CHEMMEDCHEM
COMMUNICATIONS

ChemPubSoc
Europe

DOI: 10.1002/cmdc.201400009

Design, Synthesis, and Activity Evaluation of GK/PPARγ Dual-Target-Directed Ligands as Hypoglycemic Agents

Jianxun Lu,[a] Lei Lei,[b] Yi Huan,[b] Yongqiang Li,[b] Lijing Zhang,[b] Zhufang Shen,[b] Wenxiang Hu,[a] and Zhiqiang Feng*[b]

Based on the multi-target strategy to treat type 2 diabetes mellitus (T2DM), glucokinase/peroxisome proliferator-activated receptor γ (GK/PPARγ) dual-target molecules were constructed by the rational combination of pharmacophores from known GK activators and PPARγ agonists. A series of dual-target agents were designed and synthesized, and their capacities to induce GK and PPARγ transcriptional activity were evaluated. Three of these compounds showed particularly high potency toward GK, moderate activity toward PPARγ, and their structure–activity relationships were preliminarily analyzed. The putative binding modes of one of the most promising compounds were also explored by molecular docking simulations with GK and PPARγ.

Type 2 diabetes mellitus (T2DM) is a progressive heterogeneous disease that affected more than 366 million patients worldwide in 2011. It is characterized by insulin resistance, excessive hepatic glucose production, and decreased glucose-triggered insulin secretion from pancreatic β cells. Although a number of T2DM-pathogenesis-targeting drugs have been marketed as anti-diabetic therapies, no single current available drug is capable of achieving sustained blood glucose control. Modulating multiple targets in the biological network simultaneously is recognized as being beneficial for treating a range of diseases, including cancer and T2DM.[1] At present, with the development of molecular biology, pathophysiology, and pharmacology, it has become feasible to develop multi-target therapies for T2DM to improve efficacy and to minimize side effects.[2] There is therefore a need for new and effective broad-spectrum pharmacological agents in this regard.

Glucokinase (GK) catalyzes the phosphorylation of glucose to glucose-6-phosphate as the first step in glycolysis, playing a crucial role in maintaining normoglycemia.[3] GK is predominantly expressed in the liver and pancreas. In pancreatic islets, GK catalyzes the rate-limiting step in glucose-stimulated insulin secretion, whereas in the liver, GK is required for glucose metabolism and glycogen synthesis.[4] GK therefore contributes to whole-body glucose disposal and has been suggested to act as the glucose sensor, playing an important role in both insulin secretion and hepatic glucose metabolism.[5] In recent years, several amide GK activators (Figure 1), including RO-28-1675 (Roche),[6] piragliatin (Roche),[7] GKA22 (AstraZeneca),[8] AZD-1656 (AstraZeneca),[9] LY2121260 (Lilly),[10] and PSN010 (Prosidion/OSI)[11] have been disclosed, and some of these compounds have entered clinical trials.

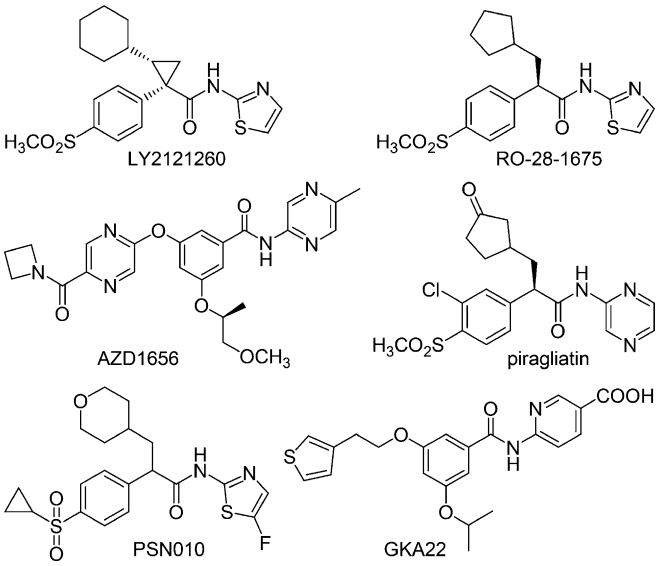


Figure 1. Representative structures of glucokinase activators.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily and are present as three isoforms: PPARα, PPARγ, and PPARβ. PPARγ is specifically expressed in adipose tissue and is a vital regulator of glucose and lipid metabolism. Rosiglitazone and pioglitazone (Figure 2) are the only approved PPARγ agonists for reversing insulin resistance and are currently in use for treating T2DM as insulin sensitizers.[12]

