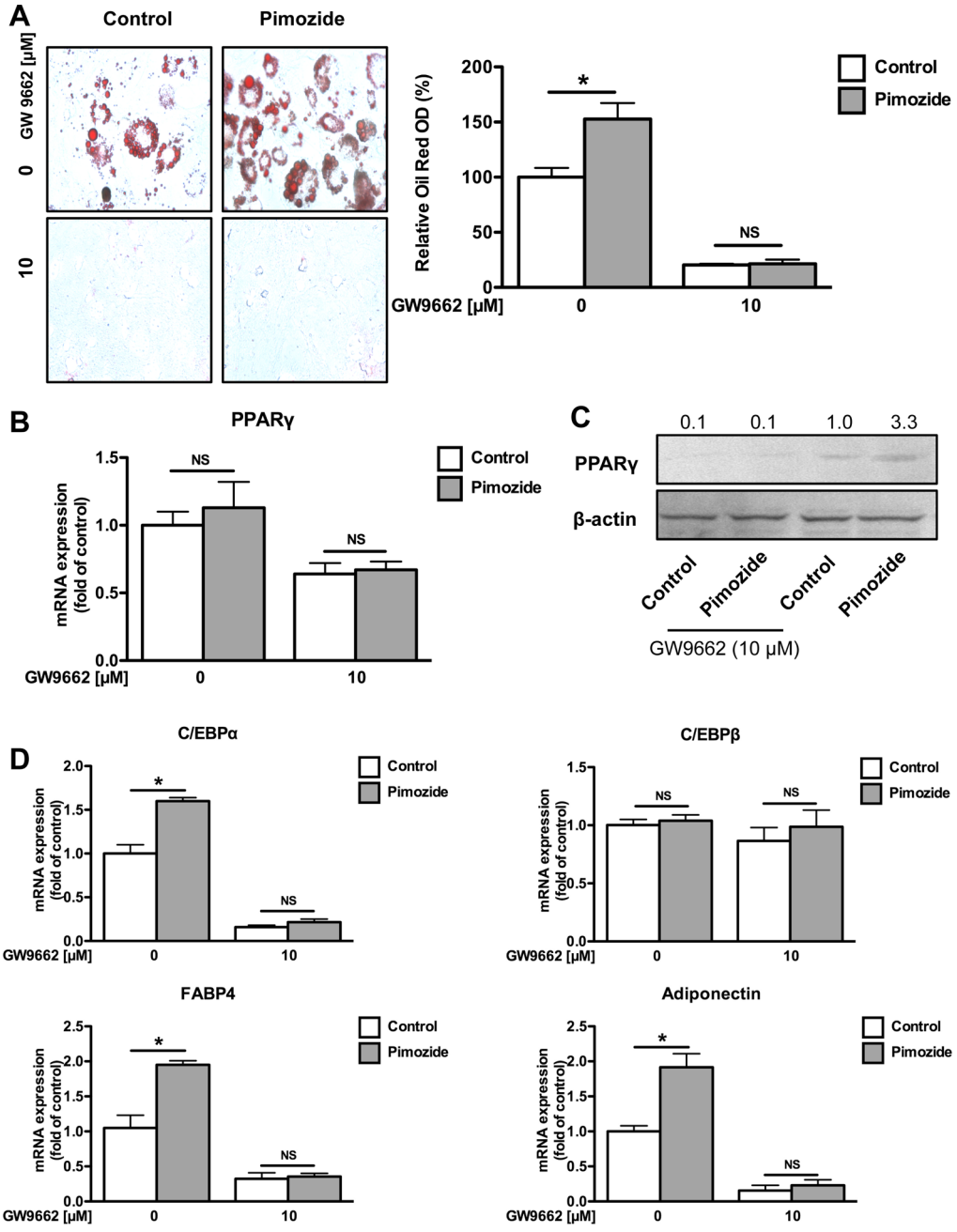

Figure 4. Pimozide induces adipogenesis through the upregulation of PPARγ protein expression. Confluent preadipocytes (day 0) were treated with pimozide and cultured for 6 days in the absence or in the presence of PPARγ inhibitor GW9662 (10 μM). (A) At day 6, 3T3-L1 cells were stained with red oil and photographed macroscopically (A, left panel); then the stained red oil was washed by isopropanol and quantitative analysis of lipid droplet was determined by absorbance values of red oil (A, right panel). Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test. *$P < 0.05$, compared with the control group. (B) At day 6, the cells were harvested and the total mRNA was collected for reverse transcription and quantitative PCR to measure mRNA levels of PPARγ. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test. (C) At day 6, the cells were harvested and the total protein was collected for Western blot to measure PPARγ protein level. Numbers above bands correspond to the fold change compared with