Based on the multi-target strategy to treat T2DM, this study was aimed at the design of an agonist that can activate both GK and PPARγ simultaneously. A wide range of biological effects from this dual-target agonist was expected, including promotion of insulin secretion and hepatic glucose metabolism, and restoration of insulin sensitization. A series of de-

[a] Dr. J. Lu, Dr. W. Hu
Capital Normal University
105 North Xisanhuan Road, Beijing, 100048 (China)

[b] L. Lei, Y. Huan, Y. Li, Dr. L. Zhang, Z. Shen, Z. Feng
Beijing Key Laboratory of Active Substance Discovery
and Druggability Evaluation, Institute of Materia Medica
Chinese Academy of Medical Sciences & Peking Union Medical College
100050 (China)
E-mail: fengzq@imm.ac.cn

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201400009.

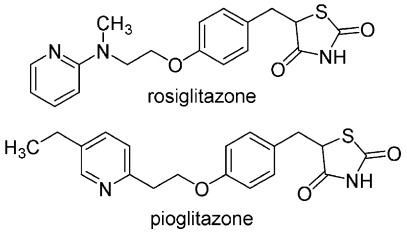


Figure 2. Structures of marketed PPARγ agonists rosiglitazone and pioglitazone.

signed dual-target ligand structures were synthesized, their activities in GK activation and PPARγ transcriptional activation were estimated, and the structure–activity relationship was analyzed.

Although complex diseases such as T2DM can benefit from complex treatment that modulates multiple targets, designing dual-target ligands with predefined biological profiles presents a new challenge. Dual-target ligand design has attracted much recent attention.[13] For target structure-based design, sequential docking and multiple cross-docking are straightforward strategies, which are all computationally demanding. Compared with this, ligand-based dual-target ligand design takes advantage of the chemical structures of known ligands, and attempts to combine them. The chances of deriving compounds that are active for one of the targets are high, while it is difficult to retain another desirable activity at the same time. Our rational design of GK/PPARγ dual-target ligands was mainly ligand-based, while molecular docking of the designed compounds to both GK and PPARγ was also performed to compare their interaction affinities. We started from the fusion of 2-amino-5-(4-methyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-methylthiazol-2-yl)benzamide (Figure 3), a GK activator reported by researchers at Merck-Banyu,[14] and 5-((2,4-dioxothiazolidin-5-yl)-methyl)-2-methoxy-*N*-(4-(trifluoromethyl)benzyl)benzamide or MK-767 (Figure 3), a PPARγ agonist also developed at Merck-Banyu.[15,16] Although these two compounds are different, they are similar in overall shape. They share the same benzamide scaffold and have definite pharmacophore structure: an alkaryl amidine for the GK activator, and a 2,4-thiazolidinedione moiety as a PPARγ agonist. The dual-target ligands were constructed by combining 2-(4-((2,4-dioxothiazolidin-5-yl)methyl)-phenyl)acetyl or 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-phenyl)acetyl and various 2-amino-nitrogen heteroaromatic systems. Molecular docking of these compounds to GK and PPARγ was carried out to select the ligand structure with good matching and high binding energy for both GK and PPARγ.

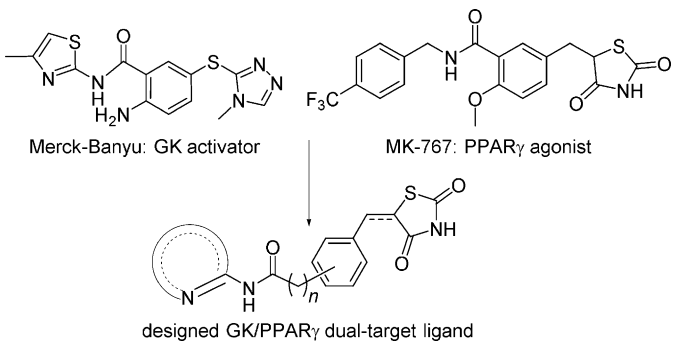
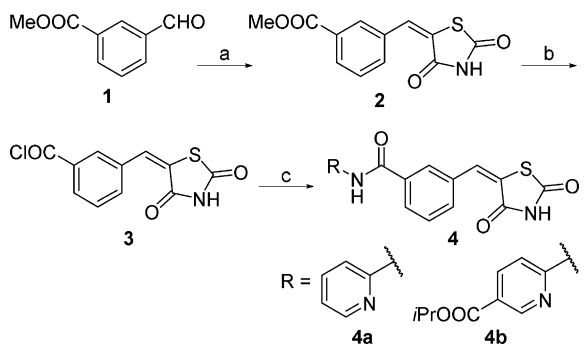


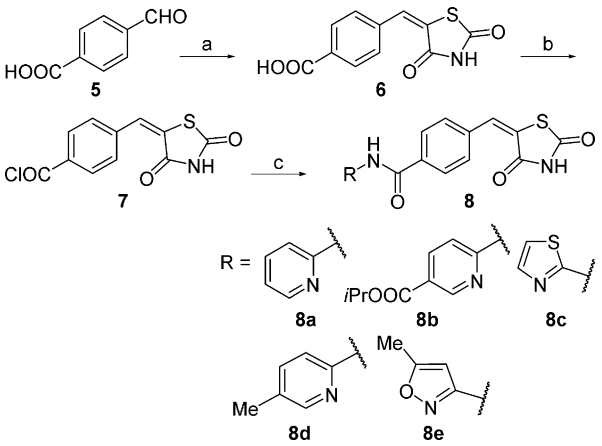
Figure 3. Design of GK/PPARγ dual-target ligands from a GK activator and a PPARγ agonist.

Synthesis of (*E*)-3-((2,4-dioxothiazolidin-5-ylidene)methyl benzamide derivatives **4a** and **4b** was carried out as depicted in Scheme 1. The intermediate **2**, from the condensation of 3-methoxyformyl benzaldehyde **1** and 2,4-thiazolidinedione, was hydrolyzed by lithium hydroxide and treated with oxalyl dichloride to afford compound **3**. Compound **3** was then treated with pyridin-2-amine and isopropyl-6-aminonicotinate to give target compounds **4a** and **4b**, respectively. (*E*)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzamide derivatives **8a**–**e** were prepared from 4-formylbenzoic acid **5**, avoiding the basic hydrolysis step, as depicted in Scheme 2.

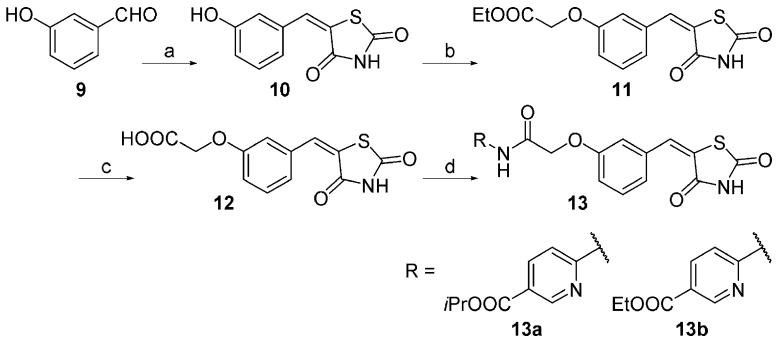


Scheme 1. *Reagents and conditions*: a) thiazolidinedione, piperidine, AcOH, toluene, reflux, 4 h; b) LiOH, MeOH, reflux, 5 h, toluene, $(COCl)_2$, reflux, 8 h; c) EDCI, DIPEA, DMF, 8 h.

(*E*)-2-(3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide derivatives **13a**,**b** were synthesized as depicted in Scheme 3. The condensation of 3-hydroxybenzaldehyde (**9**)



Scheme 2. *Reagents and conditions*: a) thiazolidinedione, piperidine, AcOH, toluene, reflux, 4 h; b) LiOH, MeOH, reflux, 5 h, toluene, $(COCl)_2$, reflux, 8 h; c) DIEPA, DMF, 8 h.