the control. (D) At day 6, the cells were harvested and the total mRNA was collected for reverse transcription and quantitative PCR to measure mRNA levels of PPARγ target genes. Results were repeated at least three times from three independent experiments and presented as mean ± SD, $n = 3$. The results were statistically analyzed by one-way ANOVA test.

demonstrated that pimozide induces adipogenesis through the upregulation of PPARγ protein expression.

To further verify the role of FABP4 in pimozide-induced adipogenesis, we constructed 3T3-L1 cells overexpressing FABP4 to test whether pimozide induces adipogenesis through the inhibition of FABP4. Upon induction of differentiation, we found that 3T3-L1/FABP4 adipocytes accumulated a smaller number of lipid droplets compared with those accumulated in wild-type adipocytes. Furthermore, the induction of lipid accumulation by pimozide was not observed in 3T3-L1/FABP4 adipocytes (Figure 5A).

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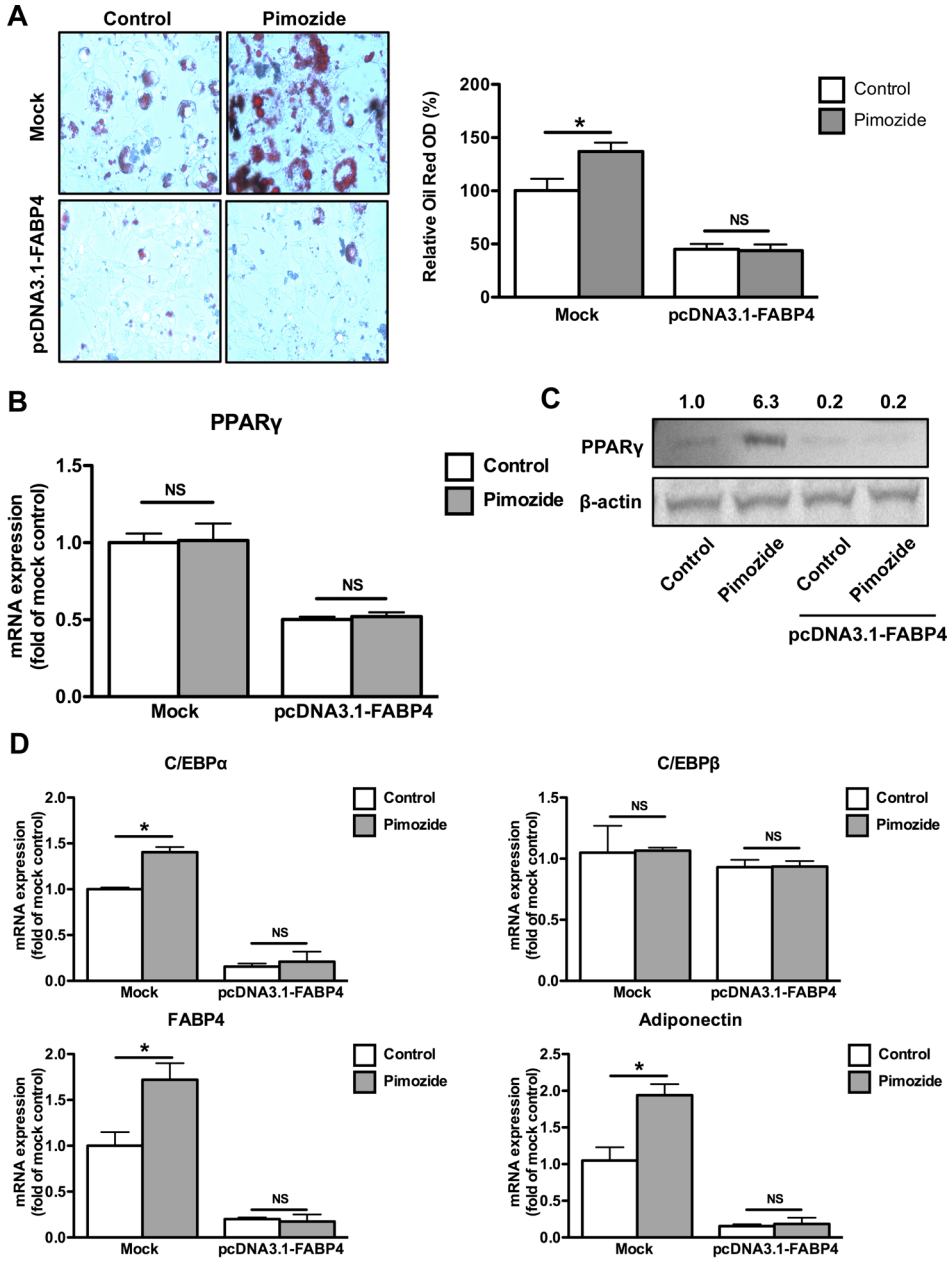