Scheme 3. *Reagents and conditions*: a) thiazolidinedione, piperidine, AcOH, toluene, reflux, 4 h; b) ethyl chloroacetate, acetone, K_2CO_3, reflux, 8 h; c) LiOH, THF, reflux, 8 h; d) EDCI, DIPEA, DMF, 8 h.

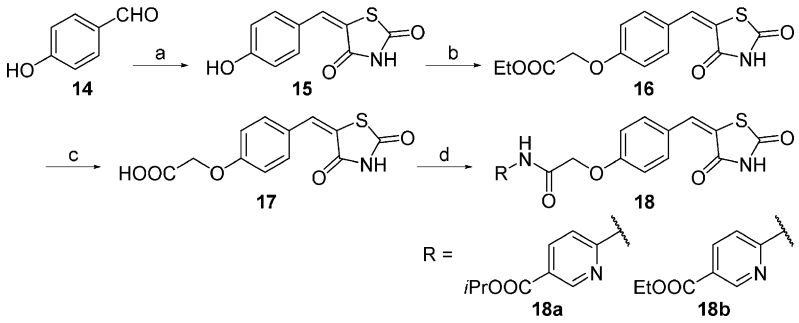
and 2,4-thiazolidinedione in toluene afforded intermediate **10**, which was then treated with ethyl 2-bromoacetic acid to provide compound **11**, followed by hydrolysis to produce compound **12**. Treatment with isopropyl 6-aminonicotinate or ethyl 6-aminonicotinate in the presence of EDCI and DIPEA afforded the desired target compounds **13a**,**b** as depicted in Scheme 3. (*E*)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide derivatives **18a**,**b** were prepared from 4-hydroxybenzaldehyde **14**, as depicted in Scheme 4.

Synthesis of (*E*)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-nitrophenoxy)acetamide derivatives **23a**,**b** was carried out as shown in Scheme 5. The condensation of 4-chloro-3-nitrobenzaldehyde (**19**) and 2,4-thiazolidinedione in toluene afforded the intermediate **20**, which was then treated with ethyl 2-bromoacetic acid to provide compound **21**. Compound **21** was hydrolyzed by lithium hydroxide to afford intermediate **22**. Then an intermolecular condensation between compound **22** and isopropyl 6-aminonicotinate or ethyl 6-aminonicotinate yielded target compounds **23a**,**b**.

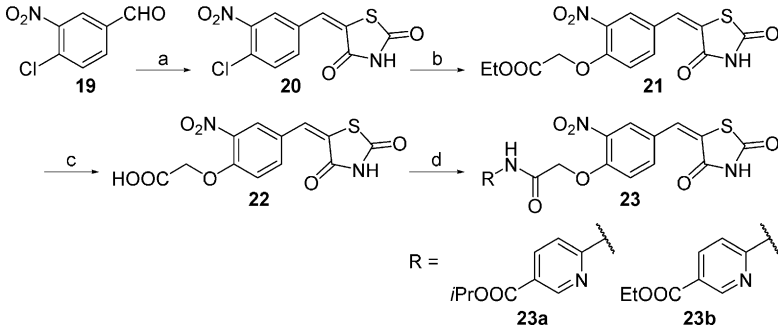
The preparation of (*E*)-2-(2-nitro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)pheny)acetamide derivatives **27a**–**l** and (*E*)-2-(2-amino-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl)acetamide derivatives **28a**–**c** was carried out as depicted in Scheme 6. The treatment of (*E*)-5-(4-chloro-3-nitrobenzylidene)thiazolidine-2,4-dione (**20**) with diethyl malonate and sodium hydride in DMSO afforded intermediate **24**, followed by acidic hydrolysis and decarboxylation to produce intermediate **25**. This was then reacted with various 2-amino-nitrogen heteroaromatics to give the target compounds **26a**–**l**; additional reduction with Pd/C and hydrogen in methanol gave target compounds **27a**–**c**.

All target compounds prepared herein were evaluated for GK activity at 10 μM, with a glucose concentration of 4 mM. The glucose concentration simulates moderate blood glucose conditions. The GK activity of compounds is expressed as GK fold activation; compound GKA22, a GK activator developed by AstraZeneca, was used as positive control in the activity assays. Potent compounds in GK activation were selected for further testing in a PPARγ transcription cell-based assay. The effect of tested compounds on the activity of PPARγ transcription was evaluated by luciferase reporter gene assay, and results are reported relative to the activity of rosiglitazone.

To evaluate the effect of the relative positions of the GK and PPARγ pharmacophores on the benzene core in GK activation, a series of *meta*- and *para*-oriented compounds (**4a**,**b** and **8a**–**e**) were first assayed (Table 1). Compared with *meta*-oriented compounds **4a**,**b**, the corresponding *para*-oriented compounds **8a**–**b** displayed improved potency for both GK and PPARγ, and 2-aminopyridine derivatives **8a**, **8b**, and **8d** exhib-

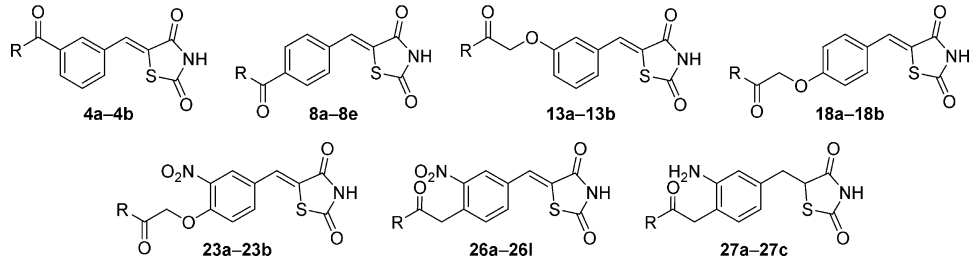


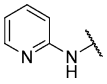
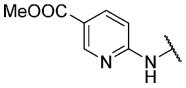
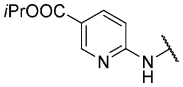
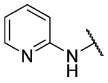
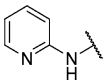
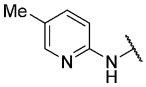
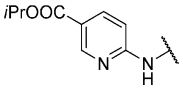
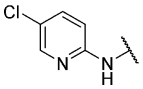
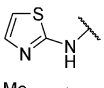
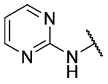
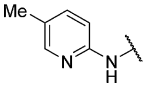
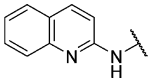
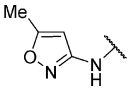
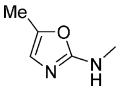
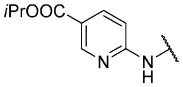
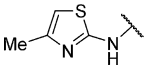
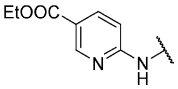
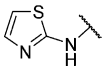
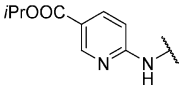
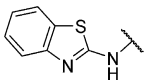
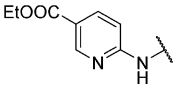
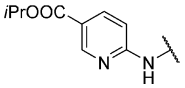
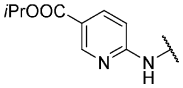
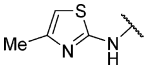
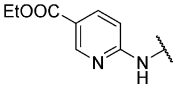
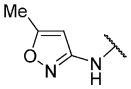
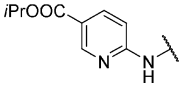
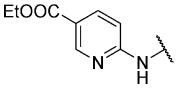
Scheme 4. *Reagents and conditions*: a) thiazolidinedione, piperidine, AcOH, toluene, reflux, 4 h; b) ethyl chloroacetate, acetone, K_2CO_3, reflux, 8 h; c) LiOH, THF, reflux, 8 h; d) EDCI, DIEPA, DMF, 8 h.