Figure 5. Overexpression of FABP4 rescues the induction of adipogenesis by pimozide in 3T3-L1 cells. Confluent preadipocytes (day 0) were transfected with pcDNA3.1-FABP4 or empty vector, and cultured for 6 days in the absence or in the presence pimozide (10 μM). (A) At day 6, 3T3-L1 cells were stained with red oil and photographed macroscopically (A, left panel); then the stained red oil was washed by isopropanol and quantitative analysis of lipid droplet was determined by absorbance values of red oil (A, right panel). Results were repeated at least three times from three independent experiments and presented as mean ± SD, n = 3. The results were statistically analyzed by one-way ANOVA test. *P < 0.05; compared with the control group. (B) At day 6, the cells were harvested and the total mRNA was collected for reverse transcription and quantitative PCR to measure PPARγ mRNA level. Results were repeated at least three times from three independent experiments and presented as mean ± SD, n = 3. The results were statistically analyzed by one-way ANOVA test. (C) At day 6, the cells were harvested and the total protein was collected for Western blot to measure PPARγ protein level. Numbers above bands correspond to the fold change compared with the control. (D) At day 6, the cells were harvested and the total mRNA was collected for reverse transcription and quantitative PCR to measure mRNA levels of PPARγ target genes. Results were repeated at least three times from three independent experiments and presented as mean ± SD, n = 3. The results were statistically analyzed by one-way ANOVA test.

Following quantitative PCR results showed that overexpression of FABP4 inhibited the upregulation of PPARγ on mRNA level during adipocyte differentiations. In addition, the treatment of pimozide did not change mRNA expression of PPARγ in both wild-type 3T3-L1 and 3T3-L1/FABP4 adipocytes (Figure 5B). Furthermore, overexpression of FABP4 attenuated the induction of PPARγ at protein levels by pimozide (Figure 5C). The upregulation of PPARγ target genes induced by pimozide was also inhibited by overexpression of FABP4 in 3T3-L1 cells. This result indicated that the induction of adipogenesis by pimozide depends on its inhibitory effect on FABP4.

Although FABP4 was reported to interact directly with PPARγ,[19] structural information on FABP4- PPARγ complex is

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still unknown. This kind of interaction was reported to trigger ubiquitination and subsequent proteasomal degradation of PPARγ. However, the interaction between FABP4 and PPARγ took place through a site distinct from the fatty acid binding pocket of FABP4.[20] Interestingly, BMS309403 directly bound to the fatty acid binding pocket of FABP4, but inhibited the interaction between FABP4 and PPARγ. In this respect, BMS309403 binding to FABP4 might lead to an allosteric regulation of FABP4, therefore resulting in the elevation of PPARγ protein expression.[20] Regarding the effects of the pimozide on 3T3-L1 adipocytes, our data suggested that pimozide binding to FABP4 might also lead to an allosteric regulation of FABP4. The allosteric regulation of FABP4 by pimozide consequently upregulated the protein expression of PPARγ, therefore resulting in the induction of adipogenesis in 3T3-L1 cells.