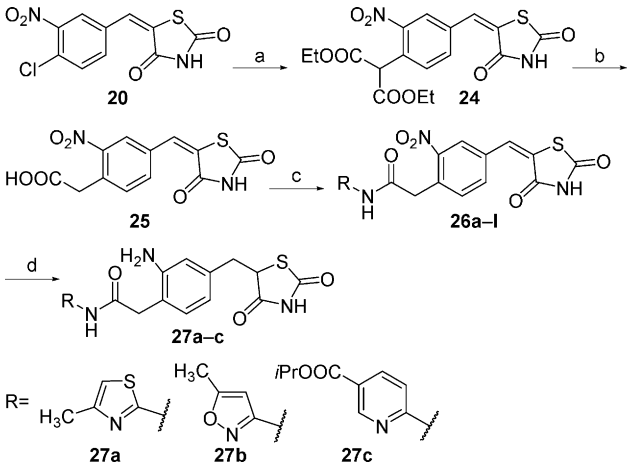
Scheme 5. *Reagents and conditions*: a) thiazolidinedione, piperidine, AcOH, toluene, reflux, 4 h; b) ethyl hydroxyacetate, acetone, K_2CO_3, reflux, 8 h; c) LiOH, THF, reflux, 8 h; d) EDCI, DIEPA, DMF, 8 h.

Table 1. Activities of synthetic target compounds toward GK and PPARγ.



Compd	R	GK [fold][a]	PPARγ [%][b]	Compd	R	GK [fold][a]	PPARγ [%][b]
4a		1.9	53	**26c**		2.4	91.3
4b		2.0	40	**26d**		2.9	47.1
8a		2.4	56	**26e**		2.9	25.6
8b		2.2	47	**26f**		2.5	46.7
8c		1.6	ND	**26g**		2.5	62
8d		1.8	ND	**26h**		2.6	38.9
8e		1.6	ND	**26i**		1.7	ND
13a		2.5	43	**26j**		2.5	55.9
13b		2.0	51	**26k**		2.3	33.9
18a		2.5	37	**26l**		2.3	34.9
18b		1.9	63	**27a**		2.8	22.8
23a		2.4	45	**27b**		3.3	17.1
23b		1.7	ND	**27c**		2.6	20.2
26a		2.9	48.0	GKA22		2.9	–
26b		2.7	47.4	rosiglitazone		–	100

[a] GK fold activation values were obtained at a Glc concentration of 4 mM. [b] The effect of test compounds (at 10 μM) on PPARγ transcription was evaluated by luciferase reporter gene assay, and values reflect the percent activity relative to that of rosiglitazone; ND: not determined. Each test was performed in triplicate, and the data in this table are the mean of triplicate measurements.



Scheme 6. *Reagents and conditions*: a) $CH_2(COOEt)_2$, NaH, 100 °C, 1 h; b) 4 N HCl, reflux, 12 h; c) EDCI, DIPEA, DMF, 12 h; d) Pd/C, H_2, MeOH, 50 psi.

ited better potency in GK activation than 2-amino-nitrogen five-member heteroaromatic ring compounds **8c** and **8e**.

Additionally, compounds **13a** and **18a**, each two bond lengths longer than the respective structures of compounds **4b** and **8b**, still showed slightly more potency than **4b** and **8b** for GK. Ethyl esters **13b** and **18b** displayed lower potency than the corresponding isopropyl esters **13a** and **18a** for GK, but higher potency than the corresponding isopropyl esters **13a** and **18a** for PPARγ, suggesting that ester size may impact the effect balance between GK and PPARγ. Furthermore, compounds **23a** and **23b**, with a nitro substituent on the benzene core of **18a** and **18b**, displayed slightly lower potency than **18a** and **18b** for GK, but slightly higher potency than **18a** and **18b** for PPARγ.

As expected, compounds **26a** and **26b**, shorter by one bond length relative to the base chain structure of compounds **23a** and **23b**, displayed conspicuously improved potency over corresponding compounds **23a** and **23b** in GK activation, and slightly improved potency for PPARγ. In addition, other 2-amino-nitrogen heteroaromatic amides exhibited improved potency for GK, such as compounds **26d** and **26e**, showing significant enhancement in potency with fold activation of 2.9, which is similar to that of GKA22, which displayed fold activation of 2.9 in our assay as well. However, compounds **27a** and **27b**, from reduced products of **26a** and **26j** at the nitro group and double bond, showed a noticeable decrease in potency for PPARγ.

For further understanding of the binding mode of the obtained dual-target active compound **26b** with these two targets, images of compound **26b** with GK and PPARγ were created by molecular docking simulations. Figure 4a suggests possible interactions between compound **26b** and GK. The compound might bind at the allosteric site of GK similar to the binding mode observed in the co-crystal structure of Merck-Banyu compound (Figure 3) in complex with GK.[14] There is also a bidentate hydrogen bond formed between the pyridine nitrogen atom and the NH group of the amide in compound **26b** with both the backbone NH and carbonyl oxygen atom of

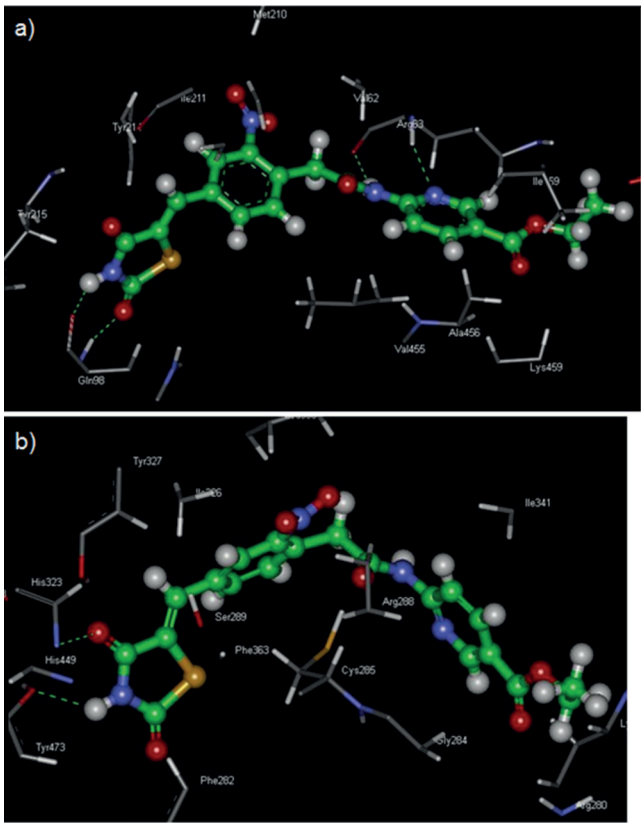


Figure 4. a) Putative binding mode of compound **26b** in GK (PDB ID: 3H1V). b) Putative binding mode of compound **26b** in PPARγ (PDB ID: 1FM6).

Arg63 on GK. The clear difference between the binding mode of compound **26b** in GK and that of the Merck-Banyu compound is that the aniline NH of Merck-Banyu additionally interacts with the hydroxy group of Tyr215 by hydrogen bonding, but the 2,4-thiazolidinedione moiety of compound **26b** can make two hydrogen bonding interactions with Gln98 of GK (Figure 4a), which may explain the potent GK activation observed. Figure 4b suggests the possible interactions between compound **26b** and PPARγ. The 2,4-thiazolidinedione moiety in compound **26b** occupies the only substantially polar cavity of the PPARγ ligand binding domain, similar to the binding mode of rosiglitazone, making a network of hydrogen bonds with the side chains of Tyr473 and His323. The main difference between the binding mode of **26b** and that of rosiglitazone may be the loss of two hydrogen bonds with His449 and Ser289 (Figure 4b). The absence of this strong interaction may be behind the significant decrease in the activity of PPARγ transcription.

In summary, we have described the ongoing development of a series of dual-target ligand compounds which can activate GK and PPARγ simultaneously. Biochemical evaluation and SAR of these compounds were also conducted preliminarily. Among these compounds, **26a**, **26b**, and **26d** showed high potency for GK, and moderate activity toward PPARγ. The possible binding modes of compound **26b** with GK and PPARγ were also explored preliminarily by molecular docking simulations. Based on these results, it can be expected that some

dual-target compounds may play a threefold role on hyperglycemia associated with T2DM. The discovery of compounds that act on two targets provides a new approach in the design of new and effective broad-spectrum anti-diabetic agents.

Acknowledgements

We gratefully acknowledge the generous support provided by the National S&T Major Special Project on Major New Drug Innovation (No. 2009zx09103-036) and the National Nature Science Foundation of China (No. 30572256).