In summary, we identified pimozide as a novel FABP4 inhibitor. The inhibitory effect of pimozide on FABP4 promoted adipocyte differentiation with the potency proportional to their propensities for weight gain. These effects in adipogenesis may propose a possible mechanism to explain obesity that is frequently observed in patients treated with pimozide.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods are given in the PDF. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖Y.W., H.-Q.L., and W.-K.L. contributed equally to this work. D.C.-C.W. and Y.W. designed research; Y.W., H.-Q.L., W.-K.L., and J.-S.H performed research; Y.W., H.-Q.L., and W.-K.L. analyzed data; W.-C.L, J.-F.Z., T.-M.I., and M.M.-Y.W. contributed reagents/materials/analysis tools; and D.C.-C.W. and Y.W. wrote the paper.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Beninger, R. J., and Phillips, A. G. (1981) The effects of pimozide during pairing on the transfer of classical conditioning to an operant discrimination. *Pharmacol., Biochem. Behav. 14*, 101−105.

(2) Friedman, J. I., Lindenmayer, J. P., Alcantara, F., Bowler, S., Parak, M., White, L., Iskander, A., Parrella, M., Adler, D. N., Tsopelas, N. D., Tsai, W. Y., Novakovic, V., Harvey, P. D., Davis, K. L., and Kaushik, S. (2011) Pimozide augmentation of clozapine inpatients with schizophrenia and schizoaffective disorder unresponsive to clozapine monotherapy. *Neuropsychopharmacology 36*, 1289−1295.

(3) Bak, M., Fransen, A., Janssen, J., van Os, J., and Drukker, M. (2014) Almost all antipsychotics result in weight gain: a meta-analysis. *PLoS One 9*, e94112.

(4) Baptista, T. (1999) Body weight gain induced by antipsychotic drugs: Mechanisms and management. *Acta Psychiatr. Scand. 100*, 3−16.

(5) Vandereycken, W., and Pierloot, R. (1982) Pimozide combined with behavior therapy in the short-term treatment of anorexia nervosa. A double-blind placebo-controlled cross-over study. *Acta Psychiatr. Scand. 66*, 445−450.

(6) Gilbert, D. L., Batterson, J. R., Sethuraman, G., and Sallee, F. R. (2004) Tic reduction with risperidone versus pimozide in a randomized, double-blind, crossover trial. *J. Am. Acad. Child Adolesc Psychiatry 43*, 206−214.

(7) Poirier, P., Giles, T. D., Bray, G. A., Hong, Y., Stern, J. S., Pi-Sunyer, F. X., Eckel, R. H., American Heart, A., and Obesity Committee of the Council on Nutrition, P. A., and Metabolism (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: An update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation 113*, 898−918.

(8) Cuerda, C., Velasco, C., Merchan-Naranjo, J., Garcia-Peris, P., and Arango, C. (2014) The effects of second-generation antipsychotics on food intake, resting energy expenditure and physical activity. *Eur. J. Clin. Nutr. 68*, 146−152.

(9) Kroeze, W. K., Hufeisen, S. J., Popadak, B. A., Renock, S. M., Steinberg, S., Ernsberger, P., Jayathilake, K., Meltzer, H. Y., and Roth, B. L. (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology 28*, 519−526.

(10) Vestri, H. S., Maianu, L., Moellering, D. R., and Garvey, W. T. (2007) Atypical antipsychotic drugs directly impair insulin action in adipocytes: Effects on glucose transport, lipogenesis, and antilipolysis. *Neuropsychopharmacology 32*, 765−772.

(11) Singh, P., Peterson, T. E., Sert-Kuniyoshi, F. H., Glenn, J. A., Davison, D. E., Romero-Corral, A., Pusalavidyasagar, S., Jensen, M. D., and Somers, V. K. (2012) Leptin signaling in adipose tissue: Role in lipid accumulation and weight gain. *Circ. Res. 111*, 599−603.

(12) Oh, J. E., Cho, Y. M., Kwak, S. N., Kim, J. H., Lee, K. W., Jung, H., Jeong, S. W., and Kwon, O. J. (2012) Inhibition of mouse brown adipocyte differentiation by second-generation antipsychotics. *Exp. Mol. Med. 44*, 545−553.

(13) Yang, L. H., Chen, T. M., Yu, S. T., and Chen, Y. H. (2007) Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacol. Res. 56*, 202−208.

(14) Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M., and Evans, R. M. (2013) PPARgamma signaling and metabolism: the good, the bad and the future. *Nat. Med. (N. Y., NY, U. S.) 19*, 557−566.

(15) Saltiel, A. R., and Olefsky, J. M. (1996) Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes 45*, 1661−1669.

(16) Fonseca, V. (2003) Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am. J. Med. 115* (Suppl 8A), 42S−48S.

(17) Furuhashi, M., Tuncman, G., Gorgun, C. Z., Makowski, L., Atsumi, G., Vaillancourt, E., Kono, K., Babaev, V. R., Fazio, S., Linton, M. F., Sulsky, R., Robl, J. A., Parker, R. A., and Hotamisligil, G. S. (2007) Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature 447*, 959−965.

(18) Damcott, C. M., Moffett, S. P., Feingold, E., Barmada, M. M., Marshall, J. A., Hamman, R. F., and Ferrell, R. E. (2004) Genetic variation in fatty acid-binding protein-4 and peroxisome proliferator-activated receptor gamma interactively influence insulin sensitivity and body composition in males. *Metab., Clin. Exp. 53*, 303−309.

(19) Adida, A., and Spener, F. (2006) Adipocyte-type fatty acid-binding protein as inter-compartmental shuttle for peroxisome proliferator activated receptor gamma agonists in cultured cell. *Biochim. Biophys. Acta 1761*, 172−181.

(20) Garin-Shkolnik, T., Rudich, A., Hotamisligil, G. S., and Rubinstein, M. (2014) FABP4 attenuates PPARgamma and adipo-

genesis and is inversely correlated with PPARgamma in adipose tissues. *Diabetes 63*, 900−911.
(21) Wang, Y., Law, W. K., Hu, J. S., Lin, H. Q., Ip, T. M., and Wan, D. C. (2014) Discovery of FDA-Approved Drugs as Inhibitors of Fatty Acid Binding Protein 4 Using Molecular Docking Screening. *J. Chem. Inf. Model. 54*, 3046−3050.
(22) Ringom, R., Axen, E., Uppenberg, J., Lundback, T., Rondahl, L., and Barf, T. (2004) Substituted benzylamino-6-(trifluoromethyl)-pyrimidin-4(1H)-ones: A novel class of selective human A-FABP inhibitors. *Bioorg. Med. Chem. Lett. 14*, 4449−4452.
(23) Lehmann, F., Haile, S., Axen, E., Medina, C., Uppenberg, J., Svensson, S., Lundback, T., Rondahl, L., and Barf, T. (2004) Discovery of inhibitors of human adipocyte fatty acid-binding protein, a potential type 2 diabetes target. *Bioorg. Med. Chem. Lett. 14*, 4445−4448.
(24) Sulsky, R., Magnin, D. R., Huang, Y., Simpkins, L., Taunk, P., Patel, M., Zhu, Y., Stouch, T. R., Bassolino-Klimas, D., Parker, R., Harrity, T., Stoffel, R., Taylor, D. S., Lavoie, T. B., Kish, K., Jacobson, B. L., Sheriff, S., Adam, L. P., Ewing, W. R., and Robl, J. A. (2007) Potent and selective biphenyl azole inhibitors of adipocyte fatty acid binding protein (aFABP). *Bioorg. Med. Chem. Lett. 17*, 3511−3515.
(25) Barf, T., Lehmann, F., Hammer, K., Haile, S., Axen, E., Medina, C., Uppenberg, J., Svensson, S., Rondahl, L., and Lundback, T. (2009) N-Benzyl-indolo carboxylic acids: Design and synthesis of potent and selective adipocyte fatty-acid binding protein (A-FABP) inhibitors. *Bioorg. Med. Chem. Lett. 19*, 1745−1748.
(26) Cai, H. Y., Wang, T., Zhao, J. C., Sun, P., Yan, G. R., Ding, H. P., Li, Y. X., Wang, H. Y., Zhu, W. L., and Chen, K. X. (2013) Benzbromarone, an old uricosuric drug, inhibits human fatty acid binding protein 4 in vitro and lowers the blood glucose level in db/db mice. *Acta Pharmacol. Sin. 34*, 1397−1402.