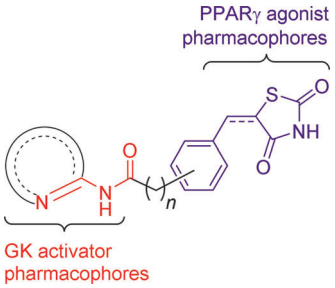
Keywords: activators · agonists · dual targeting · glucokinase · PPAR · receptors

[1] R. Morphy, C. Kay, Z. Rankovic, *Drug Discovery Today* **2004**, *9*, 641 – 651.
[2] P. Csermely, V. Agoston, S. Pongor, *Trends Pharmacol. Sci.* **2005**, *26*, 178 – 182.
[3] F. M. Matschinsky, *Diabetes* **1996**, *45*, 223 – 241.
[4] F. M. Matschinsky, *Diabetes* **2002**, *51*, S394 – S404.
[5] F. M. Matschinsky, *Nat. Rev. Drug Discovery* **2009**, *8*, 399 – 416.
[6] J. Grimsby, R. Sarabu, W. L. Corbett, N. E. Haynes, F. T. Bizzarro, J. W. Coffey, K. R. Guertin, D. W. Hilliard, R. F. Kester, P. E. Mahaney, L. Marcus, L. D. Qi, C. L. Spence, J. Tengi, M. A. Magnuson, C. A. Chu, M. T. Dvorozniak, F. M. Matschinsky, J. F. Grippo, *Science* **2003**, *301*, 370 – 373.
[7] J. Zhi, S. Zhai, M. E. Mulligan, J. Grimsby, C. Arbet-Engels, M. Boldrin, R. Balena, *Diabetologia* **2008**, *51 (Suppl. 1)*, S372.
[8] J. M. Bowler, K. L. Hervert, M. L. Kearley, B. G. Miller, *ACS Med. Chem. Lett.* **2013**, *4*, 580 – 584.
[9] R. Sarabu, J. Grimsby, *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 631 – 637.
[10] A. M. Efanov, D. G. Barrett, M. B. Brenner, S. L. Briggs, A. Delaunois, J. D. Durbin, U. Giese, H. H. Guo, M. Radloff, G. S. Gil, S. Sewing, Y. Wang, A. Weichert, A. Zaliani, J. Gromada, *Endocrinology* **2005**, *146*, 3696 – 3701.
[11] M. C. T. Fyfe, J. R. White, A. Taylor, R. Chatfield, E. Wargent, R. L. Printz, T. Sulpice, J. G. McCormack, M. J. Procter, C. Reynet, P. S. Widdowson, P. Wong-Kai-In, *Diabetologia* **2007**, *50*, 1277 – 1287.
[12] M. A. Malesker, *Pharmacotherapy* **2008**, *28*, 193 – 206.
[13] A. L. Hopkins, *Nat. Chem. Biol.* **2008**, *4*, 682 – 690.
[14] K. Kamata, M. Mitsuya, T. Nishimura, J. I. Eiki, Y. Nagata, *Structure* **2004**, *12*, 429 – 438.
[15] A. Rubenstrunk, R. Hanf, D. W. Hum, J. C. Fruchart, B. Staels, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2007**, *1771*, 1065 – 1081.
[16] A. C. Calkin, M. C. Thomas, *Curr. Opin. Invest. Drugs* **2003**, *4*, 444 – 448.

Received: January 2, 2014
Published online on ■■ ■■, 0000

COMMUNICATIONS

Two birds, one stone: GK/PPARγ dual-target ligands were constructed by rational combination of pharmacophores from known GK activators and PPARγ agonists. Their ability to induce GK and PPARγ transcriptional activity were evaluated, and the putative binding modes of one of the most promising compounds were also explored by molecular docking simulations with GK and PPARγ.



*J. Lu, L. Lei, Y. Huan, Y. Li, L. Zhang, Z. Shen, W. Hu, Z. Feng**

■■ – ■■

Design, Synthesis, and Activity Evaluation of GK/PPARγ Dual-Target-Directed Ligands as Hypoglycemic Agents